# TRANSACTIONS of the NATIONAL ACADEMY OF SCIENCE AND TECHNOLOGY

Philippines

# ABSTRACTS of PAPERS Presented during the 31st NAST Annual Scientific Meeting

Active Aging: Preparing for Quality Life

8-9 July 2009

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# 31st ANNUAL SCIENTIFIC MEETING

Active Aging: Preparing for Quality Life

8-9 July 2009; The Manila Hotel

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# MAPPING OF THE Rf GENE OF A CYTOPLASMIC MALE STERILE LINE OF RICE (Oryza sativa L.) DEVELOPED FROM A MUTAGENIZED SOURCE

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The cytoplasmic-genetic male sterility (CMS) and fertility restoration system has proven to be the most effective method in hybrid rice breeding. CMS system is controlled by the interaction of cytoplasmic genes and Rf genes in the nucleus. The inheritance and location of the Rf gene governing the pollen fertility restoration of IR73328A, a CMS-WA line with a mutagenized cytoplasm source, were determined in this study. The BC, population from a IR73328A/IR73330-83-1-2R/IR73328A cross consisting of 202 individuals were evaluated for both pollen and spikelet fertility. The segregation for pollen fertility of the population followed the 1 sterile: 3 fertile ratio expected for duplicate gene action in a backcross population. Bulked segregant analysis was performed to screen for polymorphic SSR markers to be utilized in the construction of the molecular map of the two Rf genes. The DNA of 20 completely sterile individuals (0-1% pollen fertility) and 20 fully fertile individuals (80-100%) from the population were pooled together to constitute the sterile and fertile bulks, respectively. The data from polymorphic markers along with the pollen fertility data were used to construct a linkage map with Mapmaker/Exp Ver. 3. Subsequent interval mapping done with Mapmaker/OTL revealed that one of the genes, Rf-4, is on the long arm of chromosome 10. It was determined to be flanked by SSR markers, RM6132 and RM171, in chromosome 10. It was detected to be 3.0 cM from RM6132 and 1.5 cM from RM171 (LOD = 13.08). The other Rf gene could not detected by the set of polymorphic markers used in this study. It was also confirmed that the STS marker S10019/BstUI was reliable for identifying a restorer line possessing the Rf-4 gene. Results of this study indicate that the genetic mechanism of fertility restoration in the mutagenized CMS-WA line, IR73328A, does not differ from that observed in other CMS-WA lines.

**Keywords**: Cytoplasmic male sterility, fertility restorer, duplicate gene action, interval mapping, linkage map, bulked segregant analysis

# GENETIC MAPPING OF TUNGRO RESISTANCE AND INTROGRESSION INTO NEW RICE VARIETIES

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Many of the new rice varieties, while high-yielding and often of acceptable grain and eating qualities, succumb to tungro, a devastating viral disease of rice causing staggering if not complete yield loss. Incorporating tungro resistance is an appropriate breeding target to secure farmers' yields with these varieties especially in tungro hotspot areas. The tungro resistance found in the Indian landrace, ARC11554, was localized on chromosome 4 flanked by markers RM8213 and RM3471 through quantitative trait loci (QTL) analysis. Marker-assisted breeding was carried out by crossing four varieties with ARC11554, followed by backcrossing with the recurrent parents to produce BC<sub>1</sub>F<sub>1</sub> seeds. Two BC<sub>2</sub>F<sub>1</sub> plants from ARC11554 x PJ25, three from ARC11554 x RC15, three from ARC11554 x RC130 and 1 from ARC11554 x PJ7 were heterozygous for RM8213 and RM3471 and further advanced to BC,F,. Genotyping was again performed and a few heterozygous plants were selected. Their BC,F, families were screened for tungro reaction and characterized for important morpho-agronomic traits. A number of tungro resistant near-isogenic lines (NIL) were obtained with either strong or poor morpho-agronomic resemblance to the respective original recurrent parents. This indicates that the resistance gene in ARC11554 was transmitted through marker selection. Based on morphoagronomic evaluation, the selected lines of PJ7, PJ25 and Rc15 denotes high similarity with the recurrent parent in number of days of heading and maturity, plant height, culm length, total tillers, unproductive tillers and productive tillers. With the Rc130-derived progeny, there were lines closely comparable to Rc130 while some were still dissimilar. These results demonstrate the successful application of molecular markers in transferring genes to outstanding varieties with minimal, deferred phenotyping, which is especially useful for traits with tedious screening procedures such as tungro

reaction. Future varieties that are susceptible to tungro can be similarly conferred by such resistance through marker-aided strategy.

Keywords: MAS, OTL, ARC11554, backcrossing

AS-3

# QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS FOR IRON TOXICITY TOLERANCE IN RICE (Orvza sativa L.)

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Iron toxicity is a major problem of the coastal areas in the tropics and also some specific regions like lowland areas of western Africa. As much as 60% of the lowland rice area in West and Central Africa are at risk with iron toxicity and could result to an average yield loss of 50%. In severe cases, it could contribute up to 90-100% yield reduction depending on the intensity of the toxicity and tolerance level of the rice cultivar. Understanding the genetic basis of iron toxicity tolerance in rice is a fundamental task for breeders and molecular biologists to develop new rice varieties with more iron toxicity tolerance characters. The study aims to investigate genetic factors controlling tolerance to Fe<sup>2+</sup> toxicity through investigation of the iron toxicity tolerance mechanism and identification of markers linked to iron toxicity tolerance in rice. A set of 350 F<sub>2</sub> individuals derived from a cross between Suakoko 8 (tolerant) and Bao Thai (sensitive) comprised the mapping population. The population was screened and evaluated for iron toxicity tolerance in the Phytotron at IRRI using six descriptive traits. A total of 720 simple sequence repeat markers covering the whole genome were used for the parental survey. Ninety-three markers (15%) were found to be polymorphic between the 2 parents and were used to screen the F, progenies Twelve putative OTLs for iron toxicity tolerance were detected through single marker analysis and interval mapping The identified markers located in chromosomes 3, 4, 5, 8 and 10 explain a small percentage of the total phenotypic variation as exhibited by the low individual LOD scores (2.51-4.48) The identification of 12 QTLs could help provide greater

understanding of the genetic basis contributing to iron toxicity.

**Keywords**: rice, iron toxicity, mapping population, QTL, markers

AS-4

# QUANTITATIVE RESISTANCE LOCI (QRL) AGAINST BACTERIAL BLIGHT (Xanthomonas oryza pv. oryzae) AND LEAF BLAST (Pyricularia orazae Sacc.) AND QUANTITATIVE TRAIT LOCI (QTL) FOR GRAIN QUALITIES IN RICE (Oryza sativa L.)

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A total of 174 BC, introgression lines (ILs) originally selected for drought tolerance and grain quality traits, derived from crosses IR64/Binam and Teqing/Binam were used to map quantitative resistance loci (QRL) against bacterial blight, Xanthomonas oryzae pv. oryzae (Xoo) and leaf blast, Pyricularia oryzae. Artificial inoculation was done under screenhouse conditions using Xoo races 6 and 9 and P. Oryzae isolates M64-1-3-9-1 and P06-6. Polymorphic SSR (simple sequence repeats) markers (182) were used for genotypic analysis. Three ILs from IR64/Binam population exhibited complete resistance (CR) to race 6, which was associated with six markers. Genotypic data indicated that the presence of donor alleles at all 6 loci is required for resistance, suggesting a strong epistasis between or among alleles at 6 loci. Twenty-eight ILs from IR64/Binam population exhibited CR to race 9. In the Teging/Binam population, partial resistance (PR) to race 6 was associated with four markers and 73 showed CR to race 9. One QRL for CR and two QRL for susceptibility to race 9 were identified. Two QRL from IR64 x Binam and one QRL from Teging x Binam population were identified as new QRL against M64-1-3-9-1. Three QRL from IR64 x Binam and one QRL from Teging x Binam population against P06-6 were identified. Quantitative trait loci (QTL) for grain quality traits (GQ) were mapped as well. Sixteen new OTL were associated with grain elongation.

Aroma and gelatinization temperature were contributed by Binam, with 60 and 21 putative QTL identified, respectively. Six marker loci were associated with GO traits and BB ORL for both race 6 and 9. More in depth exploration on the effect of all diseases to GO traits can be done to identify which specific QRL and QTL are interacting. This can be achieved by backcrossing the selected ILs to the recurrent parent or ultimately by cloning the QRL.

Keywords: quantitative trait loci, quantitative resistance loci, bacterial blight, leaf blast, grain quality

### **AS-5**

# MAPPING QUANTITATIVE TRAIT LOCI ASSOCIATED WITH RESISTANCE TO PREHARVEST SPROUTING IN WHEAT

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Preharvest sprouting (PHS) is the precocious germination of the grains in the spike following physiological maturity. In wheat, the main problem associated with PHS is reduction in end-product quality. White wheats are the most susceptible class of wheat to PHS whereas the red wheats have high levels of resistance due to the pleiotropic effect of the red color genes with dormancy. However, recent studies reported that several sources of resistance to PHS are available in white wheat germplasm. Our objective was to map quantitative trait loci (QTL) associated with PHS resistance in a recombinant inbred population of 94 lines from a cross between Grandin\*5/ND614-A, an elite hard white spring wheat susceptible to PHS and NY6432-18/Clark's Cream 40-1, a soft white winter wheat selected for its high level of PHS resistance. Multiple intervals mapping analysis revealed seven QTL for PHS based on the combined data across eight environments over three years (2005-2007). The QTL on chromosomes 2D, 5A and 7A had LOD score  $\geq 2.5$  and were not associated with QTL for plant

height and heading date. These three OTL jointly explained 39.4% of the phenotypic variation for PHS. Other QTL were found on chromosomes 1B, 4B, 6A and 6D that explained 2.1% to 14.2% of the phenotypic variation. A significant QTL x QTL interaction was found between the chromosomal regions in 1B and 2D that explained 5.5% of the phenotypic variation. Some of the QTL found in this study may aid in marker-assisted breeding for improvement of PHS resistance in wheat.

Keywords: preharvest sprouting, MAS, marker-aided selection, wheat, SSR

AS-6

GENETIC DIVERSITY ANALYSIS USING SSR MARKERS OF VARIETIES OF RICE (Oryza sativa L.) FROM INDONESIA AND ARAKAN, COTABATO, PHILIPPINES IN RELATION TO RESISTANCE TO RICE BLAST PATHOGEN (Magnaporthe grisea) RACES FOUND IN THE PHILIPPINES

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Genetic diversity of the traditional and improved traditional varieties of rice (Oryza sativa L.) from Indonesia, as well as, varieties from Arakan, Cotabato, Philippines was screened using Simple Sequence Repeat (SSR) markers. A total of 30 SSR markers were used for the Indonesian rice varieties, and 33 markers were used for the Arakan rice varieties. The polymorphic information content (PIC) value of the markers ranged from 0 to 0.855 for Indonesian varieties, while the PIC value range in the Arakan varieties was from 0 (RM504) to 0.814 (RM507). A phenotypic tree plot was constructed based on diseased leaf area response (DLA) to nine rice blast races in the Philippines using the Numerical Taxonomy System (NTSYS) software. The phenotypic similarity within Indonesian varieties ranged from 0.08 to 1, while the Arakan estimate for phenotypic similarity ranged from 0.09 to 0.89. Based on the genetic tree plot constructed, the estimate for genetic similarity ranged from 0.24 to 0.91 (between Sirendah 7A and

Sirendah 7B) and 0.21 to 0.94 (between Dinorado 35 and Dinorado 36) for Indonesian and Arakan varieties, respectively. These figures suggest that both germplasm are phenotypically and genetically diverse. Using the Statistical Analysis System (SAS) software, the stepwise regression analysis between the SSR markers and phenotypic response for each rice blast race was calculated. The data showed that 59 alleles in the Indonesian rice germplasm, and 91 alleles in the Arakan rice varieties amplified by the SSR markers were significantly correlated to rice blast resistance at 5% level of significance.

Keywords: Oryza sativa L., genetic diversity, SSR markers, rice blast resistance, phenotypic similarity, genetic tree plot

AS-7

# DETECTION OF RICE TUNGRO VIRUSES BY CONVENTIONAL REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR)

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Tungro is the most destructive viral disease of rice. The disease shows chlorotic leaf and dwarfing symptoms that dramatically reduce yield. Tungro is primarily caused by rice tungo spherical virus (RTSV) interacting with rice tungro bacilliform virus (RTBV). RTSV has a ribonucleic acid (RNA) genome while RTBV has a deoxyribonucleic acid (DNA) genome. Accurate detection of viral infection is imperative in screening for resistance in breeding materials. RTBV infection is amenable to detection by polymerase chain reaction (PCR), which can amplify *in-vitro* parts of its DNA genome. Through the use of reverse transcriptase (RT), parts of the RTSV RNA genome can be converted to cDNA, which then becomes amplifiable by PCR. Multiplexing or adding virus-specific primers in the same reaction – called multiplex or mRT-PCR – makes it possible to detect both viruses simultaneously. Furthermore, unlike the serological method Enzyme Linked Immunosorbent Assay (ELISA), RT-PCR has great advantage when it comes to procedure, time and efficiency. Our laboratory has succeeded in mRT-PCR detection of RTBV and RTSV in infected samples using commercial kits. This study now demonstrates that conventional RT-PCR with separate reagents can also efficiently amplify RTBV and RTSV genomic fragments from infected samples. CP1F1/R1 is the most suitable primer for RTBV detection, producing the virus-specific 0.62 kb band in infected samples. On the other hand, CP3F1/R1 was the most consistent pair in producing the RTSV-specific 0.55 kb band in infected samples. The best annealing temperature was 56°C for both RTBV and RTSV primers. Based on Kappa value analysis, RT-PCR is comparable to ELISA for RTBV detection, but it is superior to ELISA for RTSV detection. Therefore, the use of conventional, separate PCR reagents proved effective in screening for the tungro viruses, thus rendering RT-PCR more affordable. The optimal procedure can now be applied to routine tungro screening of breeding materials.

Keywords: ELISA, RTBV, RTSV, Multiplex PCR

AS-8

# MOLECULAR AND PHENOTYPIC STUDIES OF RESISTANCE GENES INTROGRESSED FROM WILD TOMATO (Lycopersicon chilense) TO CULTIVATED TOMATO (Lycopersicon esculentum) AGAINST TOMATO LEAF CURL VIRUS ISOLATE FROM THE PHILIPPINES

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The Tomato leaf curl virus disease caused by the whitefly-transmitted geminivirus (WTG) seriously affects production and cultivation of tomato worldwide. Since no cultivar of tomato ever displayed resistance to leaf curl diseases, breeding efforts towards resistance were focused on utilizing wild tomato genetic resources. However, progress in breeding for resistance is slow because of the complex genetics of resistance and its variable expression to different isolates of the virus from different geographical areas.

Mapping for resistance genes against WTG and marker development have been done for marker-assisted breeding for ToLCV resistance. Three introgression regions in tomato line FLA456-4 derived from wild relative Lycopersicon chilense were associated for resistance against WTG in Indonesia. The regions are putatively located in chromosomes 3, 6, and 11. The effectivity of these genetic factors was evaluated against *Tomato leaf* curl virus Philippines strain (ToLCPV), specifically with Los Baños isolate. By phenotypic characterization of the F, introgression families of FLA456-4 x CLN1466J cross, these regions were validated; with emphasis in chromosomes 3 and 11. Introgression lines that carry combinations of the three L. chilense-derived genomic segments from FLA456-4 are represented in these F3 families.

A (FLA456-4 x Super Apollo)F, population was also used for molecular characterization of the resistance genes against ToLCPV. In this mapping population, the introgressed regions in chromosome 3 and 6 were validated based on strong association (Likelihood Ratio 19.0 at  $P \le 0.001$ ) between the molecular markers that tag the L. chilense segments and the disease response of the population expressed as area under the disease progress curve (AUDPC) against ToLCPV-Los Baños isolate. By composite interval mapping, three quantitative trait loci (QTL) for resistance were precisely mapped with the CAPS markers for L. chilense and a candidate sequence for plant resistance gene, RGA marker TO-32. The QTL with the largest effect (71.9%) was mapped in chromosome 6. Results of this study validate the whitefly transmitted geminivirus (WTG) resistance in tomato derived from L. chilense, in particular, the introgressed segments in chromosome 3 and 6 against ToLCPV-Los Baños isolate.

Keywords; tomato, Tomato leaf curl virus, resistance, mapping, molecular marker

# TRANSGENIC RICE PLANTS OVEREXPRESSING HOST TRANSCRIPTION FACTORS Rf2A AND Rf2B ARE TOLERANT TO TUNGRO DISEASE

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Rice tungro disease (RTD) causes tremendous losses in the Philippines and other rice growing countries. Recent molecular studies have revealed interactions between specific regions of the rice tungro bacilliform virus (RTBV) promoter and host proteins resulting in the development of disease symptoms. One plausible explanation for this observation is that the endogenous transcription factors, called RF2a and RF2b, become sequestered by the viruses during infection and their limiting status disrupts plant development. To test this hypothesis, four Taipei 309-derived transgenic rice lines over-expressing RF2a or RF2b together with wild type Taipei 309, resistant check Matatag 6 and susceptible check TN1 were infected with viruliferous green leafhopper 25 days after sowing. Symptom development and ELISA indices for the two viruses were monitored daily until the eighth day and subsequently at 5-day intervals up to 55 days after inoculation (DAI). Matatag 6 maintained a steady almost undetectable RTBV titer up to 55 DAI. For all the other entries, a generally increasing trend in the RTBV titer that peaked at 30-45 DAI was observed. At 55 DAI, RTBV titers were comparable to the 10 DAI levels. For RTSV, titers were also barely detectable in Matatag 6 but there was a slow upward trend for all the other entries. In terms of disease incidence, both TN1 and wild type Taipei 309 exhibited severe symptoms. The transgenic lines exhibited less severe symptoms and at certain points some lines were comparable to Matatag 6. Most of the transgenic plants recovered from tungro damage at 30 DAI. Among the four transgenic lines, RbMT6 had the highest number of healthy plants at 34.6% as opposed to 26.1% in Matatag 6, the resistant The improved resistance of transgenic lines suggests that overexpressing RF2a and RF2b transcription factors in the plant may reduce the symptoms associated with RTD.

Keywords: rice tungro disease, ELISA, transcription factors, transgenic rice, gene overexpression

AS-10

# TOWARDS THE DEVELOPMENT OF CORN GENOTYPES FOR HIGH NITROGEN USE EFFICIENCY UNDER DROUGHT CONDITIONS

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The damage due to drought can be devastating; in comproduction, this could amount to billions of pesos. Large losses can be avoided by developing corn varieties that have the combined abilities of producing high yield, and efficient absorption and utilization of nutrients under water-limited conditions. The first step towards this goal is to develop a selection technique and corn populations with the desired traits.

To evaluate and select corn genotypes with drought tolerance and high nitrogen use efficiency, corn seedlings were grown for 3 to 4 weeks in sand culture in the greenhouse. Succeeding experiments, under field conditions in Laguna and Ilocos Norte, measured the grain yields of selected com genotypes. Results of field experiments were correlated with those from the greenhouse trials. Treatments were different levels of nitrogen fertilizer (0, 60 and 120 kg N ha') and water stress (irrigated and non-irrigated).

A protocol for rapid greenhouse screening at seedling stage was developed that was able to identify genotypes with high shoot length, shoot weight, root length, root weight, total N-uptake and total dry matter yield after drought treatment. These genotypes were also found to have relatively higher grain yield when grown under field drought conditions. Under intermediate degree of drought, they also have higher nitrogen use efficiency potential. The selected white corn genotypes are CML 8, P6-1-3, (P6-1-3 x CML 337), (CML 8 x CBR/PDC 2-5-2-4-5) and, while the yellow com genotypes are SMCE, (95-6 x Pi 23), (CLOO331 x Pi 23).

The performance of the drought-tolerant F1 hybrids which were generated will be validated on-farm before their recommendation for variety release.

**Keywords**: corn, screening, water stress, nitrogen use efficiency, drought, limited water

### **AS-11**

# DEVELOPMENT OF HIGH YIELDING AND BUNCHY TOP VIRUS RESISTANT ABACA (Musa textilis Nee.) CULTIVARS

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Abaca (*Musa textilis* Nee.), a plant endemic to the Philippines, is the source of fiber known internationally as Manila hemp. It is often used as raw material for cordage, clothing, various handicrafts, specialty papers such as currency notes, filter papers, stencil papers, tea bags among others. The abaca industry is a major dollar earner of the country. Due to the current concern for biodegradable products and forest conservation, it is expected that the abaca industry will continue to flourish in both domestic and international markets. With the advent of new uses of abaca, the crop will be extensively utilized for more industrial applications because it is a natural and superior material. However, the abaca industry is still relying solely on traditional varieties for its survival. One reason for the decline in the abaca industry is the limited attention devoted to varietal improvement. The old abaca varieties have become susceptible to bunchy top virus disease. This study aims to develop abaca varieties with high fiber quality and resistance to abaca bunchy top virus through conventional breeding.

In 1981, the Institute of Plant Breeding (IPB), resumed work on the abaca breeding program started at the Division of Plant Breeding in the late 1950's. Crosses between abaca and its wild relatives were produced. In 1986, six F1 hybrids between *Pacol* and abaca were released. These hybrids have resistance to bunchy top virus but of inferior fiber quality. To recover the superior fiber qualities of abaca, several backcross (BC1) lines were generated but crossing work was ended due to unavailability of funds. It was only in 2006, that the breeding work was continued although to a limited

extent, and several BC1 crosses were evaluated. BC2 progenies, produced from cross-pollinating four promising backcross lines (BC1) and two abaca varieties as recurrent parents were established in the field for screening of virus disease resistance and evaluation of fiber quality. Four BC2 populations were generated from the following crosses: 'BC1-19 X Abuab', 'BC1-20 X Abuab', 'BC1-21 X Abuab' and 'BC1-19 X Musa tex 51'. One hundred sixty six (166) inoculated seedlings (12 from BC1-19 X Abuab; 132 from BC1-20 X Abuab', and 22 from 'BC1-19 X Musa tex 51) were selected for field planting based on bunchy-top virus resistance, plant vigor and resemblance of morphological characters to true abaca. Characterization of agro-morphological traits was initiated on mature BC2 plants. The BC2 progenies were compared to the abaca parent on the following traits: plant height, plant diameter, fresh weight of stem, number of leaf sheaths and dry weight of fiber. Other visible traits indicative of resemblance to the true abaca were also observed. Abaca fiber samples were collected from each of the characterized BC2 plants and processed for determination of fiber quality. Data on fiber length, fiber recovery and fiber breaking load of BC2 selections were gathered. Superior BC2 segregants exhibiting good fiber qualities and desirable agro-morphological traits were selected for multilocation testing and clonal propagation.

Keywords: Abaca (Musa textilis Nee.), Abaca bunchy top virus, Abaca fibers, BC1- first backcross progeny, BC2- second backcross progeny

AS-12

# DEVELOPMENT OF IMPROVED VARIETIES OF MUNGBEAN (NSIC Mg14 AND NSIC Mg15)

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Mungbean is one of the popular and important 4crops in the Philippines. It can be processed into noodles, "togue", delicacies, novelty products etc. Its importance in agriculture and industry becomes the basis to conduct varietal development at BPI-LBNCRDC.

Promising lines of mungbean were field evaluated under the Preliminary Yield Trial (PYT) in 1996 to 2000 and General Yield Trial (GYT) in 1997 to 2004. Potential varieties from the GYT were further screened in different regions of the country under the National Cooperative Trial (NCT) in 2002 to 2006. Evaluation was conducted to screen and develop varieties with high bean yield, early and uniform maturity, resistant to lodging/shattering and resistant to natural occurrence of pests.

Based on the outstanding performance on-station/across locations, two (2) varieties were approved and released by the National Seed Industry Council (NSIC) in 2004 and 2007 as seedboard varieties for commercial production. Lines EGM 93-266 was approved in 2004 as NSIC Mg14 with local name "Kulabo" and EGM 93-293 as NSIC Mg15 locally named as "Kinang" in 2007.

A total of 217.45 kg breeder and foundation seeds of NSIC Mg14 and NSIC Mg15 were distributed by BPI-LBNCRDC in different regions of the country. Bulk of the distribution amounting to 168.95 kg was recorded in Region 4 particularly in Los Baños, Laguna.

**Keywords:** preliminary yield trial, general yield trial, national cooperative trial, national seed industry council, varietal improvement

### AS-13

# TOWARDS ESTABLISHMENT OF MUTANT TOMATO GERMPLASM

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Tomato (Lycopersicon esculentum L.) is one of the popular and important vegetable crops grown worldwide. It is the most important crop in the fresh and processed vegetable market. Current breeding efforts are geared towards the incorporation of disease resistance genes, enhanced quality traits and other important traits required by the tomato crop to sustain productivity under biotic and abiotic limiting conditions. As sources for genetic stocks, breeding materials are resourced from within the Lycopersicon and wild relatives.

Large M1 populations of tomato H7996 were established using physical (Cobalt 60 gamma ray) and chemical (ethylmethane sulfonate, EMS) mutagens. The mutant germplasm will be used as a rich source of genetic materials to intensify crop improvement and genetic studies in tomato.

From 5000 mutagenized seeds each using gamma ray (600 Gy) and EMS (1%), a total of 465 - Gy and 1012 - EMS M1 seedlings had been transplanted. Only 380-Gy and 633-EMS M1 plants produced fruits. Three (3) fruits from different normal branches and three (3) fruits from visible mutant branch were separately sampled to derive the M2 generation of tomato mutants. Visible mutants were identified in 67-Gy and 146-EMS M1 plants. However, only 16-Gy and 60-EMS M1 plants produced fruits. The most common dominant and visible mutations observed in the M, screening were monopodial, compact, short internodes, multi-branch plant type, light yellow and ghost leaf coloration, tiny and long pedicel leaf morphology and small or short plant size. Morphological characterization of the M2 mutant families is on-going.

**Keywords:** tomato mutants, gamma ray irradiation, EMS

### AS-14

# TISSUE CULTURED AVOCADO (Persea americana MILLER): A JOURNEY FROM LABORATORY TO FIELD

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Avocado (Persea americana Miller) ranks fifth in terms of world production of tropical fruits next to banana, mango, pineapple and papaya (Faylon et al. 2006). Worldwide attention is now focused on genetically improving the avocado by plant biotechnological techniques particularly in vitro mutation and selection and genetic engineering.

Hundreds of potential mutant and variant avocado lines from 'Semil', 'Mainit' and other seedling trees were produced from somatic embryos (SE) and mature zygotic embryos following tissue culture and gamma irradiation at the Institute of Plant Breeding, College of Agriculture, UPLB (Avenido *et al.* 2005). However, subsequent seedling establishment in the greenhouse has been delayed by the absence of roots and slow shoot growth among the SE-derived regenerants. Micro-grafting improved shoot growth but was tedious. This study was conducted to develop an improved system of rooting, hardening and potting out to maximize survival and greenhouse establishment of avocado regenerants.

An alternative technique was developed by rooting *in vitro* regenerated shoots. Highest rooting percentage (50%) was in Barba and Pateña's medium, then Murashige and Skoog's medium (25%) and last, the Woody Plant medium (15%). Rooting was doubled from 38% to 62% and was induced earlier (i.e., 17 days) when more vigorous shoot regenerants were used. When rooted plantlets were transplanted, a 100% survival was obtained using a modified hydroponics system in a lighted (50 µE m<sup>-2</sup> s<sup>-1</sup>) airconditioned growth room. Moreover, average survival rates of 83% and 92% were obtained among the untreated and IBA-treated regenerants, respectively. With the successful rooting and transplant of regenerants to soil, a complete micropropagation protocol for avocado was established.

A total of seventeen (17) avocado regenerants have been successfully potted out and established under screen house conditions. Of these tissue culture-derived avocado plants, 6 were produced through somatic embryogenesis from cv. 'Semil' and 'Mainit' strain while 11 were derived via shoot organogenesis from 'Mainit', 'Semil', 'San Felix' and 'Calauan' avocado. Moreover, 5 of these plants were produced from gamma-irradiated somatic embryos and shoot cultures (5 and 20 Gy) while the rest were derived from non-irradiated cultures. These avocado lines whose average heights range from 7.0 to 23.5 (ave.=14.2 cm) are now ready for field planting and subsequent evaluation for horticultural characters including mutant genetic screening.

**Keywords:** avocado, *ex vitro* establishment, fruit crop improvement, micropropagation, mutant induction, somatic embryogenesis, tissue culture

# KINETICS OF IRON IN THE DEVELOPMENT OF RAPID SCREENING TECHNIQUE FOR IRON TOXICITY TOLERANCE IN RICE

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The establishment of reproducible, efficient and reliable screening technique is a pre-requisite in developing iron-toxicity tolerant cultivars. An experiment was conducted under controlled conditions at International Rice Research Institute (IRRI) Phytotron facilities to standardize screening protocol in rice. Kinetics of iron transformation in different treatment combinations and plant's response served as bases for selection. About 300 ppm of reduced iron (Fe<sup>22</sup>) in the form of ferrous sulfate (FeSO<sub>4</sub>) is optimum to differentiate the tolerant from the sensitive rice cultivars. An acidic environment (pH 4.0) and the presence of chelator EDTA maintained considerable level of excess iron in the solution for five days. The screening technique was validated using 20 genotypes with known reaction to Fe toxicity. The genotypes were both screened in the phytotron using the standardized protocol and were also evaluated in iron toxic field. Variation in response to iron stress was observed among the genotypes in the phytotron that corresponded well to field observations. Leaf bronzing scores measured 4 weeks after stress application in the phytotron showed a high correlation with the field bronzing score as well as grain yield in an iron toxic field. Hence, the use of culture solution-based standardized screening could be used as high throughput technique to identify Fe-toxicity tolerant genotypes in breeding programs.

**Keywords:** rice, iron concentration, pH, EDTA, iron toxicity, screening technique.

# CO-INOCULATION OF MUNGBEAN WITH RHIZOBIA AND PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) IMPROVES NODULATION, DRY MATTER YIELD AND NITROGEN UPTAKE

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Selection of superior strains of rhizobia and breeding of legumes for enhanced biological nitrogen fixation (BNF) are the common strategies utilized in improving nodulation and BNF in legumes. Recent findings however, showed the role of helper bacteria in promoting better nodulation and nitrogen fixation

A field experiment was conducted at the Institute of Plant Breeding-Crop Science Cluster, UPLB to evaluate the effect of co-inoculating rhizobia (Bradyrhizobium sp.) and plant growth promoting rhizobacteria (PGPR) on nodulation, dry matter yield, and nitrogen uptake of five mungbean varieties. The experiment used the split-plot design with three replications. Results showed that in general, mungbean plants that were inoculated with both rhizobia and PGPR had significantly higher primary nodule number and primary nodule dry weight, dry matter yield, and nitrogen uptake than plants treated with single inoculation of rhizobia. Dry matter yield and nitrogen uptake of mungbean inoculated with PGPR alone and dual inoculation of rhizobia and PGPR were found comparable. Dual inoculation with rhizobia and PGPR increased nitrogen uptake by 33% and primary nodule number formed by 50% when compared with plants inoculated with rhizobia Treatment-variety interaction was not obtained in terms of the above parameters evaluated. The 5 mungbean varieties evaluated differed only in the number of nodules formed. Nodule occupancy study using agglutination technique showed that the proportion of crown root nodules occupied by the introduced Bradyrhizobium sp. strain was not affected by rhizobia and PGPR coinoculation.

The substantial increase in the amount of nitrogen uptake due to rhizobia and PGPR co-inoculation indicate an increase in nitrogen fixation activity with co-inoculation. The results of this study have significant implication in improving legumes productivity in a sustainable manner. Hence, further evaluation of rhizobia

and PGPR coinoculation under different soil types and cropping systems is being recommended.

Keywords: dual inoculation, rhizobia, PGPR, mungbean, nodulation, nitrogen uptake

### **AS-17**

# INFLUENCE OF BORON-POTASSIUM FERTILIZER APPLICATION ON THE OCCURRENCE OF CRACKING IN CARROTS (Daucus carrota Linn)

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The experiment was conducted in Banooy, Baculongan Norte, Buguias, Benguet Philippines occupying a total land area of 500 square meters where the experiment was laid out. Plot size was 1 x 5 square meters. The type of soil is clay loam with a pH value of 5.0. Final soil properties (pH, organic matter, nitrogen, boron and potassium) were significantly affected by the application of boron, potassium and their combination.

Yield of carrots was significantly affected by the application of boron and potassium and their combination, Higher yield was obtained on carrots applied with 3.0 to 4.5 ppm B and plants applied with 105 kg/ha to 140 kg/ha  $K_0O$ . Total yield is highest at 3.0 ppm B + 140 kg/ha  $K_0O$ . Marketable yield was significantly affected by boron and potassium application. Highest marketable yield was obtained on carrots applied with 3.0 ppm B. Significant higher yield was noted in plants applied with 140 kg/ha K<sub>2</sub>O. Application of 3.0 ppm B with 105 kg/ha K<sub>2</sub>O produced the highest marketable yield.

Significant differences on the number and percentage of cracked roots were observed as influenced by boron and potassium application. Higher number and percentage of cracked roots were noted on carrots applied with higher rates of potassium while lower count and percentage of cracked roots were affected by higher rates of boron application.

**Keywords:** cracking, fertilizer application, influence, occurrence, boronpotassium

# FERTILIZER MANAGEMENT OF ARABICA COFFEE DURING REJUVENATION

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The old age of coffee trees coupled with poor cultural practices contributed to drastic decline of the average yield in the region, thus rejuvenation was recommended. Long term fertilizer studies on Arabica coffee grown under pine were conducted at the Benguet State University Agroforestry project. Established Arabica coffee under pine of 18 – 20 years old were subjected to rejuvenation by bending and topping. New shoots sprouted from bended trees and were pruned to 6 sprouts per tree. The trees were applied with four kinds of organic fertilizers at a rate of 3 kilograms per tree once a year. Berries were harvested three years after rejuvenation and were evaluated for five consecutive years. Yield response of bended and topped coffee to different kinds of organic fertilizer varies every year of harvest. Application of goat manure yielded the heaviest fresh berries and green beans during the third year of harvest. However, yield varied during the succeeding years where combined application of 14-14-14 + chicken manure yielded the heaviest. Soil pH, organic matter, nitrogen and phosphorus contents of the soil were improved after three years of fertilizer treatment particularly from application of organic fertilizers. Another experiment using younger coffee trees were applied with different ratio of inorganic fertilizer and chicken manure was also done. Coffee bean yield was high from application of Chicken manure plus 1:2:1 inorganic ratio.

**Keywords**: Arabica coffee, Bending, Goat manure, Agroforestry

# ENHANCING PLANT GROWTH USING BIOFERTILIZERS AND BENEFICIAL INDIGENOUS MICROBES GROWN ON ACIDIC INFERTILE SOIL

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Biological farming is an emerging technology that uses beneficial microorganisms to replace chemical fertilizers and pesticides, resulting in cleaner and healthier environment. This study was conducted to assess the efficiency of several biofertilizers developed by BIOTECH-UPLB and beneficial indigenous microbes collected from established trees. Corn (Zea mays) and mungbean (Vigna radiata) were used as test plants. They were inoculated with several concoctions of beneficial indigenous microbes (BIMs), biofertilizers Mykovam and Bio-N (for corn) while for mungbean, Nitroplus and several combinations with Mykovam.

Generally, plants grew better in the marginal soil when inoculated with the biofertilizers Mykovam, Bio-N and Nitroplus and their combinations (Mykovam + Bio-N). BIM inoculation ranked second to the biofertilizers, while the uninoculated control plants had the poorest growth. In the mungbean experiment, Mykovam and Nitroplus inoculation either alone or combined gave the best shoot dry weight (83-156%) and 92-124% higher N uptake. In the first corn experiment (Corn A), Mykovam + Bio-N gave 63% higher total dry weight than the control. In the second corn experiment (Corn B), inoculation of Mykovam + BIMs 1 and 2 gave heavier total dry weight by 99-126%, bigger leaf area by 89-114%, and higher nitrogen uptake by 177-214% compared to the control plants.

Better growth of plants with microbial inoculation suggested possible synergistic interaction of the mycorrhizal fungi in Mykovam and the bacteria present in Bio-N, Nitroplus and the rhizosphere soil. We recommend the application of the results of this study to farmers in similar marginal areas.

Keywords: biofertilizers, Mykovam, corn, munghean, acid soils

# INDIGENOUS PRACTICES AND TECHNOLOGIES TOWARD A SUSTAINABLE ORGANIC RICE PRODUCTION SYSTEM

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A study was conducted to document the indigenous practices and technologies in selected rice-based farming areas in Southern Luzon. These include the unique practices belonging to a specific community or local group that the people in a given community have developed over time and still continue to develop; those based on experiences, often tested over years of use, adapted to local culture and environment, and as their basis for natural resource management.

The study showed that the sustainability of rice production systems depends on the ability of the environment or natural resources to continuously render its ecological services. These services provided to the rice sector include the current use of land and water resources, both as inputs to production and as receiver of wastes. The problems and approaches for sustainable organic rice production were documented based on these observations. Incorporating rice straw and plant materials into rice paddy soil, crop and animal wastes applied as organic fertilizers, and organic materials for seedling establishment are common practices employed over long period of time. The rice-duck technology, the simultaneous raising of rice and ducks in the same piece of land, is known for control of golden snails. Through the integration of ducks in rice farming, several advantages are realized (1) utilization of available family labor, (2) additional high protein food, (3) soil improvement due to fecal excretion, and (4) biological control of snails, weeds and insects. Rats and birds are also major pests in rice farms. But instead of using chemical poisoned-baits, several farmers employ innovative practices. The results of this study suggested that the intensification of rice production must not be undertaken through ecologically destructive approaches.

Keywords: rice technologies, indigenous practices, organic, ricc production, fertilizers, pest control

### ORGANIC MATTER RESIDUE MANAGEMENT FOR THE IMPROVEMENT OF SOIL QUALITY IN LOWLAND RICE **SYSTEMS**

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Soil productivity, biological diversity and impacts on the surrounding environment of the agricultural systems provide a more complete description of a 'quality' soil resource as a dynamic living system. Soil quality is an assessment of how well soil performs all of its functions now, and how those functions are being preserved for future use. A long-term project was conducted on a low-fertility clayey soil (Aquandic Epiaqualf) at IRRI-U.P. Los Baños Experiment Station. The objectives of this soil research are to develop strategies to improve soil functions and identify soil characteristics as indicators of soil quality.

Large increases in rice yields have been demonstrated with the application of fertilizer, rice straws, and the use of multi-purpose tree species, such as Gliricidia sepium and Macaranga tanarius. A combination of low-cost technologies resulted in increases in yield, long-term restoration and subsequent maintenance of soil resource base. The applications of fertilizer and plant residues which decompose rapidly had an immediate impact on rice yields. In earlier years of the experiment, there was no impact of the incorporation of organic materials. However, positive changes became evident in subsequent years. Rice yield respond reflects the beneficial effect of the organic materials on nutrient utilization efficiency which may also be related to improved soil fertility and microbial activity when residues were applied. The initial benefit of the application of G. sepium appeared to be related to its nutrient composition and decomposition rate. A change from high-input and chemically-intensive agriculture to a more sustainable form of agriculture is not only desirable, but also necessary. Management of organic materials that focus on improving soil health and conserving the remaining soil resources are realized. Careful management of residue quality and quantity, combined with judicious use of inorganic fertilizers, allows management of organic matter and nutrient dynamics to produce a 'quality' soil that should prove to be more sustainable.

Keywords: decomposition rate, plant residues, soil organic matter, fertilizers, lowland rice

# IN SITU COMPOSITING OF RICE STRAW USING EMBASED INOCULANT AND CHICKEN MANURE

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Rice straw management is considered an important aspect in sustaining long-term fertility of soil in rice cropping systems in lowland paddies as it is the most available in the farm. Incorporation of fresh rice straw (RS) in the soil however initially immobilizes nutrients because of its high C:N ratio. Chicken manure (CM), on the other hand, is considered the most economically efficient type of manure due to its low C:N ratio. With the characteristics of CM and its availability in the farm, it could be a good decomposing partner, substituting the function of commercial inoculants that are still more expensive and not readily available in the market.

This study aimed to compare the effect of CM and EM-based inoculant in rice straw decomposition A 60-day pot experiment was conducted to determine the mineralization of N, P, and K, as well as Fe, Zn, and Mn in the soil. The treatments were (1) Control (untreated), (2) RS at the rate of 10t/ha (3) RS with CM as inoculant at the rate of 3:10 or 3 tons of CM to 10 tons of RS (RS+CM), and (4) RS with EM-based inoculant at the rate of 1:10 (RS+EMB).

The dynamics of NH<sub>4</sub>-N is significantly higher in RS+CM compared with RS and RS+EMB. RS, with or without inoculants depressed NH<sub>4</sub>-N concentration in the soil. The mineralization of N in RS+CM started at 48 days after incorporation (48DAI) while that of RS and RS+EMB was not observed within sampling period. Phosphorus and potassium concentration in RS+CM were consistently higher than in RS and RSEM. Incorporating RS alone or in combination with CM or EMB, gave significantly higher Fe, Zn, and Mn than the Control but not among the RS treatments. Generally, CM is more effective in releasing plant-available nutrients from rice straw than the EMB.

**Keywords**: Chicken manure, Immobilization, Microbial inoculant, Mineralization, Paddy rice soils, Rice straw

### MICROBIAL BIOMASS AS INDICATOR OF ORGANIC FERTILIZER MINERALIZATION IN PADDY SOIL

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Immobilization of nutrients in soil microbial biomass occurs during intensive microbial activity as a result of substrate-derived nutrients from plant or animal residues. Fluctuations in the size of the microbial biomass pool can, therefore, be considered as an indicator of increasing or decreasing nutrient availability. The nutrients immobilized in the cells of the soil microbial biomass are eventually made available for plant uptake by mineralization (Lynch 1983; Inubushi and Watanabe 1986).

This study aimed to: (1) determine the effect of applying different organic fertilizers such as rice straw (RS), rice straw with inoculant (RSEM), chicken manure (CM), wild sunflower (WSF), and commercial organic fertilizer (COF) in soil microbial biomass; (2) compare the dynamics of soil microbial biomass in pot and field experiment; and (3) determined the dynamics of soil microbial biomass in paddy soil transplanted with rice.

Microbial C:N ratio is greater in the pot experiment than in the field experiment. The mineralization of RS was observed at 28 days after incorporation (28 DAI) in the pot experiment while 21 DAI in the field experiment. Partial immobilization of RS in the pot experiment occurred at 42DAI. RSEM mineralized at 28DAI in the pot and 14DAI in the field experiment. Partial immobilization was observed in the field set up at 21DAI. Mineralization of CM and WSF was observed at 7DAI both in the pot and field experiment. Immobilization in CM and WSF occurs at 35DAI in the pot and 14DAI in the field set up. COF mineralized at 35DAI in the pot and 28DAI in the field. Dynamics of N<sub>Bio</sub> showed a decreasing pattern regardless of the treatments in transplanted rice soil. C<sub>Bio</sub> on the other hand increased at the first week of transplanting then gradually decreased until 49 days after transplanting (49DAT).

Keywords: Immobilization, inorganic fertilizer, microbial C:N ratio, mineralization, organic fertilizer, paddy soils

# SURVEY OF ALTERNATE HOST PLANTS OF THE ASIAN CORN BORER, Ostrinia furnacalis (GUENEE): MARAMAIS, Trypsacum laxum, IN PANGASINAN

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Bt corn planting in the different regions of the country is increasing in area as it is becoming popular as an effective control against the Asian corn borer (ACB), and aside from being remarkably profitable for farmers in Central Luzon. Records of the Bureau of Plant Industry have shown that many farmers are already planting corn after rice and even rice farmers are shifting to corn farming in the last two years especially in Pangasinan. However, farmers should be reminded that Bt corn planting is not only looking at profits but also following the IRM requirement built-in for this technology. By disregarding this crucial requirement, it is possible that ACB resistance will become a problem in the future.

The presence of weeds and non-crops is very important in an area because they play a crucial role in an IRM program for Bt corn. These are the naturally growing weed species or cultivated crops within and around the cornfield and they serve as alternate host plants of ACB. When these weeds or non-crops are abundant, they will serve as natural refuge for the Asian corn borer. To prevent the development of resistance, the importance of refuge (as a source of susceptible population that will dilute resistant borer developing from Bt corn) is vital. Survey of the alternate host plants of the ACB is an on-going study. During our project survey in Pangasinan, we found that maramais, *Trypsacum laxum*, is abundant in Tayug and Sta. Maria. This plant is also found in other provinces. In the survey, it was observed that maramais is an alternate host of ACB. Larvae and pupae were dissected from the plants and adults were found abundant in areas where they

were growing. This study aims to further evaluate the potential of maramais as an alternate host of ACB in greenhouse and laboratory conditions and determine its agronomic traits when planted in Los Banos conditions.

**Keywords**: maramais, *Trypsacum laxum*, Bt corn, IRM, alternate host plant, Asian corn borer, *Ostrinia furnacalis* 

### **AS-25**

### INVASION OF THE BUFF COCONUT MEALYBUG (Nipaecoccus nipae) IN UP LOS BAÑOS CAMPUS, ELUCIDATION OF THE CONFOUNDED "MEALYBUG BURN" DAMAGE AND PRACTICAL CONTROL

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The buff coconut mealybug (BCM), Nipaecoccus nipae, is already all over the lower and the upper campuses at the UP Los Baños, College, Laguna. It is alarming as nothing is being done on the continuous destruction of this newly introduced pest. The most affected are the ornamental palms that are the dominant landscape species around the campus. If left unabated, it is possible that the plant diversity inside the campus will be threatened. It is the aim of this paper to present the current infestation of BCM around the UPLB campus and to further elucidate the "mealybug burn" damage. It will also recommend measures to conserve the remaining species or encourage the replanting of resistant native palm species.

According to the survey conducted by Lit et al (2006), this invasive BCM is already present in 64 plant species of agricultural and forest trees representing 14 plant families. This polyphagous pest was first noticed in 2001 on palm species, Araceae, specifically on palmera (Chrysalidocarpus lutescens) and the two species of fish-tail palms (Pugahan', Caryota

cumingii and 'Takipan', C. rumphiana). In 2002, the mealybug infestation was observed on royal palm (Roystonea regia), coconut (Cocos nucifera) and bachia (Dieffenbachia sp.). These spread to several plant families like Lauraceae (avocado), Sapotaceae (caimito, chico) and Annonaceae (guyabano, atis) in 2003. The most resistant were those of the anahaw, buri and other thick-leaf palms belonging to the palm tribe Coryphoideae. The beautiful lines of royal palms, the scattered coconut trees and the disappearing fish-tail and bamboo palms in the lower campus were most affected.

BCM usually invades the older leaves first, continue to feed and reproduce then later transfer to younger leaves. Severe damage is observed when the lower leaves are already covered with sooty molds. As a result of continuous sucking (feeding) coupled with the presence of sooty molds, senescence is hastened resulting to wilting and drying of the leaves. The infestation level is most severe during the dry season and the population build-up is very fast due to the absence of natural enemies. Not even the superytyphoon "Milenyo" had arrested the population of this pest and they further continue to affect the vegetation around the campus after two years.

**Keywords**: buff coconut mealybug, *Nipaecoccus nipae*, exotic palms, *Caryota cumingii*, *Cocos nucifera*, *Roystonea regia*, mealybug burn, sooty molds

### AS-26

### SOME BUTTERFLIES OF THE WESTERN SIDE OF MT. ARAYAT, PAMPANGA

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We previously studied the butterflies of Mt. Arayat from 1994 – 1995 and 1997-1998 on the eastern slopewhich is in the town of Arayat. This time we accumulated field data from the other side of Mt. Arayat which is the western slope at the town of Magalang from 2001 to 2008. This protracted inventory contributes to the generation of biodiversity data at Mt. Arayat.

We used the modified Polard technique to observe and record butterflies in two sites. Some of the butterflies fauna of the western side of Mt. Arayat starting from the Pampanga Agricultural College up to Brgys. Sto Niño and Lower Ayala are as follows: 7 families, 21 genera and 28 species. These represent the lowland species. The butterfly fauna from Upper Ayala up to White Rocks or almost two thirds of Mt Arayat's height are: 7 families, 34 genera and 35 species. Midway to this extinct volcano is a small patch of forest followed by pole sitao and gabi fields and cogon areas. This small patch of forest contained more species than those found in the lowland. Obviously the rich diversity of host plants in this patch of forest supported a greater number of butterfly fauna.

The knowledge on where to find a particular species of butterfly is invaluable for resource management by the local populace. They can map the species location and use this for local butterfly treks for additional livelihood. It can serve as an initial input for a community based ecotourism blue print. An inventory of the complete taxonomic richness of the western side of Mt Arayat can be added.

Butterflies, Mt. Arayat, western side. Magalang, forest, Keywords: diversity, patch, livelihood.

AS-27

### THE FECUNDITY AND FERTILITY OF FIELD COLLECTED AND CORN STALK COLLECTED ASIAN CORN BORER, Ostrinia furnacalis GUENEE

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The mass rearing of the Asian corn borer (ACB) for laboratory studies such as bio-assays depends largely on the availability of field collected gravid ACB females. Corn stalks are cut immediately by farmers and the land is prepared again for quick turn around practice. This ensures continuous planting with a short duration for stalks to remain in the field. The time to collect adult ACB is thereby shortened. The random cutting of stalks from the ground level and bringing these in the laboratory can partially solve this problem on short supply of ACB adults.

We compared the fecundity and fertility of field collected ACB's and those that emerge from randomly collected corn stalks from Barangay Sanatiago, Lubao, Pampanga from May to November 2007. The corn variety is SG75 which is susceptible to ACB attack. Five hundred seventy six female ACB's were field collected while 1,322 females and 1,697 males were collected from netted corn stalks that were placed inside mosquito nets. The percentage fecundity and fertility of field collected ACB's were 90.4% and 89.33% while the stalk collected were 86.01% and 75.19% respectively.

The use of corn stalks as alternative collection for ACB's is a viable alternative where quick turn around is done.

**Keywords**: fecundity, fertility, Asian corn borer, field collected, stalk, corn.

AS-28

# HIGH INCIDENCE OF LAND SNAILS MORTALITY AT MT. ARAYAT, PAMPANGA: AN EVIDENCE OF GLOBAL WARMING?

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An unusually high mortality of three landsnails namely; *Helicostyla ovoidea* (Bruguire) *Cyclophorus appendiculatus* Pfiefer and *Hemitrichiella segitera* (Sowerby) was documented at the Magalang side of Mt. Arayat, Pampanga from March to June 2008. We initially observed half buried bleached dead snails from an upturned root of ipil-ipil, *Leucaena leucocephla* in March 2008. Further observation around this site showed various stages of these three snail species that were dead or dying. By June 2008 we noticed more snails dying even though they were already near and around the source of potable water in the watershed. The total mortality in decreasing order from a one square meter sample replicated four times in four different locations are as follows: *H. segitera* (100) > *C. appendiculatus* (34) > *H. ovoidea* (26).

The warm weather could have trigered the occurrence of disease causing microorganisms that led to the death of the said snails irrespective of

ages and sexes. It has been reported that 2008 is the 10° warmest year on record. Also the nine warmest years occurred from 1998-2008. Residents at Bgy. Sto Nino, Magalang and farmers near the observation sites mentioned unusual dry warm weather and lack of rainfall during late 2007 up to early 2008.

**Keywords** Mt. Arayat, snails, mortality, global warming, disease

AS-29

# PESTNET: AN INEXPENSIVE DIAGNOSTICS AND PEST MANAGEMENT ADVISORY TOOL FOR CROP PROTECTION PRACTITIONERS IN THE PHILIPPINES

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An email network that provides the pest diagnostic is now in place. PestNet is an e-mail network that helps people in the Pacific and South East Asia obtain rapid advice and information on plant protection. It links the Pacific and South East Asian regions with plant protection specialists worldwide and is free to members.

The Philippines have used the services of the PestNet on various occasions such as the identification of a new invasive pest of com, the management of the coconut leaf beetle, Brontispa longissima and others. We also provided identification services for some of the unknowns that were posted by subscribers.

The PestNet is a convenient, fast, and reliable system for the diagnostics and pest management advisory needs of crop protectionists in the Philippines. Most of the scientists who provide the above assistance are likewise willing to receive specimen for further validation. Hence an array of experiences and knowledge on various topics like quarantine, surveillance, biodiversity, invasive, species, events like workshops, etc. aside from diagnostics and pest management advisories are available. The PestNet is an excellent way to network on matters that pertain to plant health and crop protection.

**Keywords**: PestNet, identification, pest management advisory, validation, plant health, crop protection, Philippines

AS-30

### SEVEN NEW ENDEMIC SPECIES OF Hova R. Br. (APOCYNACEAE) FROM THE PHILIPPINES

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Hoyas, commonly called 'wax plant' or 'porcelain plants' can be found throughout the different islands of the Philippines. Our country is considered a center of diversity for hoyas due to the number of different species that can be found and is still waiting to be discovered. Most of the Philippine species are endemic. However, a number of them are indigenous and can also be found in neighboring places such as Borneo and Malaya. This is especially true for those species discovered in Palawan.

Seven new Philippine endemic species of *Hoya R. Br.* (Apocynacea) were collected and described. Three are from Quezon Province namely: Hova benvergarai Kloppenburg et Siar from Dolores, Quezon, H. lazaroi Kloppenburg et Siar from Mt. Banahaw, and H. soligamiana Kloppenburg, Siar et Cajano from Atimonan. Four of the new endemic hoya species were from Laguna Province namely: Hoya annjacanoae Kloppenburg et Siar, H. aurantiaca Kloppenburg, Siar et Cajano, H., lucardenasiana Kloppenburg, Siar et Cajano and H. landgrantensis Kloppenburg, Siar et Cajano, all from Barangay Kapatalan, Siniloan, Laguna Province,. These seven new taxa bring the number of described Philippine Hoya species to 68.

**Keywords**: Hoya, Hoya benvergarai Kloppenburg et Siar, H. anncajanoae Kloppenburg et Siar, H. lazaroi Kloppeburg et Siar, H. Kloppenburg, Siar et Cajano, H. lucardenasiana Kloppenburg, Siar et Cajano, H. landgrantensis Kloppenburg, Siar et Cajano, H. soligamiana Kloppenburg, Siar et Cajano

## DEVELOPMENT OF PEDOTRANSFER FUNCTIONS FOR PREDICTING WATER RETENTION CURVE OF PHILIPPINE SOILS USING ARTIFICIAL NEURAL NETWORK ANALYSIS

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Soil hydraulic functions involving the soil water retention curve and hydraulic conductivity are key input for models simulating water and chemical transport in soil. However, these properties are not easily obtainable because measuring them is resource intensive. One approach to address this data gap is through the development of pedotransfer functions. Thus, this study was carried out to developed soil water retention curve pedotransfer functions(SWRC-PTFs) using artificial neural network (ANN) to predict water retention properties from basic soil physical properties namely sand, silt and clay percentages, bulk density and geometric mean particle-size diameter.

Results showed that the developed PTFs were adequate in predicting water retention curves of Philippine soils. Among the soil properties, bulk density was an important input variable in the model as it reflects the effect of soil structure on the flow of soil water. Its inclusion in the model increased prediction of water retention by about 24%. All the developed PTFs account more than 91% of the total variation of soil water retention with coefficient of efficiency (EF) ranging from 0.49 to 0.62, Likewise, the new PTFs had relative improvement (RI) of more than 50% over Rosetta and more than 60% over Neuro-m in predicting water retention of Philippine soils.

The PTFs developed in this study provide improved relationships for estimating the water retention curves of Philippine soils from soil texture and related properties, and as such may prove useful in studies dealing with assessment of physical soil quality (i.e., contaminant transport) and other hydrological and agricultural problems.

**Keywords**: Artificial neural network, Pedotransfer function, Philippine soils. Soil water retention curve

**AS-32** 

# PREDICTING THE EFFECTS OF LAND USE ON RUNOFF AND SEDIMENT YIELD IN SELECTED SUB-WATERSHEDS OF THE MANUPALI RIVER USING THE ArcSWAT MODEL

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The quantitative prediction of environmental impacts of land use changes in watersheds could serve as basis for developing sound watershed management schemes, especially for Philippine watersheds with agroforestry systems. ArcSWAT, a river basin scale model developed to quantify the impact of land management practices on water, sediment, and agricultural chemical yields, was parameterized and calibrated in selected Manupali River sub-watersheds with an aggregate area of 200 ha to simulate the effects of land use on runoff volumes, sediment yield, and streamflows.

Calibration results showed that ArcSWAT can adequately predict peaks and temporal variation of runoff volumes and sediment yields with Nash and Sutcliffe coefficient (NSE) ranging from 0.77 to 0.83 and 0.55 to 0.80, respectively. Simulation of land use change scenarios using the calibrated model showed that runoff volume and sediment yield increase by 3% to 14% and 200% to 273%, respectively, when 50% of the pasture area and grasslands are converted to agricultural lands. Consequently, this results in the decrease of baseflow by 2.8% to 3.3%, with the higher value indicating a condition of the watershed without soil conservation intervention. More seriously, an increase of 15% to 32% in runoff volume occurs when the whole sub-watershed is converted to agricultural land. This accounts for 39% to 45% of the annual rainfall to be lost as surface runoff.

While simulation results are subject to further validation, this study has demonstrated that the Soil and Water Assessment Tool (SWAT) model can be a useful tool for modeling the impact of land use changes in Philippine watersheds.

Keywords: Land use change, runoff, sediment yield, SWAT modeling

**AS-33** 

### FIELD GROWTH RESPONSES OF THREE TREE SPECIES TO MYCORRHIZA AND FERTILIZERS IN THE BIOREMEDIATION OF A MINE WASTE DUMP

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Mine spoils have sub-marginal conditions and entail costly rehabilitation. This study evaluated the potentials of mycorrhiza, fertilizers and tolerant species in the bioremediation of a mine waste dump in Antamok, Itogon, Benguet.

Seedlings of Alnus maritima Marsh Nutt., Casuarina equisetifolia Forst., and Eucalyptus camaldulensis Dehn, were subjected to mycorrhizal inoculants and planted in the mine spoil. Four fertilizer treatments: 120g compost, 0g NPK, 5g NPK and 20g NPK were also applied. Height and diameter increments were measured until the 10th month. Other parameters measured include biomass, N. P. K. Cd, Cr, Mn and Fe uptake.

Results showed that, except for Alnus, seedlings planted in the mine spoil responded positively to mycorrhiza. Likewise, Agoho and eucalyptus responded positively to 20g NPK while Alnus responded positively to 5g NPK. Alnus had the most robust growth owing to its early N-fixation which may have affected its response to mycorrhiza and fertilizers. Delayed growth and nodulation were observed in Agoho possibly due to lower tolerance to

heavy metals and poor site condition. The mine spoil's heavy metals such as Cd, Cr, and Au, Fe and Mn were abnormally high but the trees planted survived. All species used were relative excluders of Cd and Cr. Only Alnus excludes Mn while Agoho and eucalyptus tolerates high content of Mn and Fe. Both nutrient and heavy metal uptake indicate the positive role of mycorrhiza and fertilizers in increasing plant tolerance to nutritional imbalance and heavy metal toxicity.

**Keywords**: Alnus maritima, Casuarina equisetifolia, Eucalyptus camaldulensis, bioremediation, mine waste dump, mycorrhiza, fertilizers, heavy metals uptake.

### AS-34

# CARBON STORAGE AND SEQUESTRATION POTENTIAL OF UPLAND AND MANGROVE FOREST ECOSYSTEM IN BINAHAAN WATERSHED AND PADRE BURGOS QUEZON

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Conservation of mature forests and mangrove stands is being eyed as an effective means to mitigate climate change. As the latest assessment report of the Intergovernmental Panel on Climate Change (IPCC) suggests, the global mean temperature has dramatically increased over the past decade and may spell major disasters to our ecosystem. Protection of the remaining forests is, therefore, crucial. Statistics shows that we are losing in a global scale around 6 million ha of primary forest and 3,500 ha of mangrove areas annually. This problem therefore entails a gigantic loss in forest biomass which could have been very helpful sequestering the atmospheric carbons. Given this backdrop, it is therefore relevant to assess the how much carbon does our primary forest and mature mangroves produce so that we could have a gauge for valuing it as an important resource. To measure the biomass and carbon density values, a nested plot method developed by ICRAF-ASB Program and allometric equations developed by Brown (1997) and Komiyama et.al. (2005) were employed. Results showed that the mature

secondary forest stand in Binahaan has a larger amount of sequestered carbon than the mangrove forest. The biomass and carbon density for this ecosystem is around 245.5 tons/ha and 128.9 tons/ha respectively, while the amount of carbon it sequesters every year is roughly 14 to 27 tons/ha. On the other hand, the estimates of biomass and carbon density for the mature Rhizophora mangrove site in Padre Burgos were 153.0 tons/ha and 92.4 tons/ha respectively with a yearly carbon sequestration rate of 0.47 tons/ha/yr. Despite the fact that mangrove forests have lower storage and sequestration capacity, forest protection should still remain as the foremost goal of the government and local communities in order to maximize not only the full potential of sequestering carbon but also for providing a healthy habitat to marine resources. On the other hand, proper silvicultural management should also be an imperative in managing our upland forests in order to improve its vigorous conditions in mitigating climate change.

**Keywords**: carbon storage and sequestration, climate change, upland forest, mangrove forest

### **AS-35**

### EFFECTS OF FOREST GAPS ON FOLIAGE INSECT DIVERSITY IN THE PERMANENT FIELD LABORATORY AREAS (PFLAS) IN MT. MAKILING FOREST RESERVE

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The effects of forest gap on the diversity of soil insects were studied on three different sites at the Mt. Makiling Forest Reserve from May to July 2005. Soil litter insects from 1-kg soil samples were extracted through Berlese method. Specimens were identified up to family level and various diversity indices were computed.

A total of 66 species of soil insects, belonging to 42 families was sampled from the three study sites without gap while only 54 species in 37 families were collected in areas with gaps. Statistical analysis showed that differences in species diversity level between areas with gap and without gap, in general, were significant at P≤0.5. Thus, results indicate that areas without gaps have higher diversity compared to areas with gap. On the other hand, areas with gap had higher dominance had high population density of some soil insects such as Family Poduridae and Family Formicidae. These species of soil insects were able to tolerate the disturbance in the area. However, Gap 3 had high soil insect diversity due to the fallen logs and damage seedlings which were inhabited by different kinds of soil insects. Presence of other soil arthropods such as Class Arachnida, Class Diplopoda, Class Chilopoda, Class Crustacea and Class Symphyla were also observed in the study areas.

Diversity and abundance of soil insects vary with the occurrence of disturbances (forest gap). Artificial or man-made forest gap such as land clearings decrease the number of soil insect species. On the other hand, natural tree fall gaps caused by storms and diseases increase the diversity of soil insects by making the habitat heterogeneous.

**Keywords**: artificial gaps, man-made gaps, foliage insect diversity, forest gaps, natural tree fall gaps

**AS-36** 

# SURVEY OF TREES USING ArcGIS OFFSET LINE AND DIGICAM TREE MEASUREMENT TECHNIQUES

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An innovative tree measurement technique combined with GIS was developed as part of a GIS-based tree inventory project at UP Los Banos. The ArcGIS Offset Line and Digicam Tree Measurement Techniques were used to map and build a database for the Pili Drive, a 1.4 km road lined with Pili (Canarium ovatum Engl.) trees. Found at the University of the Philippines Los Baños, the road is rich with historical, aesthetic, recreational and environmental significance. Measurements of individual trees are the main information in the database. We used ArcGIS COGO (coordinate geometry) functionality to plot tree locations gathered from a line offset survey. The method allowed the survey and mapping of 234 trees by a two-man crew in just two days. For tree measurements, we demonstrated a technique we call

"Digicam Tree Measurement". An ordinary digital camera was used to take photographs from where measurements of tree heights and crown diameters were derived. We found that the technique was faster (P=0.01) and easier (70% of the job is done in the office) than the usual method of tree measurement, i.e., measuring height by abney level and tree crown by projecting crown edges to the ground. The accuracy is comparable with that of the usual method both in terms of height (P=0.64) and crown diameter (P=0.72) measurements. Added benefits include archiving of photographs for future use such as biomass change measurement. However, the techniques are suitable only for roadside and other low-density tree planting. They are very useful for management of urban trees.

Keywords: tree measurement technique, GIS, COGO

**AS-37** 

# SILVICAL CHARACTERISTICS OF PURE AND MIXED STANDS IN PERMANENT FIELD LABORATORY AREA (PFLA 3), MT. MAKILING FOREST RESERVE

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The study was conducted to describe the current structure of tree species and regeneration dynamics in relation to the prevailing environmental conditions in PFLA 3 secondary forest. The focus was to describe and compare the overstorey structure of the two stands i.e. pure mahogany and mixed-species; assess the diversity and distribution of the regeneration under these two stands; and evaluate the prevailing site conditions such as soil characteristics and nutrients, air temperature and light intensity.

Results show that there is mean increment of 1.05 cm from 2006 to 2008 or an equivalent of 0.53 cm increment per year in the Pure stand. While in the Mixed stand a mean increment of 1.75 cm was observed or an equivalent of 0.88 cm increase in diameter per year. Majority of the trees in both stands were at least 9 meters in height. Tree heights ranged from 3.5 meters - 27 meters and 2.0 meters - 22 meters for mixed stand and mahogany stand, respectively. The species Tamayuan (Strombosia philippinensis) and large leaf Mahogany (Swietenia macrophylla King) dominate the upper canopy in the Mixed stand and Pure stand, respectively. On the hand, a total of 20 species of regenerations were found growing under the mahogany dominated forest of PFLA3. Mahogany species had the highest total number of regeneration which comprises 33.51 % of the total regeneration count in the two stands. This was followed by Hagimit and Tamayuan with a percent regeneration of 22.16 and 20.62%, respectively. The prevailing site conditions that were measured showed the following results: bulk density, 0.934g/cm<sup>3</sup>; soil pH, 6.8; NPK, generally low; temperature, 31.19°C; and relative humidity, 73.65%.

Although the two stands are occupying the same geographic location they exhibit variations in the different parameters measured. Hence, a more rational and convincing explanation of such variation may be gleaned from the comparison of the present site characterization studies and initial conditions or in the following measurements. Thus, in the absence of baseline information, it is suggested that a follow-up study maybe helpful to further understand site-specific variations associated with prevailing environmental conditions. Since the study is primarily descriptive, a more scientific account or measurements of these variations is suggested for future studies to understand the relationship and degree of influence of the different environmental factors to stand formation and regeneration pattern.

Keywords: Mixed stands, Pure stands, Regeneration Dynamics, Regeneration Patterns, Secondary Forest, Silvical Characteristics

### IMPROVING THE ENVIRONMENT AND LIVING CONDITIONS OF THE RURAL POOR: POTENTIALS OF REFORESTATION CDM IN THE MANGROVE COMMUNITIES OF SAN JUAN, BATANGAS

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In the context global warming, carbon sequestration receives a considerable attention now because it provides opportunities to improve the environment and the living condition of the rural communities as well. Under the Kyoto Protocol, a flexibility provision called Clean Development Mechanism (CDM) could provide reliable sources of income and livelihood to rural poor. Highly industrialized countries (Annex 1 countries) could collaborate with developing countries such as the Philippines in investing on different environmental projects including alternative fuel production and reforestation to help them meet their carbon emission reduction targets. In the latest assessment report of the Intergovernmental Panel on Climate Change (IPCC), global mean temperature has dramatically increased over the past decades thus mitigation projects was noted to be at their fast pace. Greater attention is focused on rehabilitating tropical forests like mangrove forests to offset carbon emissions. Thus, this study was done to assess the carbon storage potential of the mangrove forest in the Mun. of San Juan in Batangas. A nested plot method developed by ICRAF-ASB Program and allometric equations developed by Komiyama et al. (2005) were employed to estimate the above-ground biomass and carbon density of the two stands: Rhizophora-dominated stand in Barangay Poctol; and Avicennia-dominated stand in Barangav Catmon. The total carbon storage in the Rhizophoradominated stand is 115.45 Mg/ha. Bakawan babae (Rhizophora mucronata) and Tabigi (Xylocaropus granatum) were recorded as the most dominant species in the area that have the largest contribution in sequestering of carbon. On the other hand, the Avicennia-dominated has a larger amount of total carbon stored with 141.71 Mg/ha. Notably, Bungalon (Avicennia marina) showed as the most effective species in the stand as far as carbon storage capacity is concern. Other species co-dominating Bungalon include Tangal (Ceriops tagal), Malatangal (Ceriops decandra) and Piapi (Avicennia marian var. rumphiana). At the sediment layer, soil carbon density was also high. Estimates showed that Avicennia stand remained to have a larger

sequestration capacity than Rhizophora with 15.92 Mg/ha and 11.95 Mg/ha, respectively. Overall, both Rhizophora and Avicennia stands exhibited good carbon storage potential. This therefore entails an economic advantage for rural communities as huge amount of carbon credits can be produced and traded through reforestation and CDM. Proper forest management is likewise an essential task of both the government and local communities in order to sustain and improve the capacity of mangroves in mitigating the impacts of climate change.

**Keywords**: carbon sequestration, reforestation, CDM, *Rhizophora*, Avicennia

AS-39

### HULL-IMPOSED DORMANCY IN RICE

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Seed dormancy in rice is considered a valuable trait particularly in tropical areas as it serves as a mechanism to prevent germination in the field as the crop lodges in standing water. Factors such as rudimentary or physiologically immature embryos, impermeable seed coats and the presence of endogenous germination inhibitors are recognized as causing seed dormancy. Few studies have been conducted to indicate that impermeable seed coats are involved in rice dormancy which prompted the conduct of this study. In this study, eight rice varieties were used to determine seed hull impose dormancy in rice. Seed samples were subjected to dormancy breaking treatments: soaking in water for 24 h, clipping the seed tip, dehulling and heat treatment (50°C) for 5 days. Seed germination, vigor, coleoptile and radicle length were observed and measured. The eight rice varieties showed varying degree of dormancy (7-83% germination). Results showed that seed dehulling (85-95% germination) and heat treatment (83-99% germination) were effective in breaking seed dormancy in all the varieties tested. Vigor was also high in seeds subjected to dehulling and heat treatment. However, longer radicle (52-74 mm) was observed in soaked seeds and in the control whereas longer coleoptile was observed in heat treated and soaked seeds. The study indicates that removal of seed hull and subjecting the seeds to 50°C improved germination. High germination in dehulled compared to undehulled seeds seems to indicated that dormancy in rice is seed hull-imposed.

**Keywords**: rice, seed dormancy, seed hull, germination, scarification

AS-40

### QUANTITATIVE AND QUALITATIVE ASSESSMENT OF CORN POSTHARVEST LOSSES

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The project aimed to update baseline data on corn postharvest losses specifically in Isabela, Bukidnon and South Cotabato with the ultimate objective of providing the basis for formulating sound and tenable loss reduction programs. Postharvest practices were identified and losses were measured quantitatively and qualitatively.

Majority (85%) of the farmers in Isabela, Bukidnon and Koronadal, South Cotabato manually harvest, mechanically shell and sundry dry the grains before marketing to the local traders/assemblers, wholesalers or hog raisers. Corn grains are dried using concrete pavements (MPDP), barangay roads or highways. In Banga, South Cotabato, around 90% of the corn farmers harvest the corn immature and sell it as earcorn right after harvest. Shelling and drying operations are done at the local traders/assemblers, wholesalers or feedmillers levels.

Total postharvest losses were recorded at 7.15%, 7.35% and 5.98% in Isabela, Bukidnon and South Cotabato respectively. Drying loss comprised 63,23% of the total losses. Climatic condition, varietal characteristics and machine factors were associated with postharvest losses.

Corn samples from the three provinces, except for the dry season in Isabela, have moldy grains beyond the 0.10% level set by Bureau of Agriculture and Food Product Standards (BAFPS).

High incidence of aflatoxin contamination (above 20ppb) in corn was detected in Isabela at the on-farm level during the rainy season. Moreover, corn grains at the trader level were highly contaminated with aflatoxin in Isabela and Bukidnon. Non-detectable aflatoxin was recorded in corn samples from South Cotabato.

Continuous awareness campaign on how to lessen and/or prevent postharvest losses, and inclusion of postharvest loss control measures as component under the government corn program can reduce postharvest losses and increase domestic supply of corn.

**Keywords**: Postharvest technologies, postharvest losses, loss reduction, loss assessment, mechanical dryers

### **AS-41**

# WILD GENETIC USEFUL MUSHROOM RESOURCES OF CENTRAL LUZON, AS SOURCES OF NUTRICEUTICALS

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With our intention of harnessing the economic potential of wild useful mushroom resources in Central Luzon as sources of nutriceuticals, wild useful mushrooms were rescued and pure cultures were obtained. The rescued mycelia of Ganoderma lucidum and Auricularia polytricha were deposited at the Mushroom Gene Bank of the Center for Tropical Mushroom Research. Their mycelial growth performances and their antibacterial properties were evaluated. Tissue culture technique was adopted in rescuing the mycelia of the above mentioned mushrooms. The rescued mycelia of each strain were evaluated and compared for their ability to grow efficiently on indigenous culture media. Also, their ability to inhibit the growth of both gram positive and negative bacteria was tested using their immobilized mycelial discs against Escherichia coli and Staphylococcus aureus. Eight strains each of G. lucidum and A. polytricha were evaluated. All the evaluated wild strains of useful mushrooms exhibited antibacterial activity with varying degrees of mycelial growth performances. Significant findings gathered in this investigation regarding their ability to inhibit the growth of bacteria proved that these strains are novel sources of nutriccuticals.

**Keywords**: Auricularia polytricha, Ganoderma lucidum, mushroom nutriceuticals, useful mushrooms, wild mushrooms

### HEAT PUMP DRYING OF ONION (Allium cepa)

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A heat pump drying system was used to determine the drying kinetics of onion. Onion is a heat sensitive crop requiring favourable dehydration process to preserve its organoleptic characteristics.

Drying kinetics of onion was described using the different stages of drying: initial stage, falling rate and at the constant rate. Results showed that onions had a constant drying rate at the falling rate, that is, at an operating temperature of 50.0°C and relative humidity of 15.0%.

Dehydration results also showed that moisture content of onion was from 83.0 % to 15.0% at moisture reduction rate of 0.029 kg/hr dried for 33.0 hours. Drying time and moisture loss was highly significant with R<sup>2</sup>=0.97.

Favourable drying, thermal and dehumidifying efficiencies of the system contributed to a dehydrated onion with reduced colour degradation safe for storage.

Keywords: heat pump drier, latent heat, sensible heat, dehumidification efficiency, thermal, efficiency

#### **AS-43**

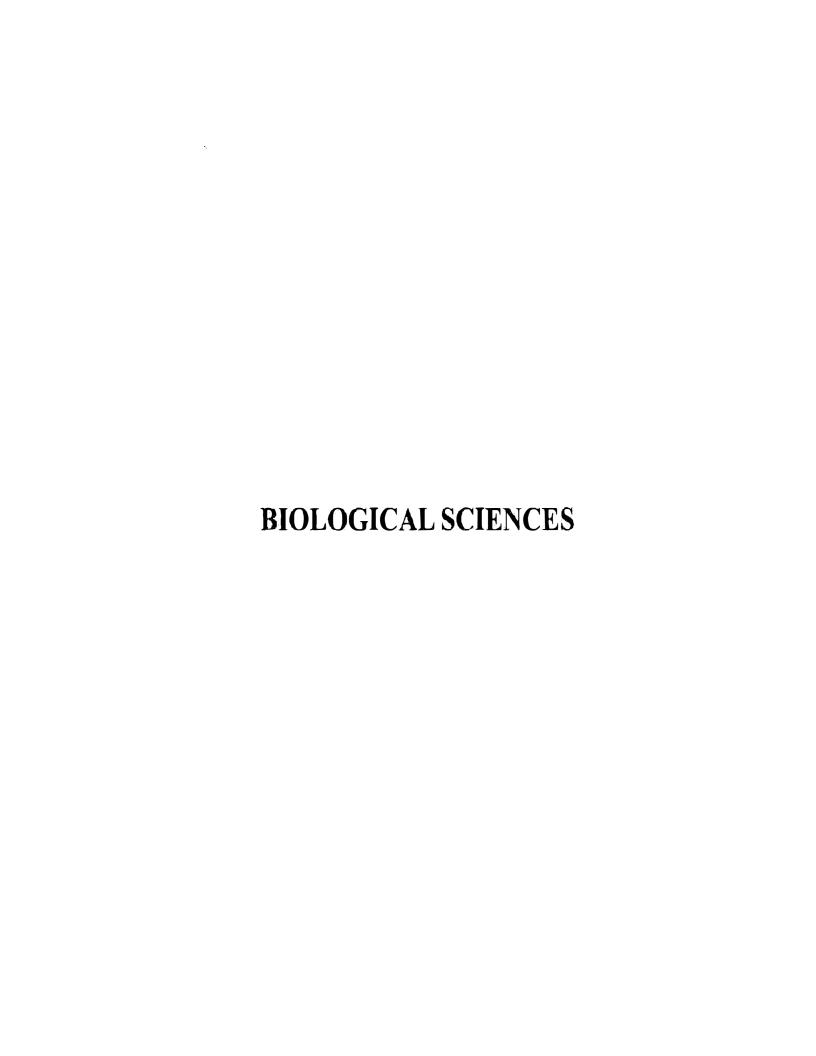
#### MULTIPLEX POLYMERASE CHAIN REACTION FOR SIMULTANEOUS DETECTION OF MAJOR MASTITIS-CAUSING PATHOGENS IN BUFFALO MILK

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Clinical mastitis is one of the major diseases in the dairy sector and its incidence increases with lactation number or age. To efficiently treat and minimize its effect on dairy industry, a sensitive, rapid, and specific test for identifying the mastitis-causing pathogens is required. PCR-based method provides a promising option compared to bacteriological method. Recently, a more improved PCR method is being applied to simultaneously detect various pathogens in one assay. The use of the multiplex PCR (mPCR) capable of detecting simultaneously four of the most common mastitiscausing pathogens in milk including Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uberis was tested directly from DNA isolates of buffalo milk. Twenty five milk samples tested for mPCR were based on California Mastitis Test (CMT). Sensitivity of mPCR was assessed by comparing the results from bacteriological culture. Fifty six percent (56%) matches the results from both methods while 44% were detected positive in mPCR but no growth in the culture. This study suggests that mPCR can be a practical tool for rapidly diagnosing mastitiscausing pathogens in milk. This would mean significant improvements in the disease control and accurate decision for treatment.

**Keywords**: Buffalo milk, Mastitis, Multiplex PCR (mPCR), California Mastitis Test (CMT)



#### SWEET SORGHUM JAGGERY AS ALTERNATIVE MEDIA FOR THE PRODUCTION OF COMMERCIAL YEAST

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Yeast plays a vital role in a number of industrial products especially in the production of wine and bakery products. Its production, however, entails a sizeable amount due to the expensive culture media used. The need to look for alternative, reasonably priced culture media is crucial to lessen the production cost.

Sweet sorghum produces juice of high sugar content, which can be extracted through milling. The juice then is subjected to high temperature for two or more hours to produce jaggery, a substance that can be a good substitute for table sugar.

This study used sweet sorghum jaggery as an alternative culture media for yeast production. Several percent jaggery were used in the culture studies. Results show that 15% jaggery is the optimum concentration for the highest percent production and recovery. Sweet sorghum jaggery contains the necessary nutrient requirements needed for the growth and development of yeast. Sweet sorghum jaggery can be a very affordable media for commercial production of yeast

Keywords: Sweet Sorghum, Jaggery, Yeasts, Culture Media

#### PHENOTYPIC DISTINCTION OF ENTEROBACTERIAL FLORA OF HOUSEFLIES (Musca domestica L.)

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The synanthropic nature of houseflies (Musca domestica L.) allows them to be closely associated with humans. Their presence in residential and food settings poses a significant health threat to human beings as they are potential vectors of several pathogenic microorganisms. investigated the antibiotic resistance profiles of enterococci from houseflies collected in eating establishments around the vicinity of Mindanao State University-Iligan Institute of Technology, Iligan City. presumptively identified Enterococcus sp. isolated from the stomach contents of thirty-eight houseflies were assayed for their antimicrobial resistance making use of the Kirby-Bauer disc diffusion method. Twenty (83%) of the isolates showed phenotypic resistance to erythromycin while non-susceptibility was also exhibited against kanamycin (58%), tetracycline (54%), and chloramphenicol (21%). Ciprofloxacin was the most effective antibiotic having the highest number of susceptible isolates (83%) and thus the least number of resistant strains against it. Eleven isolates exhibited resistance to at least three antibiotics and were characterized as multidrugresistant Enterococcus. The isolation of these antibiotic resistant Enterococcus strains from the houseflies suggests that these organisms may play a role in the transmission of antimicrobial-resistant bacteria in the community. Resistance of enterococci to multiple antibiotics leaves few alternatives for disease management as fewer drugs would be available to combat the infection caused by the resistant strain. Detection of these strains would impel constant monitoring of houseflies in the locality to manage the prevalence of enterococcal infections.

**Keywords:** houseflies, Kirby-Bauer disc diffusion method, multidrugresistant Enterococcus

#### BIODEGRADATION OF BANANA STALKS AND SWEET SORGHUM BAGASSE UNDER SOLID STATE CULTURE OF Pleurotus sajor caju

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Banana stalk fiber (BSF) is the dried fibrous waste generated after the banana fruits are harvested while sweet sorghum bagasse (SSB) is the fibrous residue after the juice has been extracted from the sweet sorghum stalks. In this study, BSF and SSB were processed under solid state culture conditions with Pleurotus ostreatus and the effect of this fungal culturing on the biodegradation of these lignocellulosic residues was evaluated. Fungal growth was carried out at 50% moisture level for 30 days. carbohydrate, reducing sugar, monosaccharide glucose and fructose of the water hydrolysate of the fungal-treated and untreated samples were analyzed using colorimetric, somogys, nelson, and HPLC procedures, respectively.

The treated BSF and SSB gave a total sugar of 60.47 and 52.37 % respectively, while only 14.8 and 11.1% were observed in their untreated counterparts. Glucose was only detected in the hydrolysate of the treated samples where SSB gave 0.053g/L and BSF showed 0.042 g/L. Xylose was not detected in either of the treated and untreated samples. The test on reducing sugar confirmed that most of the water soluble sugar in the hydrolysate are oligosacharides (partially degraded cellulose). Lignin content of the two samples was significantly reduced by the fungus.

The results showed the potential of the edible fungi in degrading lignocellulose materials for cellulose ethanol production.

Keywords: Lignocellulosic, biodegradation, BSF, SSB

# BANANA ROOT ENDOPHYTES: POTENTIAL BIOCONTROL AGENTS FOR VASCULAR WILT DISEASE?

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Banana (*Musa* spp.) is considered one of the major agricultural crops in the Philippines. However, it is continuously being plagued by pests and diseases of microbial origin, e.g. the fungus Fusarium oxysporum, the causative agent of vascular wilt disease, and may result in huge economic losses. Application of agrochemicals, though effective, often results in environmental problems and health hazards. Thus, the use of endophytic fungi as biological control agents poses a novel and promising alternatives for plant disease management. Our research study then aims to test locally isolated banana root endophytes (BRE) for their potential as biological control agents against F. oxysporum. A total of seventy-five BREs were isolated from root samples of mature, asymptomatic banana cultivars collected from different sites in Manila and Quezon City, Highest species richness (d) was observed from plants collected from Lagro (d=8.89) and Commonwealth Avenue (d=8.50) in Quezon City. Twenty-five selected isolates were then screened for their antagonistic interactions against F. oxysporum using the dual culture method. Only three inhibited the test fungus either on contact (BRE18 and BRE 71) or at a distance (BRE 14). Morphocultural characterization identified them as those belonging to the genus Aspergillus. Further antagonistic testing showed that the three BREs significantly reduced the colony radial growth of F. oxysporum. Extraction of secondary metabolites was further conducted to test the antifungal properties of the three BREs. Crude culture extracts failed to inhibit the fungus using the paper disk diffusion assay. However, it showed remarkable decrease in spore germination. Thus, the isolated banana root endophytes (BRE) showed their potential as biocontrol agents.

**Keywords**: *F. oxysporum*, *Aspergillus* sp., fungal endophytes, biocontrol, *Musa* sp.

#### **Bacillus subtilis 168 ENDOGLUCANASE BINDS TO** CHEMICAL SOILS UNDER DIVERSE CONDITIONS

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The bglC gene of Bacillus subtilis 168 was cloned and the BglC protein was characterized. The endoglucanase was active in a wide pH range (3-12), retaining 50-60% of its activity even at the extreme pH values. The protein exhibited highest adsorption to montmorillonite followed by kaolinite then sea sand. There was visual evidence of enzyme binding to the soil surface using a BglC-Green Fluorescent Protein fusion. BglC binding to montmorillonite was shown to be negatively affected by low pH (pH 3) but not pH 10-11. Binding of the protein to kaolinite was not pH-sensitive. The enzyme's binding and activity were not affected by up to 1M NaCl. Our results provide further evidence for enzyme-soil binding via electrostatic interactions between the positively charged protein molecule and the negatively-charged soil surface.

**Keywords**: bglC, endoglucanase, soil binding, chemical soils (montmorillonite, kaolinite, sea sand)

#### SITE-DIRECTED MUTAGENESIS IDENTIFIES PUTATIVE SOIL-BINDING RESIDUES OF Bacillus subtilis 168 **ENDOGLUCANASE**

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The bglC gene of Bacillus subtilis 168 was cloned. Conserved amino acids that are positively-charged and accessible on the surface of the native Bacillus subtilis BglC endoglucanase were substituted with negativelycharged or neutral amino acids by site-directed mutagenesis with the aim of identifying residues in the putative soil binding domain. Lysine residues at positions 38, 177, and 249 seem to have significant roles in enzyme binding to soil as suggested by the decreased binding abilities of the mutant proteins. We hypothesize that the localized electrostatic changes due to the amino acid substitutions resulted in the decreased affinity of the protein for the negatively-charged soil surface. Further study is required to elucidate the precise role of each residue and the nature of the protein-soil interaction.

**Keywords**: BglC, endoglucanse, site-directed mutagenesis, soil-binding domain, chemical soils (montmorillonite, kaolinite)

#### MARINE FUNGI FROM Kappaphycus Alvarezii AND K. Strigtum: POTENTIAL CAUSATIVE AGENTS OF ICE-ICE DISEASE IN FARMED SEAWEEDS

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Farmed seaweeds have been infected by ice-ice disease for over 30 years and have resulted to severe production and revenue losses. Previous studies showed the occurrence of the disease following infection of pathogenic marine bacteria and environmental stress. No studies, however, relate the disease with marine fungi. Our research study then aims to isolate and characterize marine fungi from healthy and ice-ice-infected Kappaphycus species and asses their ability to induce the disease. Two species of Kappaphycus, K. alvarezii and K. striatum (green and orange varieties), were collected from Calatagan, Batangas. Following washing with sterile artificial seawater and cultivation of collected algae on the culture media Malt Extract Agar (MEAS) and Potato Carrot Agar (PCAS) supplemented with 33 g/L marine salts, 18 morphospecies of marine-derived fungi (MDF) were isolated. Highest fungal diversity (H=2.4) were then observed on infected than in healthy seaweeds. Among the Kappaphycus species, K striatum (orange variety) had the highest incidence of MDF with 67 fungal isolates while K striatum (green variety) had the lowest incidence with only 17 fungal strains. Most MDF isolates grew better in the presence of marine salts. The ability of the isolated marine fungi to produce carrageenolytic and cellulolytic enzymes and to utilize various components of red algae was also tested. Results showed that three and ten MDF isolates exhibited carrageenolytic and cellulolytic activity, respectively. Most isolates utilized different algal substrates, i.e. carrageenan, agar, and cellulose, but highest fungal biomass was obtained from several isolates grown in carrageenan. Among the ten fungal isolates assayed for their ability to induce "ice-ice" disease, infection with 3 MDFs (I, P, and F) produced iceice symptoms (thallus bleaching) in healthy, non-axenic cultures of K. alvarezii. Thus, marine-derived fungi may play a role in the induction of the

ice-ice disease in nature.

**Keywords**: marine-derived fungi, icc-icc disease, pathogenicity, scaweeds, *Kappaphycus* sp.

BS-8

#### DEGRADATION OF PYRENE, FLUORENE AND FLUORANTHENE: THE ROLE OF LIGNIN PEROXIDASE AND MANGANESE PEROXIDASE

FROM Ganoderma lucidum

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Reports on the medicinal properties of Ganoderma lucidum arc extensive, yet little is known of its ligninolytic system and bioremediation potential. The most ubiquitous ligninolytic enzymes produced by this fungus, lignin peroxidase (LiP) and manganese peroxidase (MnP) cultured in N-rich and N-poor media, respectively, were explored for their ability to polycyclic aromatic hydrocarbons pyrene, fluorene and fluoranthene. Mycelia collected after 3 d culture were incubated for 21 in both N-rich and N-poor media containing Polyaromatic hydrocarbons (PAHs) (20 mg fluoranthene, 2.5 mg pyrene and fluorene, respectively) with shaking at 30 °C. Metabolites were extracted with ethyl acetate and analyzed by Fourier Transform Infrared Spectroscopy (FTIR) and Thin Layer Chromatography (TLC). Results showed that crude LiP and MnP have the ability to distort the aromatic rings of fluorene, thus transforming C=C to C CH indicating a reduction in its aromaticity. In contrast, LiP and MnP were able to reduce the amounts of pyrene and fluoranthene but were not capable of their transformation. Analysis by TLC revealed the presence of these PAH metabolites but were not yet further characterized.

**Keywords:** Ganoderma lucidum, lignin peroxidase, manganese peroxidase, bioremediation potential, polycyclic aromatic hydrocarbons, lignin degrading activity

#### PROBIOTIC PROPERTY OF LOCALLY-ISOLATED LACTIC ACID BACTERIA AND DEVELOPMENT OF SOYA-BASED PROBIOTIC PRODUCT

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Functional probiotic foods may help prevent food-borne diseases and gut disorders, enhance the immune system and fight colon cancer and heart diseases. This study was done to characterize the probiotic properties of local bacteriocinogenic lactic acid bacteria (LAB) isolates, evaluate their anti-obesity and hypoglycemic effects in mice, and assess a soya milk-based probiotic drink.

Acid and bile tolerance tests were done on Lactobacillus fermentum 4B1 and Lb. pentosus 3G3 in comparison with Lb. paracasei IY9, a commercial probiotic isolate. Only 4B1 was able to recover after being exposed to pH 2 artificial gastric juice (AGJ) for 3 hours and transferred to pH 8 simulated intestinal fluid (SIF). However, at pH 3 AGJ, all strains were still viable and recovered in SIF.

Oral administration of the LAB isolates for 14 days showed that the blood glucose levels of mice fed with 4B1 and 3G3 decreased but not with 1Y9. The control group (normal diet) and high fat non-lactobacillus group showed increased blood glucose and body weight, respectively.

A formulation consisting of 150 ml 10% skim milk, 150 ml low sugar soymilk (4% sugar), 7.5 g white sugar, 7.5 g muscovado and 25 ml pineapple syrup, was most acceptable for a proposed probiotic drink on the basis of palatability and probiotic bacterial growth. This formulation was stable for 14 days with 3G3, 1Y9 and Lb. plantarum BS but only 2 days for 4B1. Sensory evaluation, in terms of sourness, sweetness, color, appearance and flavor, showed that the drink with BS was most acceptable, but not significantly different (P= 0.05%) from the drinks with 3G3, 4B1 and the uninoculated formulation. On the other hand the drink with 1Y9 was least acceptable and significantly different. The slight beany flavor of the fermented soy milk, though mixed with skim milk, still poses a challenge.

**Keywords**: Lactobacilli, Probiotic Soya Milk, Anti-obesity, Hypoglycemic

# BIOLUMINESCENT BACTERIA AS BIOINDICATOR OF MARINE ENVIRONMENTAL STRESS IN ILIGAN CITY

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Iligan City, the industrial city of the south, houses several industrial units. However, industrial progress poses tremendous threats to environmental health. The need to manage balance between industrialism and environmentalism should be prioritized so as to promote good environmental quality amidst industrialized development. Water quality along coastal areas adjacent to industrial sewages in Iligan City was monitored for toxic chemical contamination via bioindicators. Bacterial bioindicators were used in detecting environmental stress and contamination. Free living bioluminescent bacteria (Vibrio sp.) isolated from oxeye scad (Selar boops) and cuttlefish (Sepia sp.) were evaluated in terms of its ability as a bioindicator. The water samples obtained from selected sites near industrial units (cement factory, oil depot and chemical manufacturing company) were evaluated using a bioluminescence reduction and inhibition assay. Water samples showed high rate of decline in light intensity upon the first four hours of inoculation; about 56% in samples adjacent to cement factory, 65% in samples close to fuel oil depots and 82% in samples within the vicinity of a chemical manufacturing company. Variances among light intensity reduction rates were highly significant, thus the concentration of contaminants within sampling areas can be determined based on the reaction of bioluminescent bacteria. Results of the bioluminescence reduction and inhibition assay suggest that the water samples collected nearby industrial units were chemically contaminated and proved to be biohazardous due to rapid reduction of bioluminescence. Finally, the use of bioluminescent bacteria provides a simple, fast, inexpensive and efficient alternative environmental monitoring technique, thus it should be considered for regular detection of contaminants and evaluation of water quality.

**Keywords:** bioindicator, bioluminescent bacteria, *Vibrio* sp., bioluminescent reduction and inhibition assay, contamination

#### OPTIMIZATION OF FERMENTATION MEDIUM FOR THE PRODUCTION OF BIOACTIVE COMPOUND BY Streptomyces sp.

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The antimicrobial market is large and had a global sales of US\$ 23 Billion in 1996 (Rossner and Scott, 1996). The Streptomycetes produced around 55% of the 12,000 antibiotics known in 1995. Antibiotics can be antibacterial, antifungal or even anti-cancer agents, hence, the need to continue the search for antimicrobial compounds. Fermentation medium plays a significant role in the production of any antimicrobial compound. Components of the medium must enhance growth of the producing organism at the same time yield the highest amount of antimicrobials.

Streptomycete C1 was evaluated for its ability to produce an antimicrobial compound using three fermentation media: yeast malt broth (YMB), Croatian fermentation medium (CFM) and cassava medium (CM) after seven days of incubation with shaking. Three test organisms, namely, Staphylococcus aureus 1823, Escherichia coli 1825 and Salmonella sp. were used to evaluate the different media using cup cylinder assay. C1 grown in CFM gave the highest zone of inhibition among the media evaluated in all test organisms: 20.69 mm (S. aureus); 22.26 mm, (E. coli) and 21.79 mm, (Salmonella sp), in all media evaluated. On the other hand, C1 grown in either YMB and CM gave similar zones of inhibition on organisms.

Different concentrations of the cornstarch (10, 3 and 2% w/v) component of CFM were tested to optimize the growth and antimicrobial production of C1. CFM with 3% w/v cornstarch gave the highest production as shown by the larger zone of inhibition against S. aureus (22.06 mm.) and E. coli (22.58 mm.). The C1 broth extracted with ethyl acetate, evaporated to dryness and dissolved with methanol showed growth inhibition against the three test organisms.

Modified CFM is presently being utilized as production medium for the production of our bioactive compounds against Methicillin Resistant Staphylococcus aureus (MRSA).

**Keywords:** Antimicrobials, Croatian fermentation medium, cylinder cup assay, MRSA and Streptomyces.

#### IDENTIFICATION OF BACTERIA ISOLATED FROM STANDING AND DRIPPING WATER OF BULALON CAVE, BURDEOS, POLILLO ISLANDS, QUEZON PROVINCE

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Cave microbiology and its possible contribution to microbial diversity in the Philippines have not been given favorable attention at present and are almost neglected. It has been recognized that microbial contribution to cave ecology, mineral formation and ecosystem bioenergetics are some of the salient information that might be drawn from the taxonomic studies of microbial communities specifically from caves in the Philippines. Hence, preliminary assessment on the bacterial diversity of caves in the Philippines was conducted based on the samples collected from Bulalon Cave in the Municipality of Burdeos, Polillo Island, Quezon Province. Standing water from streams and underwater passages as well as dripping water were collected and analyzed following standard collection and isolation procedures. Representative bacterial isolates were then purified and characterized using the conventional methods. Cultural, morphological, physiological and biochemical tests revealed significant presence of Escherichia coli, Salmonella sp. and Pseudomonas sp. Furthermore, results suggest that the water inside the cave is highly contaminated with these microorganisms, either indigenous (resident) or transient from fecal wastes of common cave inhabitants, bats and birds, as well as those of human origin. Though they are common water-borne bacteria, their growth and adaptation in a subterranean environment of extreme and/or near-starvation conditions are indeed interesting facets of microbial diversity. Thus, characterization of bacterial strains is currently being further performed to explore taxonomic and biotechnological significance.

**Keywords**: cave microbiology, bacterial diversity, standing and dripping water, resident and transient microorganisms, subterranean environment

<sup>\*</sup>Part of UPLB Basic Research Project 88-D70-00

#### SIMPLE AND RAPID SCREENING OF ANTIMICROBIALS IN FEED SAMPLES

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The presence of antimicrobials in animal feeds and its ingredients is of great concern to the animal industry. The development of new microbial strains with antibiotic resistance poses a threat not only to animals but to consumers as well. Hence, a simple and rapid screening of antimicrobials in feed samples adapted from spot-on-lawn antimicrobial assay (U. S. .Pharmacopoeia, 1990) was conducted.

Ninety percent (90%) of 30 feed samples tested for antimicrobials against Salmonella typhimurium or Salmonella enteritidis were found positive, while one out of three feed samples tested for antimicrobials against Escherichia coli was found positive. Three different feed samples were found negative for antimicrobials against Candida albicans, Saccharomyces cerevisiae, Aspergillus flavus and Aspergillus parasiticus. It was also found that increasing the moisture content of feeds increased the availability of antimicrobials in the culture medium resulting in greater zone of inhibition.

Feed samples found to contain antimicrobials based on this simple method were further tested by artificial inoculation with Salmonella enteritidis and Salmonella typhimurium. Feed sample tested to be free of antimicrobials was used as control. Growth of these pathogens was monitored by cultural method and by PCR-based detection kit developed at BIOTECH. Results from this trial confirmed the preliminary evaluation obtained from the simple spot-on-lawn assay method.

With the growing concern of antibiotic resistance in farm animals for food production and antibiotic carry-over in humans, this simple and rapid detection of antimicrobials in feed could facilitate the screening of antimicrobials in feeds before use by the animal industry,

Keywords: antibiotic resistance, zone of inhibition, animal feeds, antimicrobials, spot-on-lawn antimicrobial assay

# EFFECT OF MYKOVAM, BENEFICIAL INDIGENOUS MICROBES AND COMPOST FOR IMPROVED GROWTH OF Terminalia catappa IN AN ACIDIC INFERTILE SOIL

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Terminalia catappa, commonly called Talisay, is a very promising tree for mass production due to its rich oil content and multiple medicinal uses of almost all parts of the plant. This study explored the establishment of Talisay in an acidic marginal grassland soil in the nursery with the help of mycorrhizal fungi, beneficial indigenous microbes and compost as soil amendments in addition to chemical fertilizers.

Results showed that application of compost plus chemical fertilizer (CF)(T6) gave significantly (p<0.05) the highest shoot fresh weight (FW) at 8.41 g/plant, widest leaf area at 319.2 m<sup>2</sup>, giving 30% increase in shoot FW and 28% in leaf area over the CF alone. Treatments T4 (BIM+CF), T5 (Mykovam +BIM+CF) and T6 (Compost+CF) gave similarly the highest total fresh weight or 11.99 g/plant, giving 61% increase over the CF plants alone. The treatment T4 (BIM +CF) gave significantly the second highest shoot FW, root dry weight (DW), and total DW. Treatments T7 (MYK + BIM +Com+CF) significantly (p<0.05) the highest root DW, total DW and the second highest leaf area. This is equivalent to a 40 % increase in Total DW over the CF plants and 70 % increase over the uninoculated control plants. The highest moisture content (MC) was observed in T6 (compost + CF) with a 244% moisture content, followed by T5 (Mykovam + BIM + CF) with 209% MC. The uninoculated plants had the lowest MC at 165% only and all parameters measured. It is recommended that best treatments be used in the reforestation of marginal grasslands to enhance survival and growth of plants.

Keywords: Talisay, BIM, biofertilizers, compost, Mykovam

#### LABORATORY SCALE BIOREMEDIATION OF COPPER-CONTAINING WASTEWATER FROM GOLD SMELTING USING BIOGENIC HYDROGEN SULFIDE

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The need for an economical, environment friendly and efficient treatment technology for heavy metal contaminated wastewater led to studies on bioremediation technologies. One of the promising agents is the Hydrogen sulfide (H,S) producers.

Application of biogenic H<sub>2</sub>S for the bioremediation of Cu<sup>2</sup>contaminated wastewater from gold smelting showed a 99.94 % removal of Cu<sup>2</sup> and recovery of 0.64 g of Copper sulfide (CuS). The concentration of Cu<sup>20</sup> was reduced from 1434 ppm to 0.885 ppm based on the analysis of Spectroquant NOVA 60, Parameters that were used include incubation of the sulfate reducing bacterial culture for three days at 36°C, use of 1875 mL Sulfate Reducing Medium (SRM), N<sub>2</sub> sparging of the bacterial culture every 2 min (total of 10 min) after 2 and 3 days of incubation and use of tubing with an inner diameter of 0.1 cm as inlet for H<sub>2</sub>S that will be reacted to Cu<sup>2+</sup> in the wastewater and separation of the growth chamber and the precipitation vessel. SRB-21 isolated from sediment samples from Mogpog, Marinduque was used in this study.

Excess H<sub>2</sub>S produced from 1875 mL bacterial culture was able to reduce the concentration of Cu<sup>2+</sup> in 100 mL of the wastewater from 1434 ppm to 677.95 ppm. This corresponds to an additional 53.72% Cu<sup>24</sup> precipitation and recovery of 0.16 g CuS.

This study showed that bioremediation technology based on biogenic H,S can clean up industrial wastewater contaminated with Cu<sup>2</sup>, recover Cu<sup>2+</sup> in the form of CuS, and the laboratory scale bioreactor system used is an effective treatment system for Cu<sup>23</sup> from wastewater of gold smelting.

Keywords: biogenic H<sub>2</sub>S, bioremediation, copper, gold smelting, sulfate reducing bacteria

#### ARSENIC AND MERCURY CONCENTRATIONS OF THE WATERS AND JANITOR FISHES (Pterygoplichthys spp.) IN THE MARIKINA RIVER, PHILIPPINES

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The Marikina River is an important river basin situated at the eastern side of Metro Manila, Philippines. Since the late 1990s, the Marikina River has been plagued by an invasive species, the janitor fish (Pterygoplichthys spp.). Despite this adversity, it continues to provide resources for people who are continuously dependent on the river for their livelihood. However, the Marikina River, like other rivers in the world, is also exposed to heavy metals emitted mostly from anthropogenic sources.

Historically, arsenic and mercury are used extensively for the benefits it provide but continuous use of these metals bring about numerous environmental and health implications that are of great concern. The study aims to assess the total arsenic and total mercury concentrations of the waters obtained and janitor fishes caught from the upstream and downstream areas of the Marikina River in June 2008. Results were analyzed using the t test for unpaired observations at a 95% significance level.

Arsenic and mercury concentrations were detected in the janitor fishes caught and in the waters obtained from the Marikina River. The mean total arsenic and mean total mercury concentrations were 0.001 and 0.084 mg/L, respectively, and the fishes examined had mean total arsenic and total mercury concentrations of 0.015 and 0.012 mg/kg, respectively. The mean total mercury concentrations in the waters exceed the DENR permissible limit of 0.02 mg/L. No significant differences in the total arsenic and mercury concentrations in the waters and in the janitor fishes caught at the upstream and downstream areas of the Marikina River. This study provides the preliminary groundwork in assessing the extent of heavy metal pollution in the waters and janitor fishes in the Marikina River. Findings of this study may eventually guide decisions and policies on the protection of water sources and the lives of people dependent on these waterways.

**Keywords**: Arsenic, Mercury, River Basin, Janitor fish, Philippines

#### MANAGEMENT OF Vibrio INFECTIONS IN FISHERY INDUSTRY BY ANTIBIOTIC SUSCEPTIBILITY PROFILING

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Vibrio causes the most diseases and infections in the tropical fishery industry which has led to significant mortality in fishery culture. Despite employment of several chemical treatments and sanitary procedures, Vibrio infections still prevail indicating the ineffectiveness of the methods used or the possible development of antibiotic resistance of Vibrio strains. This study determines the antibiotic susceptibility profile of Vibrio isolates from Peneaus monodon (Tiger prawn) and Selar boops (Oxeve scad) against common broad-spectrum antibiotics. Vibrio strains were isolated from external body surface and intestinal content of tiger prawn and oxeve sead and were grown on Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar), a selective and differential medium for isolation of Vibrio. Phenotypic profiles of the isolates were determined by employing the Kirby-Bauer disc diffusion method. All Vibrio strains isolated from tiger prawn and oxeye scad were resistant to chloramphenical and conversely susceptible to streptomycin and erythromycin. The emergence of chloramphenicolresistant Vibrio strains indicates the need for constant monitoring of microbial profiles in local fisheries as well as establishment of guidelines regarding methods being employed in eradicating infections in the fishery industry.

Keywords: Vibrio, Peneaus monodon, Selar boops, antibiotic susceptibility profiles

#### MICROBIAL LOAD ASSESSMENT OF SOME "ONE TOWN ONE PRODUCT (OTOP)" FOOD PRODUCTS OF ILOCOS NORTE

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This study was conducted to pre-assess the OTOP food products to be packed by the collaborative mobile packaging project of NEDA-DBP-MMSU. This endeavor ensures safety of consumers and creates benchmark information on the initial microbial load and shelf life of the OTOP food products.

Microbial load assessment was conducted for the following OTOP food products of Ilocos Norte: Chichacorn, Longganisa and Bagnet. Chichacorn samples from 8 famous manufactures in Paoay were considered. Bagnet Samples from 6 producers from Batac were studied and Longganisa samples obtained from 6 entrepreneurs in Batac were obtained. The samples were inoculated in Nutricut Broth and plated in Nutrient Agar. The Total Plate Count for each sample was counted. Results show that initial readings were above acceptable readings. Results of this study relayed to the manufacturers led to better measures employed in handling and preparation of their products thus lessening microbial load.

Keywords: One Town One Product, Microbial load, Total Plate Count, Food **Products** 

#### LIPID PEROXIDATION AND PATTERNS OF CADMIUM AND LEAD ACCUMULATION IN THE VITAL ORGANS OF SUCKERMOUTH ARMORED CATFISH Pterygoplichthys pardalis CASTELNAU FROM MARIKINA RIVER

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Lipid peroxidation and the patterns of cadmium (Cd) and lead (Pb) accumulation in the gills, liver, gut and muscles of Pterygoplichthys pardalis from four sites of Marikina River were analyzed to study effects of possible metal toxicity alongside stress response. Mean Cd concentrations from all tissues studied are within permissible amounts (0.02-0.05 mg/L). Mean Pb concentrations from the muscles, gills and gut were within permissible amounts except for the liver (0.22 mg/L). Lipid peroxidation, indicative of oxidative stress was highest in the spleen, followed by the liver and gills among fish samples. Lipid peroxidation values were not correlated with Pb and Cd concentrations in fish samples and were not site-significant which suggest that other possible factors might have also contributed to such stress aside from the Pb and Cd determined in the study. Morphologic and histological observations of gills, gut and liver show general health for this invasive pollution tolerant species, confirming several adjustments and adaptations towards air-breathing, though minute pathologic changes were seen in the liver and gills. This study is the first to assess the patterns of metal accumulation in this invasive species under field conditions in relation to oxidative stress. Further studies using specific biomarkers of metal accumulation and oxidative stress are necessary to support the novel results of this study in *P. pardalis*.

**Keywords**: Lipid peroxidation, Pb and Cd bioaccumulation, janitor fish, Pterygoplichthys pardalis, oxidative stress

# SALINITY TOLERANCE OF FRESHWATER CATFISH (Clarias batrachus Linnaeus AND Clarias macrocephalus Gunther) IN AQUARIA IN THE LABORATORY OF CSCST- FISHERY AND INDUSTRIAL COLLEGE, SAN FRANCISCO, CEBU

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A plan for the cultivation of freshwater catfish in marine waters of Camotes Islands was made due to its diminishing yield as well as to improve its taste. This study was conducted to determine the reactions of the two species of freshwater catfish (*Clarias batrachus* Linnaeus and *Clarias macrocephalus* Gunther) to the dilution process in terms of swimming behaviour, breathing, color changes and the feeding behaviour and movement of the fish including its mortality and survival rates.

The Complete Randomized Design (CRD) using glass aquaria was used to prepare the culture media. There were five batches and six treatments of this study in which the control ( $T_0$ ) had a salinity range from 0-0.9 ppt;  $T_1$  is 1-5 ppt;  $T_2$  is 6-10 ppt.;  $T_3$  is 11-15 ppt.;  $T_4$  is 16-20 ppt;  $T_5$  is 21-25 ppt. Feeds given were 5% of the body weight of the fish.

Results showed that leaping and other swimming movements of both species of fish increased from 2.0 ft. in  $T_0$  to 2.3 ft in  $T_3$ ; however, it slows down to 1.4 ft. in  $T_4$ . Breathing frequency was highest in  $T_3$  (61 times in 1 hour in both species of freshwater catfish and lowest breathing rate was 29 times/hr in  $T_4$ . Color changes occurred after several days from black to dark brown in  $T_0$  to dark brown in all the rest of the treatments. Feeding consumption rates from 25 seconds in  $T_0$  increased to 30 seconds; 40 seconds and 230 seconds in  $T_1$ ,  $T_2$  and  $T_3$  respectively. In  $T_3$  only 15% of the feed given was consumed in this treatment.

Mean survival rate of *Clarias batrachus* Linnaeus and *Clarias macrocephalus* Gunther was 100% from T<sub>0</sub> to T<sub>3</sub> but a very high salinity level was fatal.

**Keywords**: Clarias batrachus Linnaeus, Clarias macrocephalus Gunther, Aquaria, Salinity Tolerance

#### PHYSIOLOGICAL SIGNALING AND ADAPTATION PROCESSES TO DROUGHT AND SALINITY STRESS INTERACTIONS IN RICE

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The effects of drought and salinity stresses in plants are being studied extensively at the physiological and molecular levels. However, most studies deal with the single effect of each stress. However, under natural conditions, a single stress factor does not exist; thus this study aimed to address the conditions that will likely approximate that of the natural environment.

A simple, repeatable system of combined drought and salinity stresses under a managed environment was established. Fully established rice seedlings of Vandana (drought tolerant), Pokkali (salt tolerant), IR-64 (drought sensitive) and IR-29 (salt sensitive) were challenged by drought, salinity and their combination. Physiological traits (e.g. osmotic adjustment, relative water content and growth characters) were used as screening parameters for drought and salinity stress interactions. Among the stresses imposed, the combined interaction of salt and drought stress was the most detrimental to all rice plants irrespective of genotypes. This was confirmed by the high relative water content (RWC %) of the leaf tissues in all cultivars under salinity stress as compared with the RWC of the various cultivars under drought and combined drought plus salinity stresses. The results were further confirmed by the low osmotic concentration of rice cultivars under salinity stress. Although different rice genotypes respond negatively to these abiotic stresses, Vandana (drought tolerant) and Pokkali (salt tolerant) were less affected by the stresses. The system established may be used by molecular biologists to search for novel genes working for both drought and salinity stresses.

**Keywords**: salinity, drought, interaction, adaptation, physiological signal

#### FRUTICOSE LICHENS FROM SELECTED SITES IN LUZON AS SOURCES OF BIOLOGICALLY ACTIVE LICHEN ACIDS

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With new and re-emerging diseases, the search for new drugs from natural sources is of urgency. Lichens, a symbiotic association between a fungus and an alga or a cyanobacterium, are known prolific sources of secondary metabolites and thus, can be tapped as new sources of biologically active natural products. Our research study then explores the antibacterial activities of lichen acids extracted from fruticose lichens. Sixty three lichen thalli were collected from different sites in Luzon Island, e.g. Bataan, Batangas, Benguet, Cavite & Laguna. Morphological characterization identified the collected fruticose lichens as Usnea baileyi (3), Ramalina dendriscoides (58), Stereocaulon massartianum (1) and Cladonia gracilis (1). The lichen thalli were then air-dried and their lichen acids extracted with acetone and tested against gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus) and gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) using the paper disk diffusion assay. Results showed that all the 45 tested extracts inhibited at least one of the test bacteria. Most of the lichen extracts were found active mainly against gram-positive bacteria. Extracts from R. dendriscoides were observed to be the most active. Selected lichen extracts also showed activity against S. aureus even at a volume of 30 µl. MIC/MBC of two lichen extracts, Rd06 and Rd42, were found to be 156 µg/ml and 2,500 µg/ml, respectively. Detection of lichen acids using TLC showed the presence of at least eight lichen acids. TLCbioautography detected barbatic acid, stictic acid, diffractaic acid, galbinic acid, norstictic acid, salazinic acid and usnic acid to be the bioactive lichen acids.

**Keywords**: fruticose lichens, lichen acids, paper disk diffusion assay, TLC, bioautography

#### LEMON GRASS (Cymbopogon citratus) EXTRACT: A PROMISING ANTIBACTERIAL JUICE

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The antibacterial property of lemon grass (Cymbopogon citratus) crude extract in formulated juice was determined in this study. Lemon grass is widely grown in some parts of Asia (Malaysia, Thailand, Philippines, etc.). It is known to have useful properties such as antiseptic and antifungal. It also contains citral and antimutagenic (Mycecerene) components. Thus, lemon grass was used in this study as an additive in the formulation of a juice drink which may benefit people because of its antibacterial value.

The antibacterial activity was analyzed using the paper disc assay method. The two test organisms used were *Staphylococcus aureus* and *Escherichia coli*. Kanamycin monosulfate was used as positive control and sterile water as negative control.

Results showed that the crude extract of lemon grass was less effective in inhibiting the growth of *S. aureus* and *E. coli* as compared to the positive control. However, when formulated into juice, lemon grass extract was found to be more effective than the kanamycin sulfate in inhibiting the growth of the two test organisms. The sensory evaluation test showed that the concentrations 20g lemon grass:200ml water and 20g lemon grass:400 ml water with honey and calamansi are the most acceptable. However, the concentration of 20g lemon grass:400 ml water with honey and calamansi is more acceptable in terms of color.

The data support the hypothesis of this study that lemon grass crude extract has antibacterial activity against *S. aureus* and *E. coli* which can be used in the formulation of lemon grass juice with antibacterial value.

**Keywords**: Cymbopogon citrates, Staphylococcus aureus, Escherichia coli, Kanamycin, antibacterial

#### ANTIMICROBIAL ACTIVITY OF Chromolaena odorata (L. f.) KING & ROBINSON AND Uncaria perrottetii (A. RICH) Merr. EXTRACTS

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Ethanol extracts of leaves of Chromolaena odorata (L. f.) King & Robinson and ethyl acetate extracts of stem bark of *Uncaria perrottetii* (A. Rich) Merr, were examined for their antimicrobial properties. C. odorata and U. perrottetii extracts were tested against bacteria, namely, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus; and fungus, Candida albicans. Potential antimicrobial compounds were obtained through solvent extraction and rotary evaporation. To determine the antibacterial and antifungal properties of the extracts, disc diffusion assay was performed and minimum inhibitory concentration (MIC) was determined. C. odorata extracts revealed antibacterial activities, inhibiting the growth of B. subtilis, S. aureus, and S. typhimurium, MIC data showed that 0.5%, 0.1%, and 0.3% were the inhibitory concentrations of the plant extract against B. subtilis, S. aureus, and S. typhimurium, respectively. Results of antimicrobial assay for U. perrottetii extracts showed that only the aqueous extract of this plant demonstrated antibacterial and antifungal activities; inhibiting B. subtilis, S. aureus, and C. albicans. MIC data showed that B. subtilis, S. aureus, and C. albicans can be inhibited at 0.3%, 0.1%, and 0.1% concentrations of the plant extract, respectively. The organic extract of *U. perrottetii* showed no activity. Preliminary phytochemical screening revealed the chemical composition of the plant extracts of C. odorata containing flavonoids, saponins, tannins, and steroids, while U. perrottetii possessing alkaloids, tannins, and leucoanthocyanin. Thus, these plant extracts can possibly be used to produce alternative forms of antimicrobials.

**Keywords**: Chromolaena odorata; Uncaria perrottetii; antimicrobial; MIC, phytochemical screening

#### PRELIMINARY SCREENING OF METHANOLIC EXTRACTS OF KALINGAG (Cinnamomum mercadoi Vidal) AND TALISAY (Terminalia catappa) AGAINST METHICILLIN RESISTANT Staphylococcus aureus (MRSA)

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In Surigao del Norte, the native Mamanuas and other barriofolks use Kalingag (Cinnamomum mercadoi Vidal) as medicine to help digestion, aid in flatulence and as an expectorant while Talisay (Terminalia catappa) is used as sudorific and antihelmenthic. The widespread use of these plants in traditional medicine led the researchers to determine its effectivity against methicillin-resistant Staphylococcus aureus (MRSA). The worldwide occurrence of MRSA is one of the most common causes of fatal infections and diseases in hospitals. The drug of last resort is vancomycin. In 2002, the emergence of vancomycin-resistant enterococci and vancomycin-resistant S. aureus is raising serious public health concerns. With the emergence of resistant organisms, it is high time to continue search for novel antibiotics that inhibit pathogens resistant to other antibiotics.

Preliminary screening of methanolic extracts of Kalingag and Talisay leaves by cup cylinder method showed that Talisay can inhibit MRSA (22mm dia.). However, Kalingag extract has lower zone of inhibition (20.70mm) against MRSA which is significantly different than the control antibiotic (Vancomycin, 1,000ppm) at 24.55mm. Thin layer chromatography showed several spots on Talisay crude extract. Moreover, bioautography plate has an active spot against MRSA.

Findings of this study suggest that Kalingag and Talisay can be a potent source of novel compounds for the treatment of infectious diseases.

Keywords: Kalingag, Talisav, vancomycin, methanolic extracts, bioautography

### THE ANTISEPTIC ACTIVITY OF Psidium guajava ESSENTIAL OIL

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The use of essential oils as disinfectants and antiseptics is not entirely new. A wide variety of antiseptic compounds in an essential oil make mutation of microorganisms extremely difficult. This study investigated the antiseptic activity of *Psidium guajava*, guava, leaf essential oil against clinical staphylococci, streptococci, Escherichia coli, Klebsiella pneumoniae, and Salmonella typhi. Its major constituents, eugenol and limonene, were also assayed against standard ATCC strains: S. aureus 25923 (Sa), E. coli 25922 (Ec), and Pseudomonas. aeruginosa 27853 (Pa). Fresh guava leaves were hydro-distilled in a condenser with a clavenger attachment. The guava leaf oil, GLO, was dehydrated with anhydrous sodium sulfate and refrigerated in amber vials. GLO was emulsified in 20% Tween 80 and distilled water (0.1:0.05:1.85), diluted twofold serially with distilled water, and mixed with Mueller Hinton agar (± 7% sheep's blood) to final concentrations of 0.5-0.0078% (v/v). Clinical isolates were identified with the Vitek system using GPI and GNI cards. Approximately 10<sup>4</sup> cfus of bacterial suspensions in the logarithmic phase was spot-inoculated on the GLO-MHA plates and allowed to dry. Plates were incubated overnight at 35°C. The minimum inhibitory concentration was read as the least GLO concentration inhibiting bacterial growth. Results indicate that growth of Staphylococcus and Streptococcus clinical isolates was best inhibited by GLO at 0.03-0.25%, v/v, and <0.0078-0.25%, respectively. Salmonella typhi was inhibited at 0.25-0.5%, and both E. coli and K. pneumoniae were not inhibited at 0.5%. Eugenol was active against Sa and Ec at 0.125%, while limonene was active against only Sa at 0.25% and did not inhibit Ec and Pa at the highest concentration used, 0.5%. The data suggest that eugenol- a phenolic alcohol, and limonene- a monoterpene could be responsible for the antiseptic property of GLO. This antiseptic property of GLO may be used to formulate topical ointments and personal hygiene products.

**Keywords**: guava leaf essential oil, antiseptic, disinfectant, minimum inhibitory concentration, eugenol, limonene

#### EVALUATION OF THE POTENCY OF GENERIC ANTICANCER DRUGS AGAINST VARIOUS HUMAN TUMOR CELL LINES USING *IN-VITRO* CELL-BASED ASSAY AND PARALLEL LINE ASSAY

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Anticancer drugs must be cytotoxic and potent enough to successfully treat tumor tissues or cells. Storage and temperature requirements must be strictly monitored and adhered to when transporting these drugs from one facility to another. Deviations from the prescribed storage and transport conditions may impact on the activity of the drug. Potency is an expression of activity of a drug in terms of concentrations required to achieve a desired effect. Relative potency is defined as the potency of any biological product expressed relative to a well-defined reference preparation. The objective of the study was to compare the *in-vitro* cytotoxic activity of generic anticancer drugs and to determine its relative potency in comparison with the originator. In this study the cytotoxic potency of six (6) anti-cancer drugs was determined in an *in-vitro* cell-based assay using different human tumor cell lines. Half-maximal inhibitory concentration (IC50) of the different drugs was obtained from an experimentally derived dose-response curve. The dose-response curve of the different oncology products tested was comparable with the originators. The cytotoxic response based on IC50 varied depending on the cell type used. The relative potency effect of the products was analyzed statistically using Parallel Line Assay (PLA version 111.2.06), which demonstrated that generic oncology products tested had similar efficacy with that of the originator. The products showed equivalent results as proven by both *in-vitro* cell-based assay and statistical analysis. *In-vitro* cell-based assay promises to be a useful and reliable method of demonstrating anticancer drug activity.

**Keywords:** IC50, MTT, PLA, potency, relative potency

#### PHYTOCHEMICAL SCREENING AND TOXICITY OF SOME GREEN LEAFY VEGETABLES CONSUMED IN BATAC, ILOCOS NORTE

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Green leafy vegetables (GLV) such as *Ipomea batatas, Ipomea aquatica* and *Corchorus olitorius* are some of the traditionally grown vegetables in Batac, Ilocos Norte. These vegetables have long been consumed as part of the native cuisines of Ilocano culture. GLV have dietary components and phytochemicals like alkaloids, cardenolides, tannins, saponins and flavonoids that play important roles in alleviating certain disorders/age-related diseases. Non-essential or toxic substances can also be present and when accumulated in the body, they give a negative effect leading.

The leaf and stem extracts of the GLV were subjected to: (1) phytochemical screening, (2) toxicity assessment considering Lethal Concentration 50 (LC<sub>50</sub>) through Brine Shrimp Toxicity Test (BSTT), and (3) calculation of the acceptable daily intake (DI) of GLV by individuals.

Phytochemical screening revealed that leaf and stem extracts of all the GLV contain alkaloids and cardenolides. Tannins were found present only in *C. olitorious* and *I. aquatica*. Whereas, saponins were found present in *C. olitorious* and flavonoids in *I. batatas*.

BSTT revealed that extracts of *Ipomea batatas* exhibited the highest toxicity against the shrimps (LC<sub>50</sub> value 14.79 µg/ml), compared with *Ipomea aquatica* (LC<sub>50</sub> value 52.48 µg/ml) and *Corchorus olitorius* extract (LC<sub>50</sub> value 69.18 µg/ml).

Based on the evaluated toxicity level, the computed acceptable Daily Intake (DI) of these GLV in mg/kg of body weight as reference for the daily diet are as follows: *Ipomea batatas* (36.98 mg/kg of body weight), *Ipomea aquatica* (131.2 mg/kg of body weight) and *Corchorus olitorius* (172.95 mg/kg of body weight).

Hence, the GLV consumed in the City of Batac have alkaloids,

cardenolides, tannins, saponins and flavonoids, two of which have antioxidant property (alkaloids and flavonoids) necessary to reduce the ageing process and cytological degenerative behavior. The exhibited toxicity of GLV extracts against the shrimps can cause acute or chronic toxicities only when consumed in large quantities, thereby, attention to consumption and daily intake must be given to avoid extensive damage to cells and its metabolism.

Keywords: Phytochemical, Toxicity, GLV, LC50, Daily Intake

**BS-29** 

MASAKUSIKAM HERBED COOKIES: INCORPORATION OF LEAVES OF MALUNGGAY (Moringa oleifera), SALUYOT (Corchorus olitorius), KULITIS (Amaranthus spinosus), SILI (Capsicum frutescens) and KAMOTE (Ipomea batatas)

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The present worsening global health situation calls for healthier functional foods. This study was done to check for the possibility and acceptability of producing nutrient enhanced cookies through the incorporation of leaves of malungay, saluyot, kulitis, sili and kamote, referred to as MaSaKuSiKam puree. Results showed that five percent (5%) of MaSaKuSiKam puree incorporation on the flour mixture was slightly preferred over the ten percent (10%) formulation. Variations in flavor were done through the incorporation of other ingredients. Thus cookies were prepared that were: 1) pure herbed (control), 2) herbed-chocolate chip flavored, and 3) herbed-carrot flavored cookies. Sensory evaluation was undertaken in terms of taste, odor, color intensity, mouth feel and general acceptability and compared with just plain (unammended cookies). Proximate analysis of crude protein and crude fiber as well as elemental analysis of iron and calcium content were done.

Statistical analysis showed no significant differences in all sensory attributes of the plain and herbed cookies. This means that herbed cookies

were accepted similarly as the plain cookies by the participants/tasters. Higher nutrient contents of the herbed cookies made them more nutritious compared to the plain cookies. Procedures on the preparation of the cookies will be elaborated. It is recommended that studies on the incorporation of MaSakuSiKam puree to other food products be done.

Keywords: cookies, herbed cookies, malungay, saluyot, kulitis

**BS-30** 

#### REDISCOVERING JOB'S TEARS

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Preparing for quality life? There is money-making leisure out of the different products derivable from the plant which the local folks would name "job's tears, adlay, ag-gey, ag-dey, tigbi, etc." The plant, considered a weed by some farmers when found growing in rice paddies, is scientifically known as Coix lachryma-jobi Linn. Belonging to the same family where rice, corn, barley, sorghum, belong (i.e. Family Poaceae/Gramineae), Coix is known to have a wide array of uses. Through interviews with the local folks who are knowledgeable on job's tears, this study documented several cultivated and wild varieties of *Coix* in three municipalities of Kapangan, Kibungan and Atok in Benguet Province. When the seeds are prepared into food (as when cooked like oatmeal, as tea or even into 'tapey' or rice wine), into ornaments (as when made into earrings, bracelets, necklace, rosaries, or curtains), or as medicine (to cure some ailments like wounds, urinary tract infections, etc.), it is remarkable that Coix plant is going to provide a lot of economic opportunities for people, both young and old alike, to prepare for quality life ahead. The study also showed that *Coix* is superior in terms of some essential nutrients and vitamins as compared with rice. Indeed, we may just have a solution at hand to the current rice shortage crisis. Rediscovering such diverse uses also would be inherent for people to protect, conserve biodiversity and propagate the plant because they realize that their livelihood and hence, survival, depend on the plant.

Keywords: Poaceae/Gramineae, job's tears, ornaments, rice wine

#### CpG OLIGODEOXYNUCLEOTIDES AS **IMMUNOMODULATORS** OF rBio t 5-INDUCED ALLERGIC REACTIONS

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Unmethylated oligodeoxynucleotides (ODN) containing CpG motifs have been implicated to play a role in shifting allergic Th2 response to a less inflammatory Th1 reaction by modulating cytokine and antibody expressions. The immunomodulatory role of CpG-ODN in the regulation of sensitization to the house dust mite Blomia tropicalis recombinant Blo t 5 allergen in BALB/c mice was determined in this study. The immune response of BALB/c mice administered with rBlot5+CpG, rBlot5 alone, or PBS were determined through the production of immunoglobulin subclasses and the cytokines IFN- and IL-4. Pre-treatment with CpG ODN resulted in elevated IFN- and rBlo t 5-specific IgG2a antibody expression. Moreover, CpG-treated mice showed significantly reduced IL-4 and IgE production compared to the Blo t 5-treated and placebo groups (p<0.05). Results obtained in this study confirmed that CpG ODN exhibit immunoprophylactic effects on rBlo t 5-iduced allergic reactions. Its use as an adjuvant in the development of highly effective vaccine and immunotherapeutic reagent against house dust mite allergy is highly recommended.

oligonucleotide, allergy, Blomia tropicalis, recombinant Keywords: allergen, immunomodulator

#### ENHANCED AUTOPHAGIC CELL DEATH IN EXPANDED POLYHISTIDINE VARIANTS OF HOXA1 REDUCES PBX1-COUPLED TRANSCRIPTIONAL ACTIVITY AND INHIBITS NEURONAL DIFFERENTIATION

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HOXA1 is a member of the homeobox gene family and is involved in early brain development. In our previous study, we identified novel variants of polyhistidine repeat tract in HOXA1 gene and showed that ectopic expression of expanded variants led to enhanced intranuclear aggregation and accelerated autophagic cell death in a time-dependent manner. Autophagy has also been reported to have an important role in ageing. Here. we further investigate the implications of polyhistidine variants on HOXA1 function. Aside from intranuclear aggregation, we observed cytosolic aggregates during the early stages of expression. Rapamycin, an autophagy inducer, resulted in decreased protein aggregation and cell death. Here, we also show an interaction between variants of HOXA1 and one of the HOX protein known cofactors, PBX1. Expanded HOXA1 variants exhibited reduced PBX1-coupled transcriptional activity through a regulatory enhancer of HOXB1. Moreover, we demonstrate that both deleted and expanded variants inhibited neurite outgrowth in retinoic acid-induced neuronal differentiation in neuroblastoma cells. These results provide further evidence that expanded polyhistidine repeats in HOXA1 enhance aggregation and cell death, resulting in impaired neuronal differentiation and cooperative binding with PBX1.

Keywords: Autophagy, Cell Death, HOXA1, Polyhistidine Repeats

#### MOLECULAR INVESTIGATION OF CYCLOTIDES IN Rubiaceae AND Cucurbitaceae

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Cyclotides are small disulfide-rich peptides that form unique cyclic structures and have a range of interesting biological activities including antihuman immunodeficiency virus (HIV) and neurotensin inhibition, antimicrobial activity and insecticidal activity. Extensive studies have been conducted in the plant family Violaceae, but remains limited for other related plant families from the tropics.

The five hundred eight peptide sequences of known cyclotides were sub-grouped into the four major cyclotide families (circulin A, kalata B1, cycloviolacin 01 and palicourein) and back-translated using the VectorNT1 10 software to generate the consensus sequence for the synthesis of representative polymerase chain reaction (PCR) primers. The genomic DNAs were isolated from selected species belonging to the families Rubiaceae and Cucurbitaceae. The PCR of ampalaya (Momordica charantia), gardenia (Gardenia pseudosidium) and squash (Cucurbita maxima) were able to yield amplicons from 80 to 200 bp, the typical size range of a putative cyclotides. The amplicons were cloned into pGEM T Easy vector and were submitted for nucleotide sequencing. Pattern matching of the nucleotide sequences revealed putative cyclotides similar to the Kalata B1 family.

**Keywords**: cyclotides, circulin A, kalata B1, cycloviolacin 01, palicourein, Rubiaceae, Cucurbitaceae

#### IDENTIFICATION OF RIBOSOME-INACTIVATING PROTEIN (RIP) IN SELECTED PLANTS FOR MEDICINAL PLANT PROPERTIES SCREENING

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Ribosomal-inactivating proteins (RIPs) are proteins that are generally found only in the leaves, seeds and latex of some plants. These proteins naturally function as part of the defense mechanism of plants against predators like bacteria, viruses and insects. These proteins have the ability to inactivate the ribosomes of such predators, hence, disrupting protein synthesis. As such, ribosomal inactivating proteins (RIPs) exhibit antiviral, antibacterial, antifungal as well as antitumour activities. These proteins are important in the field of medicine, particularly in the production of drugs against various pathogens.

This present study aimed to isolate RIPs from the seeds of Cucurbitaceae plants including Cucumis melo (melon), Cucumis sativus (cucumber), Luffa acutangula (patola) and Cucurbita maxima (squash). Protein extracts were then analyzed using SDS-PAGE. Results of the SDS-PAGE analysis showed that nearly all of the plant species had at least a protein that is close to the molecular weight of known, documented RIPs from the same plant family. Cucumis melo and Luffa acutangula both had protein bands at 25 kDa close to the 26 kDa molecular weight of pepocin; Cucumis sativus has a 30 kDa band similar in weight with a novel RIP found in Cucurbita moschata; Cucurbita maxima had a protein band with a molecular weight of 29 kDa which has the same molecular weight with TAP-29 RIP. On the other hand, two RIP genes (Curcin 1 and Curcin 2) were identified through PCR analysis using four gene specific primers among plants of the genus Jatropha (J. curcas, J. podagrica and J. pandorifolia) of the family Euphorbiaceae. Another plant species (Acalypha hispida L.) also of the family Euphorbiaceae was also found to have the Curcin 1 gene. Cloning experiments for the confirmation of the candidate Curcin genes will be further pursued.

**Keywords**: genes, proteins, defense, Cucurbitaceous, Euphorbiaceous

#### MOLECULAR CLONING OF CYSTEINE PROTEASE INHIBITORS FROM FOUR ENDEMIC PHILIPPINE PLANTS

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In the recent years, plant based cysteine protease inhibitors (CPIs) have found novel uses in the biomedical sciences such as antiviral, neurodegenerative suppressor, and anti-tumor activities. Though studies have been extensive on several plant families like Brassicaceae, Cucurbitaceae and, however, the studies on endemic Philippine plants are rare. Four endemic Philippine plants, namely: Lycopodium cernuum (Lamon-babae). Trichosanthes cucumerina Linn. (Melon-melonan), Quisqualis indica (Niyog-niyogan) and Triumfetta bartramia Linn, (Kulutkulutan) were investigated for CPIs. Three sets of specific polymerase chain reaction (PCR) nucleotide primers representing CPI sub-families were designed from Genbank published sequences and were used to isolate CPI nucleotides from the genomic DNA of the listed endemic plants. PCR amplicons of several sizes were isolated from L. cernuum, 600 and 1,500 bp; T. cucumerina, 750 bp; O. indica, 450 bp; and T. bartramia, 450 and 750 bp. The amplicons were cloned into pGEM T Easy vector and were submitted for nucleotide sequencing. The results established the first report on the presence of CPI nucleotide fragments among the tested plants species.

Keywords: cysteine protease inhibitors, polymerase chain reaction, Lycopodium, Tichocanthes, Quiscalis, Triumfetta

#### UNRAVELING SHRIMP IMMUNITY BY RNA INTERFERENCE TECHNOLOGY

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Shrimp aquaculture is an indispensable source of revenue in Southeast Asian and other developing countries. The industry however, is now plagued by a lot of disease and other disease-related problems. Understanding, therefore, the mechanism of the shrimp immune system will not only contribute to the basic knowledge of shrimp biology but will be of enormous importance in the proper management and control of shrimp diseases as well. Here, we utilized RNA interference (RNAi) technology to clucidate the function *in vivo* of immune related gene in a crustacean system.

In vivo gene silencing studies were conducted utilizing transglutaminase (TGase) and clotting protein (CP) genes. Double stranded RNAs (dsRNA) were generated in vitro using T7 Ribomax express following the manufacturer's recommendations. Shrimp of about 1-2 grams weight were injected with 1 and 10 µg of dsRNA for each gene and gene expression in relevant organs were analyzed. RT-PCR was also conducted using primers from other immune-related genes.

Expression of TGase mRNA was inhibited in gills, heart, hemocyte, hepatopancreas, intestine and lymphoid organ while the CP gene was suppressed only in gills and heart tissues on day-1 post injection with 1  $\mu$ g and 10  $\mu$ g of TGase- and CP-dsRNA, respectively. Systemic gene silencing was observed for both genes and dosages as shown by mRNA expression, blood coagulation and protein data on day-7 post injection. Suppression of antimicrobial peptides (crustine and lysozyme) and genes involved in the prophenoloxidase system (prophenlooxidase and  $\alpha$ -2 macroglobulin) following TGase and CP silencing demonstrate an association between blood coagulation and humoral biodefense genes in shrimp.

Results showed that RNAi technology is indeed a powerful tool in elucidating gene function in shrimp. Findings of this study can also serve as model for the possible prevention of viral or bacterial disease by utilizing reverse genetics technology through RNA interference.

**Keywords:** shrimp, RNA interference, transglutaminase, clotting protein, blood coagulation

BS-37

#### 3-D MODELING AND STRUCTURE OF GFP-LIKE PROTEINS FROM PHILIPPINE SOFT CORALS AND SEA ANEMONES

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Green Fluorescent Protein (GFP)-like proteins isolated from marine invertebrates, particularly from class Anthozoa, have comparable structural and biochemical features with the GFP isolated from the jellyfish Aeguorea victoria. In this study, soft coral and sea anemone samples collected from Taklong Island, Guimaras were screened for fluorescence prior to total RNA extraction and cDNA library construction. These cDNA libraries were used to amplify contiguous regions using multiple rounds of PCR and DNA sequencing. For the initial amplification of internal regions, consensusdegenerate hybrid nucleotide primers (CODEHOP) were used and RACE-PCR was done to assemble full-length cDNA sequences. Analyses of the cDNAs from soft coral (SC) and sea anemone (SA) samples show that their GFP-like proteins contain high sequence similarities with the green fluorescent proteins of Sarcophyton sp. and Heteractis sp. respectively. Translated sequences obtained from three soft coral species are each comprised of 227, 224 and 230 amino acids while those from two sea anemone samples are each made up of 228 amino acids. All samples contain the highly conserved three amino acid chromophore at the core of the \beta-can structure that is a characteristic feature of all GFPs. Moreover, modeling of the amino acid sequences using CHPmodels 2.0 and VMD 1.8.5 as well as analysis using support vector machine (SMV) learning shows that the encoded protein products can be classified as either fluorescent or chromophoric with at least 84% accuracy.

Keywords: green fluorescent proteins, soft corals, sea anemones, fluorescence, B can structure

## GENETIC DIVERSITY OF PHILIPPINE Trichomonas vaginalis ISOLATES USING THE 5.8S RIBOSOMAL RNA GENE

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Trichomoniasis, a sexually transmitted disease caused by Trichomonas vaginalis, is an important but overlooked disease occurring worldwide. Many studies report the various clinical aspects and methods to detect infection with T. vaginalis, but there are very few current reports concerning the intraspecies diversity of the organism, which may prove useful in effective epidemiological assessment of the disease. This study aimed to establish the diversity of T. vaginalis isolates in a sample population in the Philippines using the 5.8S rRNA gene and associated internal transcribed spacers (ITS), and to synthesize T. vaginalis-specific primers from the conserved regions of the DNA sequences. Vaginal and urethral swabs were collected and subjected to culture methods to identify T. vaginalis-positive samples. Crude DNA extracts of culture-positive samples were amplified through polymerase chain reaction (PCR) using primer set TFR1/2. The desired PCR products were purified and sequenced for phylogenetic analysis. Four haplotypes were identified from the sample population, each differing from the designated standard haplotype by a single nucleotide residue. The putative rRNA region is highly conserved, and BLAST searches indicate that it is unique to T. vaginalis. GC rich-regions unique to the four haplotypes were identified from the sequence alignments, and these were used to develop new T. vaginalis-specific primers. A phylogenetic tree was generated which proved the monophyly of the species under genus Trichomonas and the monophyly of the isolates used in this study. The synthesized primers were tested through PCR using the same samples, and they were found to be 100% sensitive. The results prove promising in further epidemiological studies, especially those that will make a concordance between strains and clinical states. Results can also be used as basis for the improvement of current methods in diagnosing trichomoniasis.

**Keywords**: trichomoniasis, *Trichomonas vaginalis*, polymerase chain reaction, DNA sequencing, haplotypes, phylogenetic tree

#### PRELIMINARY CYTOGENETIC CHARACTERIZATION OF THE GOLDEN BIRDWING, Troides rhadamantus (LUCAS) (LEPIDOPTERA: PAPILIONIDAE)

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In order to initially investigate the chromosomes of the golden birdwing, Troides rhadamantus (Lucas), a single male specimen was procured from a private butterfly rearing station in Bay, Laguna, Meanwhile, the eggs of this species were collected from host plants in the same butterfly farm through the use of forceps. Following cytogenetic preparation of the male testes as well as the eggs, qualitative observation of the mitotic cells and chromosomes were done. Chromosome counting was carried out through the Image J software In addition to this, an interpretative drawing of the chromosomes and a karyograin were constructed from the chromosomes at pre-metaphase.

Out of the 30 mitotic cells examined, 29 showed 2n = 30 to 39. One mitotic cell had a chromosome number equal to 41. The mode or the most frequent chromosome number was 2n = 30, while the mean and median chromosome numbers were 2n = 32.5 and 2n = 31, respectively.

**Keywords:** Chromosomes, Golden Birdwing, Karyogram, Mitotic Cell, Pre-Metaphase

#### HOST PLANTS OF PACHYRRHYNCHINE WEEVILS

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The taxonomy and distribution of pachyrrhynchine weevils are currently being studied. This particular portion of the research project focuses on the host plants of the weevils. The study was conducted on three collection sites, Polillo Island, Quezon National Park and Mt. Makiling. Pinaglubayan, Puting-bato and Burdeos, Polillo Island have forested areas that have been converted to several types of agricultural land such as coconut plantations and rice farming. The Sibulan watershed in Pinaglubayan, Polillo Quezon is one of the remaining forest patches in Polillo Island with a good forest stand. This watershed is a 200 hectare primary lowland dipterocarp forest. Quezon National Park is composed of secondary to old growth forest while Mt, Makiling has a fairly good secondary lowland forest cover.

Host plants were photographed. Unfamiliar plants that could not be identified in the field were collected and brought to a botanist for its proper identification. A total of 19 plant species were observed and identified. These belong to Family Acanthaceae, Asteraceae, Cannabaceae, Costaceae, Euphorbiaceae, Lamiaceae, Lecythidaceae, Loganiaceae, Melastomataceae, Moraceae, Olacaceae, Rubiaceae, Rutaceae and Solanaceae. Results revealed that the weevils have a wide range of host plants particularly when their habitats were altered. Plant preference data of this weevil would be helpful to determine if the insect has the potential of becoming a pest and a good biological indicator, therefore, further study is needed.

Keywords: Curculionidae, host plant, Pachyrrhynchini, weevils

#### MODULARITY AND INTEGRATION IN THE FOREWING OF THE PHILIPPINE RICE BLACK BUG (Scotinophara SPP. STÅL, HEMIPTERA: PENTATOMIDAE)

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To determine how many modules define the shape of the forewings of Philippine Rice Black Bug (Scotinophara spp. Stål), the shapes of the forewing were summarized via Procrustes analysis of a total of 150 landmarks where 10 a priori models of variational modularity in the GM forewing data were tested using the y\* (Gamma\*) test for goodness of fit (GoF) statistic by comparing the observed and expected covariance matrices. Jackknife support values for each model were also computed using y\* as the GoF statistic. The analysis was implemented based on a total of 1000 replicates, dropping 10% of the specimens per jackknife replicate, and computing 95% confidence intervals for the statistic. Results showed that the forewing of RBB is spatially organized into two modules, the corium and the clavus - membrane. It can be concluded from this study that these two modules are mutually integrated but statisfically independent from each other.

**Keywords:** RBB. Modularity, Integration, Geometric Morphometrics

#### MORPHOLOGICAL DIFFERENCES IN THE SHAPES AND VENATION OF WINGS OF SELECTED DRAGONFLY SPECIES

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Dragonflies (Odonata) are one of the most ancient and instantly recognizable groups of insects. They were originally classified in the Libellula within the Neuroptera. Classical dragonfly taxonomy is often based upon the venation of the wings. This study was conducted to analyze the morphological disparity in the wing shape and venation of five different dragonfly species namely: Pantala flavescens, Aerthriamanta brepennis, Libellula incestra, Libellula croceipennis, and Gomphus externus using Thin-Plate Spline (TPS) coupled with Correlation Analysis based on distances (CORIANDIS). These were done for triangle, anal loop and outline shapes of the left and right forewings and hindwings of the dragonflies. Results showed that the locations of the species in the "compromise" space reflect a high similarity between Aethriamanta brepennis and Libelluta incestra however; Pantala flavescens, Libellula croceipennis and Gomphus externus show disparity with each other. Furthermore, there is congruence of anal loops (left forewings and right forewings) characters in Pantala flavescens and the triangle left forewing and anal loop right forewing in Libellula croceipennis. Stacked bar graphs also showed that Pantala flavescens departs considerably from other species, which seems to be largely a function of disparity of triangle left and right forewing characters. Libelluta incestra and Libellula croceipennis show close similarity of outline left hind wing. The high similarity of the character is maybe due to the fact that these two different species belong to the same genus. Aethriamanta brepennis and Libellula croceipennis also indicate close similarity of outline left hind wing and anal loop right hind wing characters. Gomphus externus indicates high level disparity of outline left hind wing character from other dragonfly species. Results show that Pantala flavescens, Aethriamanta brepennis, Libellula croceipenis and Gomphus externus indicate disparity in their wing characters, although not all of the character differences are so distant. Pantala flavascens demonstrate high level of trait differences compare to the four dragonfly species. However, Aethriamanta brepennis

and Libellular croceipennis show close similarity of their morphology. Results of this study suggests that Thin-Plate Spline (TPS) and Correlation Analysis based on distances (CORIANDIS) can be used as a tool for morphological disparity in dragonfly wings.

**Keywords:** Dragonfly, wing venation, morphological differences

BS-43

#### OUTLINE ANALYSIS OF WING SHAPE VARIATIONS IN FOUR SPECIES OF DAMSELFLIES COLLECTED FROM A STREAM AND WATERFALLS IN ILIGAN CITY

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The higher level classification and biosystematics of damselflies (Zygoptera) rely on qualitative description of the shapes of the wings and selected wing venational elements, i.e. anal loop, fore- and hind wing triangle and subtriangle. This study was conducted to determine variations in the shapes of the wings and wing venational elements within and between populations of four species of damselflies collected from a stream and waterfalls in Iligan City. The aim of the study is to determine the reliability of these characters in ascertaining species boundaries in odonatans. The characters analyzed include the fore- and hind wing, triangle, subtriangle, and the pterostigma. The shapes of these characters were compared using the method of elliptic Fourier analysis which is used to summarize mathematically the outlines of closed biological contours. A total of three replicates were analyzed per character. Likewise, digitization was done three times to determine measurement error. Tests for significant differences in the shapes of the characters were also done using multivariate methods of analysis such as the Multivariate Analysis of Variance (MANOVA) and Procrustes ANOVA. The results showed that the wing shapes of the four damselfly species differ between sexes and significantly between the two sites. Aside from sexual dimorphism, geographic differentiation could explain the differences considering that damselflies are territorial in nature.

The results also show that elliptic Fourier analysis could be used in understanding the diversification of these species of damselflies.

Keywords: outline analysis, wing shape, damselflies

#### **BS-44**

## THE FRUIT ANATOMY OF Moringa oleifera Lam: A POTENTIAL PLANT FOR HEALTHY ACTIVE AGEING

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Moringa oleifera L. commonly called "malunggay" in the Philippines, is considered one of the most economically important species. It is probably the most useful plant in the world, since every part of the plant can be used for food, medicine, nutrition and as a source for oils. It is an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands. The ethnobotanical, nutritional and folkloric uses of the plant are voluminous, and is reported to be acceptable, based on its wide usage in different regions of the country and in Asia. Yet, there have been no known clinical human trials to quantify the effects of *Moringa*, neither is there any extensive anatomical studies on the plant. Thus this study aims to characterize and describe the anatomy of the pericarp of the fruit at different stages of development. Fruit samples of different ages were processed and sectioned using different microtechniques. The pericarp is differentiated into exocarp, mesocarp and endocarp. The exocarp of the young fruit contained an outer single layer of epidermal cells and subepidermal layers that were composed of 2-3 layers of elongated collenchyma cells and 3-5 layers of polygonal sclereid cells. The epidermis of the exocarp of a mature one is uniseriate with abundant sclerified fiber cells. The fibrous exocarp is tough and hard. The mesocarp showed the vascular bundles, the xylem and phloem. The endocarp showed irregular-shaped parenchyma cells, which differentiated into longitudinally arranged fibers at maturity. This study can used as baseline data in determining the age of malunggay fruit to be used as vegetable and possible use as dietary and industrial fiber.

**Keywords**: exocarp, mesocarp, endocarp, uniseriate, pericarp

**BS-45** 

#### PRELIMINARY TAXONOMIC AND IMAGE CATALOGUE OF COPEPOD SPECIES (CRUSTACEA, COPEPODA) FROM THE NERITIC WATERS OF NORTHERN MINDANAO. **PHILIPPINES**

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Copepods often dominate zooplankton samples and in the marine pelagic ecosystem they form a critical pivotal role in the transfer of biomass and energy from phytoplankton to fishes. Most peritic tropical copepods are very small (0.1 to 1.5mm average total length) and similar looking rendering them very difficult to identify to species level. Many species have been reared for the aquaculture live feed industry because they contain high levels of DHA, EPA, and AA poly-unsaturated fatty acids (PUFA) ideal for the increased survival of fish larvae. As part of a study aimed at screening for species that are easily reared and PUFA-rich, peritic marine copepods were collected in Iligan Bay and Mindanao Sea, identified to species level, and the whole animal photographed. The pictures and taxonomic information were made into a catalogue which became a very useful guide to quick and easy identification of species. To date, I have photographed 43 common species including: (1) Acartia negligens, (2) Acartia erythraea, (3) Pontella sp., (4) Calanopia elliptica, (5) Calocalanus pavo, (6) Candacia catula, (7) Candacia curta, (8) Canducia discaudata, (9) Canthocalanus pauper, (10) Paracandacia truncata, (11) Centropages calaninus, (12) Centropages furcatus, (13) Centropages gracilis, (14) Copilia sp., (15) Corveaeus typicus, (16) Corycaeus limbatus, (17) Corycaeus speciosus, (18) Corycaeus longistylis, (19) Cosmocalanus darwini, (20) Euchaeta marina, (21) Euchaeta media, (22) Euterpina acutifrons, (23) Labidocera acuta, (24) Labidocera pavo, (25) Labidocera minuta, (26) Labidocera detruncata, (27)

Macrosetella gracilis, (28) Microsetella disseta, (29) Oithona similis, (30) Oithona plumifera, (31) Oithona rigida, (32) Oncaea venusta, (33) Paracalanus aculeatus, (34) Parvocalanus crassirostris, (35) Pontella surrecta, (36) Pseudodiaptomus philippinensis, (37) Sapphirina sp., (38) Subeucalanus crassus, (39) Temora turbinata, (40) Temora discaudata, (41) Undinula vulgaris, (42) Calanopia thompsoni, and (43) Tortanus discaudatus. In the long term, an electronic database comprising images, taxonomic and ecological information, and genetic barcodes will be built up from this preliminary catalogue.

**Keywords**: Copepods, Crustacea, Diversity, Image catalogue, Taxonomy

**BS-46** 

#### SEXUAL DIMORPHISM AND MORPHOMETRIC DIFFERENTIATION AMONG COLORMORPHS OF THE SWORDTAIL FISH Xiphophorus helleri

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Morphological distinction through truss network analysis in fishes has been an effective tool in the study of stock identification, thus improving the biological and systematic basis of management. In this study, the truss networks of three colormorphs of swordtail fish Xiphophorus helleri were analyzed using geometric morphometrics. This study aims to present an alternative digitized method to analyze fish truss network morphometric data. Analyses includes Procrustes fitting, thin-plate spline transformation grids, partial and relative warp scores, principal component analysis, discriminant analysis and cluster analysis. The results show distinct variation in body shapes between sexes within the colormorphs (sexual dimorphism) and distinct differences between the colormorphs. Variables that contribute to truss network variation were identified. The success of classification of every individual with respect to the group cases was identified (97.8 % correctly classified and 94.2% cross validated). The results proved the efficiency of geometric morphometric analysis in understanding biological forms in organisms.

**Keywords**: sexual dimorphism, truss network, procrustes

#### SURVEY AND CENSUS OF GROUND LIZARDS IN THE PERMANENT FIELD LABORATORY AREAS (PFLAS) IN MT. MAKILING FOREST RESERVE

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This study was conducted to survey the ground lizards of the Permanent Field Laboratory Areas (PFLAs) in Mt, Makiling Forest Reserve and estimate their population size using the pitfall trapping method and Schnabel formula for Repeated Mark-Recapture Technique. Lizard fauna were compared in terms of the number of species, number and size of individuals trapped and the condition of the immediate surroundings.

Five species of scincid lizards and one gekkonid were sampled from the Permanent Field Laboratory Area I and III. These were Brachymeles gracilis, Mabuya multifascinata, Mabuya multicarinata, Sphenomorphus jogori, and Tropidophorus grayi, and Gecko gecko, respectively. PFLA III (Plot 2) harboured all these species while PFLA I (Plot 1) had only 4 species of lizards. Furthermore, the population of the lizards in PFLA III was higher than in PFLA I. PFLA III is a plantation of Mahogany, Swietenia macrophylla, with less diverse plant species, limited food resource, less litter biomass, and unhealthy interspecific interactions. The latter plot is a mixed secondary forest with high understorey, rich litter biomass, and rather diverse food resource (e.g. insects). This area, presumably, has healthy interspecific interactions, with a high prodation rate resulting in less species and population of lizards.

The estimated population of all the species of lizards in PFLA I ranged from 6 to 45 individuals while for PFLA III, it ranged from 25 to 82. Among the species, S. jagori had the highest actual count and estimated population sizc.

Keywords: Brachymeles gracilis, Gecko gecko. Mabuya multifascinata, pitfall trapping, repeated-mark-recapture technique, Sphenomorphus jogori

# SPECIES RICHNESS, ASSESSMENT AND CONSERVATION OF SOME ECONOMICALLY IMPORTANT PHILIPPINE LYCOPODS

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Lycopods are lower vascular plants with microphyllous leaves and dichotomous branching. Field exploration and herbarium examinations revealed 39 species and 3 genera of Lycopodiaceae. Of these, 2 are endangered, 9 endemic and almost all species of Lycopods are economically important. Specific economic uses and distribution of Lycopods were recorded. Many of these ornamental species collected from the wild are sold exorbitantly in commercial gardens. The forest ecosystem is the best habitat and sources of these Lycopods for collection and propagation. The practice of over-harvesting from the wild has depleted and threatened the population of Lycopods. Ex-situ conservation was initiated at Central Mindanao University Fernery to save these economic and threatened species of Lycopods. Using hanging baskets or clay pots with crushed trunk of Cyathea and chopped coconut husk, propagation of Lycopods is possible.

Keywords: Species Richness, Uses, Conservation, Lycopodiaceae

# OCCURRENCE OF CORTICOLOUS MYXOMYCETES FROM ACACIA TREES (Samanea samans Merr.) COLLECTED FROM DIFFERENT SITES IN LUZON ISLAND, PHILIPPINES

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Annually, the Philippines' deforestation rate is computed to be 1.4 % or about 89,000 hectares lost per year. This necessitates urgent assessment of the country's biodiversity. Moreover, the microbial flora in the Philippines is poorly documented. One of the less studied microflora are the myxomycetes. Myxomycetes thrive mainly in decaying substrates like litters, dungs, twigs and barks. Our research study then explores dead barks of living acacia trees from different sites in Luzon as microhabitat for myxomycete assemblages. Acacia trees are one of the most commonly grown trees in the country and thus, may harbour unique species of myxomycetes. Dead barks of living trees (5 trees per site) were then collected from the Northern Luzon Area (Sison, Alaminos, Anda and Mangataren in Pangasinan, Zambales, Isabela). Central Luzon Area (Subic, Nueva Vizcaya, Paniqui, Capaz, Tarlac, and Mt. Arayat, Pampanga), Southern Luzon Area (Los Baños in Laguna, Tagaytay) City, Batangas, Daet in Camarines Norte, Goa in Camarines Sur), and from three sites in the Metro Manila Area (Quezon City, Manila). Moist chambers (300) were set up for each of the collected samples (in triplicates) and were used to assess myxomycete diversity. Following incubation at room temperature for 8 weeks, substrates with plasmodia and/or fruiting bodies were observed. Preliminary results showed that eighty of the total moist chamber set-ups had corticolous myxomycetes. The percent myxomycete yield was low for the bark substrates, i.e. only 17 % and 8 % exhibited plasmodia and fruiting bodies, respectively. Morphological characterization of the fruiting bodies identified the myxomycetes as belonging to three genera, namely, Physarum, Didymium and Arcyria. It seems that geographical locations did not influence the number nor kinds of myxomycetes observed on the acacia bark samples. The myxomycetes associated with the acacia trees were not as diverse as expected.

**Keywords**: myxomycetes, biodiversity, *Physarum* sp., *Didymium* sp., *Arcyria* sp., acacia trees

**BS-50** 

#### FLORISTIC INVENTORY OF THE LUNETA PARK, MANILA

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A floristic inventory of the Luneta Park in Manila was conducted on January 2008. The study provides baseline information on plant resources and generates a listing of plants such as trees, shrubs, herbs, palms and vines. A total of 95 species of plants belonging to 52 families and 81 genera have been documented. There were one gymnosperm and 94 flowering plants. Of these 32 trees, 37 shrubs, 15 herbs, 7 are palms and 3 are vines. Three species are endemic to the Philippines, namely: Cycas chamberlanii. Heterosphate philippinensis and Podocarpus sp. Family Palmae/Araceae has the most number of representative species having 15 species types, followed by Family Leguminosae/Fabaceae with 7 species and Family Agavaceae and Family Araliaceae with 5 representative species each. The identified plants are economically important as source of food, medicine, and lumber, others are for ornamental uses. The gathered data will provide useful information on the survival of taxa and promote effective strategies for botanical conservation and environmental awareness.

**Keywords**: Floristic inventory, plant resources, taxa, endemic species, botanical conservation.

#### FOUR NEW RECORDS AND ONE NEW SPECIES OF PHILIPPINE MYXOMYCETES FROM HUNDRED ISLANDS AND ANDA ISLAND, PANGASINAN

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Myxomycetes are a morphologically diverse group of eukaryotic microorganisms. Their unique life cycles and fascinating fruiting bodies make them ideal model organisms for study of cellular differentiation. In the Philippines, a country known for its biodiversity, limited studies unfortunately were done on myxomycetes. Our research study thus assesses the diversity and occurrence of myxomycetes in selected islands of Hundred Islands and in Anda Island, Pangasinan, Aerial (105) and ground leaf litter (135), twigs (135) and barks (15) were collected from the islands of Camantiles, Monkey, Children, Governor, Century Park, Sison, and Shell and from Anda Island. Moist chambers (420) were set-up for each of the collected samples and were observed for plasmodia and fruiting bodies to assess their myxomycete diversity. Following incubation for 8 weeks, the moist chamber cultures yielded 34 species of myxomycetes representing 9 genera. Highest myxomycete yield was observed in Century Park Island (51 %) and with aerial leaf litter as substrate (50 %). Arcyria cinerea was the most abundant species among all island sites. Diversity assessment using the Species: Genus (S/G) ratio showed Governor's and Shell Islands as the islands with the highest taxonomic diversity. The Coefficient of Community (CC) indices revealed highest species similarity (0.53) between Governor and Children's Islands. Of the 34 species identified, five species were new records for the Philippines: Craterium microcarpum, Elaeomyxa miyazakiensis, Lepidoderma tigrinum, Perichaena pedata, and Physarum decipiens.

**Keywords**: myxomycetes, biodiversity, moist chamber set-ups, Hundred Islands, Anda Island

#### ASSESSMENT OF FISH FAUNA IN LAKE LANAO

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Lake Lanao is the second largest lake in the Philippines found in Lanao del Sur, Mindanao which has a surface area of 357 sq. km. The lake plays an important role not only for the people living near the area but also for the whole of Mindanao as it is the reservoir of the hydropower plant which supplies energy all over the Mindanao archipelago.

This study was conducted to assess the fish fauna in Lake Lanao. There are three sampling sites: Balindong, Taraka and Lumbatan Lanao del Sur. Sampling periods were started in April and ended in June, 2008. Physico-chemical parameters; water temperature, pH, and dissolved oxygen (ppm) were determined. Fish hook, fishnet and fish arrow were used to eatch the species.

Findings show that the area has normal a range of values in terms of its physico-chemical properties. These are the species that are found in the 3 sites: katulong (Hypseleotris agilis Herre), kadurog (Glosogubius guiros and G. celebius), tilapia/mampawi (Oreochromis niloticus), pait (Puntius amarus Herre), aruan (Ophicephalus striatus Bloch), odang (Macrobrachium sp.), snail (Pomacea sp.) and fry katulong.

There are also endangered species and these are: gurami, aruan, pair and baolan. It was revealed that the major reason for the drastic decline of the endemic fishes was the accidental introduction of white goby and electrid in the early 1960s. It might also be due to the use of the destructive method of fishing. It is highly recommended that the government of Lanao del Sur and Marawi City provide a program to conserve and preserve the lake and the fishes found in the lake.

Key words: fish fauna, lake Lanao, endemic fish species, endangered species

#### RELATIVE WARP ANALYSIS AND CORRELATION ANALYSIS BASED ON DISTANCES TO STUDY MORPHOLOGICAL VARIATIONS IN THE SHELL SHAPE OF Pomacea canaliculata (LAMARCK) FOUND IN ILIGAN CITY

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In terms of analysis of the forms of organisms, landmark-based geometric morphometrics are more preferred nowadays, by many systematists over the so-called traditional morphometrics. In this study, Relative Warp Analysis and Correlation Analysis based on Distances were used to study the existing morphological variations in the shell shape P. canaliculata from the three major barangays in Iligan city: Buruun, Tubod, and Mahayahay, Relative warps are factors (principal components of partial warp scores) which themselves summarize the major vectors of shape variation within samples. Correlation Analysis based on distances on the other hand, allows both looking into similarities among specimens/groups, and interpreting such similarities in terms of congruence among traits. It is also a measure of trait disparity. Comparison of samples was done by looking into the relative contributions of different traits to each species groups' distinctiveness. Results have shown close resemblance or association in terms of shell shape between female samples from Tubod and Mahayahay and male samples from Tubod and Mahayahay. This is based on the three traits being considered. P. canaliculata (M)-Buruun departs considerably from other species groups, although this seems to be largely a function of trait #1 (top portion of shell) alone. Results obtained have somehow demonstrated that subtle shape differences in shell shape can be detected by geometric morphometrics,

Keywords: Geometric morphometrics, Relative Warp, shell shape, trait disparity, Correlation Analysis

# ASSESSMENT ON THE ESTUARINE AREAS OF CAMOTES ISLANDS, CENTRAL PHILIPPINES: THEIR ECOLOGY AND PROSPECTS FOR MILKFISH (Chanos chanos) AND PRAWN (Penaeus monodon) INDUSTRY

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The three estuarine areas in Camotes Islands, Cebu, Philippines namely; Baring, San Isidro, San Francisco, Cebu, Baliis, McArthur, Tudela, Cebu, and San Isidro, Pilar, Cebu were studied in order to determine the status of bangus fry (*Chanos chanos* Forsskal) and sugpo fry (*Penaeus monodon* Fabricius) in these areas. Other species of fish caught together with them and the profile of the estuarine areas as prospects for fry industry in the Islands were also noted.

A simple skimming net locally known as *hudhud* or *tigpayan* was used to collect fry of bangus, sugpo and fry of other species. The other fishes caught with the bangus and sugpo were accounted for and determined up to species level. An interview guide was used to gather data from the fishermen in the estuarine areas.

Results showed that Sitio Baring, San Isidro, San Francisco, Cebu topped the estuarine areas in Camotes Islands in terms of abundance of fry of bangus, sugpo and other fry species, followed by Mc Arthur, Tudela, Cebu and San Isidro, Pilar, Cebu. There were 16 species of fry in Camotes Islands including bangus and sugpo: eight families of fish; 1 family of Crustacean and 1 family of shellfish.

Catch per Unit Effort (CPUE) was 30 pcs of bangus fry/ minute operation of skimming net in Baring, San Isidro, San Francisco, Cebu; 3-7 pcs of bangus per minute operation of skimming net in Baliis, Calmante, Tudela, Cebu and 1 pc of bangus fry/ 5 minutes operation of scoop net in San Isidro, Pilar, Cebu. The salinity profile of the three estuarine areas surveyed ranged from 5 ppt to 36 ppt; temperature was 28-35°C; pH is 7.3-8.1 and transparency is 6-20 inches.

Keywords: Estuarine Areas, Bangus, Sugpo, Ecology, Camotes Islands

## PRELIMINARY SURVEY OF MANGROOVES AND PINE FOREST OF MASINLOC, ZAMBALES

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Floral diversity in Masinloc, Zambales was determined by surveying the vegetation along the coastal mangrove and pine forest ecosystems in September 2008. Sampling was done using visual encounter with individual plant species that were photographed. Unidentified species were collected and verified at the UPLB Forestry Herbarium. The total number of species surveyed was 25, belonging to 18 genera and 17 families. These include 12 species of trees, 8 shrubs, 3 orchids and 2 ferms. Rhizophoraceae and Acanthaceae dominated the mangrove forest; represented by two Rhizophora (R. mucronata and R. apiculata), Ceriops decandra and three Avicennia (A. mariina var. rumphiiana, A. marina and A. officinalis). At present, mining is operational in the pine forest of Zambales where Pinus merkusii, a vulnerable pine species can be found. The taxonomic list of plants in this report is important as baseline information for biodiversity. The presence of mining operation will have a detrimental effect in the ecology and existence of the pines and therefore, conservation is necessary.

**Keywords**: floral diversity, pine forest, mangrove, mining, *Pinus merkusii* 

#### IN VIVO TOXICOLOGY ASSESSMENT OF SYSTEMICALLY INTRODUCED FUNCTIONALIZED NANOPARTICLES IN MICE

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The last few years have seen the emergence of various applications of nanomaterials in the field of biomedicine. Fluorescent semiconductor nanocrystals, or quantum dots (QDs), have proven to be extremely useful as fluorescent tags for quantitative imaging and detection. Recently, quantum dots with targeting and imaging functionalities have been used in the study of drug delivery and therapeutics. On the other hand, single-walled carbon nanotubes (SWCNTs) are members of the fullerene family of carbon which are composed of carbon atoms arranged in a single sheet of benzene rings rolled up into a tubular form. Carbon nanotubes possess unique optical, electronic, and physicochemical properties that make them useful in a diverse number of applications in different fields, including therapeutics and diagnostics in the biomedical field. Studies have been conducted exploring the use of nanotubes as sensors for detection of mutations and other molecular abnormalities, as scaffolds for tissue regenerationn, and as delivery systems for a wide range of diagnostic and therapeutic agents such as peptides, DNA, and various drugs. However, before any clinical trials can be undertaken to test these nanomaterials in various biomedical applications, their toxicological and pharmacological profiles will have to be studied completely to assess the safety of their use for therapy. We evaluated the possible toxicological effects of QDs and SWCNTs to be used for biomedical applications through systemic introduction of the nanomaterials into mice via tail vein injection. Morphological analysis of various organs, especially those of the reticuloendothelial system (RES), showed no apparent abnormalities 24 hours after introduction of the nanomaterials. Furthermore, no nanomaterials were observed in the brain, indicating that they were unable to cross the blood-brain barrier. The survival and embryonic development of systemically exposed embryos were also similar to the controls. Our results indicate that, at least for the time points observed, there were no adverse effects of the introduction of nanomaterials via the systemic route in mice, showing promise for future therapeutic applications.

Keywords: nanoparticles, quantum dots, single-walled carbon nanotubes, mouse embryos

### CHEMICAL, MATHEMATICAL AND PHYSICAL SCIENCES

## WEIGHTED HARDY-SOBOLEV INEQUALITY WITH INFINITELY MANY REMAINDER TERMS

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The study of the minimal and the extremal solutions of the quasilinear elliptic equation gained much attention in the recent years because of its applications in Magnetic and Potential Theory. Under some conditions, the analysis starts with the establishment of the existence of the minimal and the extremal solutions and then proceeds with studying their behaviors in the linearized quasilinear elliptic equations. To analyze the linearized equation at extremal solution, the classical Hardy-Sobolev inequality is not enough since it has singularity at the origin. Hence we have to essentially improve the classical result by having a weight on both sides of the inequality. This study focused on weighted type of the recent improvement of Hardy-Sobolev inequality

$$\int_{\Omega} |\nabla u(x)|^{2} dx \ge \left(\frac{n-2}{2}\right) \int_{\Omega} \frac{|u(x)|}{|x|^{2}} dx + \frac{1}{4} \int_{\Omega} \frac{|u(x)|}{|x|^{2}} \left[ A_{1}(|x|)^{2} + \left(A_{1}(|x|)A_{2}(|x|)\right)^{2} + \cdots + \left(A_{1}(|x|)A_{2}(|x|)\cdots A_{k}(|x|)\right)^{-2} dx \right] (1)$$

where  $\Omega$  is a bounded domain in  $\mathbb{R}^n$ ,  $n \ge 2$ , with  $0 \in \Omega$  and  $u \in W_0^{1,2}(\Omega)$ .

By  $W_0^{1,2}(\Omega)$  we denote the completion of  $C_0^\infty(\Omega)$  in the norm  $\|u(x)\|_{1,2,\Omega}:=$ 

$$\left(\int_{\Omega} |u(x)|^2 dx + \int_{\Omega} |\nabla u(x)|^2 dx\right)^{\frac{1}{2}}. \text{ Here we define } A_1(x) = \log \frac{R}{|x|} \text{ and }$$

 $A_k(x) := \log A_{k-1}(x)$ ,  $R \ge e_k \sup_{\Omega} |x|$  and  $e_1 := e_1, e_k := e^{e_{k-1}}$ . The result involves a weight function  $x^{\alpha}$  on both sides of (1) where the domain is a ball centered at  $x \in \mathbb{R}^n$  of radius  $\rho$  and this is denoted by  $B_{\rho}(x)$  where  $B_{\rho}(x) \subset \mathbb{R}^n$ . The result of the study is given by the theorem:

**Theorem**: Let n,  $\alpha$  and k be positive integers such that n > 2,  $k \ge 1$  and  $R \ge e_k \sup_{\Omega} |x|$ . Then the inequality

$$\int_{B_{\rho}} |\nabla u(x)|^{2} |x|^{\alpha} dx \ge \frac{(n-2)^{2} + 2\alpha (n-2)}{4} \int_{B_{\rho}} |u(x)|^{2} |x|^{\alpha-2} dx$$

$$+ \frac{1}{4} \int_{B_{\rho}} |u(x)|^{2} |x|^{\alpha-2} \left[ A_{1}(|x|)^{2} + (A_{1}(|x|)A_{2}(|x|))^{-2} + \dots + (A_{1}(|x|)A_{2}(|x|) \dots A_{k}(|x|))^{-2} \right] dx$$

holds for any  $u \in W_0^{1,2}(\Omega)$ .

**Remark**: If  $\alpha = 0$  then inequality (2) reduces to a known result given by inequality (1).

**Keywords**: Hardy Inequality, minimal and extremal functions, sharp remainder term.

#### CMPS-2

#### BIVARIATE DATA ANALYSIS USING FUZZY LOGIC APPROACH

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Bivariate Data analysis using fuzzy logic approach envisions the developing theoretical concepts that investigate the effect of fuzzy logic application in the analysis of bivariate data. This paper considers the bivariate standard normal distribution with  $\rho = 0$ .

Let the rejection region R as, 
$$R=M+N$$
, where  $M=2\pi\int_{\mu+\sigma}^{\mu+\sigma+\epsilon}\frac{1}{x^2}\frac{x^2}{xe^{-\frac{1}{2}x^2}}dx$ , the fuzzy rejection region and  $N=2\pi\int_{\mu+\sigma+\epsilon}^{\infty}\frac{1}{e^{-\frac{1}{2}x^2}}dx$  as the

absolute rejection region. Hence,

$$R = M + N = 2\pi \int_{\mu + \sigma}^{\mu + \sigma + \varepsilon} \frac{1}{xe^{-\frac{1}{2}x^2}} dx + 2\pi \int_{\mu + \sigma + \varepsilon}^{\infty} \frac{1}{xe^{-\frac{1}{2}x^2}} dx$$

In addition, fuzzy sets in three-dimensional form will be presented and incorporated in this paper.

**Keywords**: bivariate data analysis, fuzzy logic, standard normal, fuzzy rejection region, absolute rejection region, fuzzy set

#### CMPS-3

#### TWO-TAILED FUZZY HYPOTHESIS TESTING FOR NORMALLY DISTRIBUTED DATA

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This paper will apply fuzzy logic (FL) technique in developing a twotailed gray neighborhood around the boundary between the acceptance and the rejection region of a normally distributed data. This neighborhood idea will be formulated within the framework of fuzzy sets.

In applying fuzzy logic in two-tailed fuzzy hypothesis testing in a normally distributed data, we have the following results:

Define the rejection region  $S=S_1\cup S_2$  where  $S_1=\left\{T:T>a\right\}$ ,  $S_2=\left\{T:T<-a\right\}$  and  $T=\overline{X}-\theta_0$ . Each side of the rejection region is partitioned into two disjoint sets M and N defined as  $M_1=\left\{T:a\leq T\leq a+\varepsilon\right\}$ ,  $N_1=\left\{T:T>a+\varepsilon\right\}$ ,  $M_2=\left\{T:-(a+\varepsilon)\leq T\leq -a\right\}$ , and  $N_2=\left\{T:-(a+\varepsilon)< T\right\}$ , respectively. Note:  $S_1=M_1\cup N_1$ ,  $M_1\cap N_1=\phi$  and  $S_2=M_2\cup N_2$ ,  $M_2\cap N_2=\phi$ 

Construct a fuzzy set on each  $M_i$ , i=1,2 and define the size of each of the critical region  $\alpha_i$  that include fuzzy set region, thus producing a partial rejection of the hypothesis. Thus the fuzzy membership function on each of

the 
$$M_i$$
,  $i = 1,2$  is given by  $\mu_1(T) = \varphi(-a) \left[ \frac{T + a + \varepsilon}{\varepsilon} \right]$  and  $\mu_2(T) = \varphi(a) \left[ \frac{a + \varepsilon - T}{\varepsilon} \right]$ .

The effect of "fuzzification" of the rejection region on the power of the test will be investigated. Consequently, the results of this paper will serve as an initial attempt at fuzzy statistical decision theory.

**Keywords:** hypothesis testing, fuzzification, fuzzy logic, fuzzy sets, power of the test, fuzzy membership function, critical region, partial rejection

#### CMPS-4

## ELECTROCONDUCTIVE POLYTHIOPHENE/POLYESTER COMPOSITE AS e-CLOTH

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Advances in textile technology and materials science are promoting a new breed of functional fabrics and at the same time conducting polymers have proven to be very useful in the fabrication of new materials such as conducting textiles. In this study, a conducting, flexible, and mechanically stable polythiophene/ polyester (PTp/PE) patch was fabricated. The patch could be used as invigorator and bio-stimulator for fatigued and injured muscles and it could be an aid in removing cellulites.

PTp/PE was prepared by chemical polymerization with the following optimum conditions: 24 hours in diffusion bath (0.1 M thiophene monomer in chloroform) and 24 hours in polymerization bath (0.23 M ferric chloride dopant). The PTp/PE composite fabric exhibited good conductivity 2.500 x 10<sup>-3</sup> S/cm (0.027% RSD, n=3) with 4.77% PTp content (12.23% RSD, n=3). Micrographs from scanning electron microscopy (SEM) showed PTp on the surface and in the interstices of the PE fabric. Results of the elemental analysis by energy-dispersive X-ray (EDX) spectroscopy showed the presence of sulfur (11.43%) in the fabric which is indicative of polythiophene. A small and portable electronic circuit was assembled to supply electricity to the textile composite. The input voltage supplied to the

circuit was 5 volts direct current (DC). The output voltage of 3 volts was measured by connecting the output connections of the circuit to the prepared conducting patch. The efficiency of the electronic circuit was 57.4%, thus the PTp/PE patch is a promising e-cloth.

**Keywords**: *e*-cloth, bio-stimulator patch, polythiophene, polyester, composite fabric

#### CMPS-5

## CONDUCTIVE POLYMERS AS HOST MATRICES FOR THE DISPERSION OF GOLD NANOPARTICLES WITH ELECTROCATALYTIC PROPERTIES

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Electropolymerization has attracted widespread interest in producing conductive polymers such as polypyrrole (PPy) and poly(3,4ethylenedioxy)thiophene (PEDOT) with electrocatalytic and sensor This study aimed to optimize the parameters for the applications. electropolymerization of pyrrole and 3,4-ethylenedioxythiophene (EDOT) on Au polycrystalline electrode. Using cyclic voltammetric (CV) method, the monomer was polymerized anodically on Au polycrystalline electrode in HClO<sub>4</sub> electrolyte at scan rates of 10 mVs<sup>-1</sup>, 50 mVs<sup>-1</sup> and 100 mVs<sup>-1</sup>. The surface morphology of the electropolymerized film on Au substrate was characterized using Scanning Electron Microscopy (SEM) and Non-Contact Atomic Force Microscopy (NC-AFM). Incorporation of Au nanoparticles on PPv-modified or PEDOT-modified Au electrode was accomplished by electrodeposition of gold metallic particles from an aqueous solution of HAuCl, at various deposition times. The dispersion of the Au metallic particles on PPy and on PEDOT films was investigated using SEM. Optimum conditions for the electropolymerization of the conductive polymers and for the electrodeposition of the Au metallic particles were utilized to produce metallic particles / conductive polymer composite films. The electrocatalytic behavior of these composite films towards ethanol and glucose oxidation was investigated.

**Keywords**: conductive polymers, polypyrrole, polythiophene, electropolymerization, electrocatalysis, sensors, surface analysis

#### CMPS-6

#### FABRICATION OF CHITOSAN MICRON AND SUB-MICRON PARTICLES FOR DRUG SEQUESTRATION

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This study looks into the fabrication of nano-sized chitosan particles for in vivo drug sequestration. Nanoparticles, being porous in nature, can act like sponge that absorbs toxins and renders them inactive. Its high surface area to volume ratio makes it a more efficient way of flushing out toxins compared to using macromolecules. The biopolymer chitosan is the material of choice in this study because of its biocompatibility as well as abundance in the Philippines.

The particles were synthesized using precipitation and reverse microemulsion technique. Precipitation involved injecting chitosan solution into toluene and iso-octane, while the system was subjected to an ultrasound generator. The reverse microemulsion technique involved generation of chitosan nanoparticles in the reverse micelles of the surfactants Span 80 and Tween 80 in hexane. Glutaraldehyde was also used as a crosslinker.

An initial Scanning Electron Microscope (SEM) image showed that the particles generated via the precipitation method did not have well-defined surfaces. Instead, the reverse microemulsion method generated particles that were more rigid. The 10% (mol/mol) crosslinked system were of size 150-600nm. Also, Differential Scanning Calorimetry runs showed no drastic changes in sample transition temperature when the particles were subjected to a degradation test that simulated physiological conditions of pH 7.4 and 37°C. Surface area analysis of the nanoparticles is in progress using Brunauer, Emmett, and Teller (BET) analyzer. Chitosan can therefore form nanoparticles by reverse micelles technique. The fabricated particles will be tested for sequestration of the drug propatenone, overdosing of which results in coma and seizures.

**Keywords**: Chitosan, nanoparticles, reverse microemulsion, drug sequestration, propafenone

#### GLYCEROL-CROSSLINKED POLYACRYLIC ACID HYDROGELS

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Glycerol is mostly generated as a by-product from soapmaking and biodiesel production process. With the increase in biodiesel production, glycerol is expected to be a surplus and, hence, it is practical to explore its potential uses. Polyacrylic acid (PAA) hydrogels are known to have excellent bloadhesive property which allows them to adhere to mucosal linings for extended periods, releasing encapsulated medications gradually with time. The objective of this study is to explore the potential of glycerol as a crosslinker for the fabrication of PAA hydrogel.

Hydrogels were fabricated by free-radical polymerization of acrylic acid (AA) at 50°C using benzoyl peroxide as initiator and acetonitrile as solvent. The PAA formed was crosslinked with glycerol in the presence of Novozym 435 in the same reaction vessel. The effects of various reaction parameters such as (1) presence of molecular sieves, (2) monomer to solvent ratio (3) reaction time were studied systematically.

Addition of molecular sieves to the system generated higher molecular weight hydrogels as compared to the reaction in the absence of molecular sieves. The ratio of 1:1(v/v) AA:acetonitrile produced crosslinked PAA with a molecular weight of 2.1 x 10° g/mol after 6 hours of polymerization. Dilution of the system produced hydrogel of even higher molecular weight. The ratio of 1:2(v/v) AA: acetonitrile produced crosslinked PAA of 1.0 x 10'g/mol after 15 minutes of polymerization. The hydrogels swelled rapidly in deionized water. Almost 80% swelling was observed in 50 sec.

It can be concluded that glycerol can be used as crosslinker to fabricate PAA hydrogels. Moreover, since the fabricated hydrogels undergo rapid swelling, they can encapsulate water soluble drugs and can release them at a controlled rate These hydrogels will be evaluated for the controlled release of corticosteroid for the treatment of mouth ulcer.

Keywords: Polyacrylic acid, hydrogel, glycerol, crosslinking, drug delivery

#### SYNTHESIS AND CHARACTERIZATION OF CHITOSAN AND K-CARRAGEENAN IPN HYDROGEL SYSTEMS FOR TRANSDERMAL DRUG DELIVERY

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Interpenetrating polymer network (IPN) hydrogel systems were synthesized using the biopolymers—chitosan, modified chitosan and k-carrageenan, along with polyacrylic acid(PAA) as the second component for transdermal drug delivery application.

Chitosan-PAA semi-IPN hydrogel system was fabricated by polymerization of AA in the presence of the crosslinker N,N'-methylenebisacrylamide (NN'MBA) and chitosan/modified chitosan. 3-chloro-2-hydroxypropyltrimethylammonium chloride (quat 188) was used as the modifier to impart antibacterial property to chitosan with. Since  $\kappa$ -carrageenan film has poor strength, for the -carrageenan-PAA system, a full IPN was synthesized with NN'MBA-crosslinked PAA and NaOH-crosslinked  $\kappa$ -carrageenan.

IPN hydrogels were allowed to swell in deionized water. It was detected that the increase of crosslink density, slowed down the rate of swelling of the system. In the case of chitosan-PAA semi IPN, the rigidity of the hydrogel increased with the increment of the chitosan component of the system. Incorporation of Quat 188 further improved the structural integrity of the hydrogel and hence the transition temperature as revealed by differential scanning calorimetry analysis. κ-Carrageenan-PAA IPN also showed improved rigidity with the increase of κ-carrageenan content as both the systems swelled rapidly without rupturing on addition of deionized water.

Since these hydrogels can swell without rupturing, they can incorporate drugs solubilized in water and can be used as patches for transdermal drug delivery. These hydrogels are under evaluation for the incorporation and release of the drugs silver nitrate and mafenide acetate, the drugs that are used for the treatment of wound due to burn.

**Keywords**: Chitosan, polyacrylic acid, interpenetrating network, κ-carrageenan, Quat 188

#### FABRICATION OF ANODIC ALUMINA OXIDE TEMPLATES AND THEIR USE FOR THE ELECTROSYNTHESIS OF POLYTHIOPHENE NANOWIRES

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Nanowires of polythiophene (PTp) were electrochemically synthesized using the pores of the prepared anodic alumina oxide (AAO) templates. The anodization experiments were performed using Al foil to create alumina pores having pore diameters of 150-250 nm. The electrochemical polymerization synthesis of PTp nanowires was carried out using a potentiostatic condition in a three-electrode system. electropolymerization conditions were 0.1 M thiophene (Tp) monomer, 0.1 M tetrabutylammonium perchlorate (TBAClO<sub>4</sub>) dopant, in 10.00 mL acetonitrile, at a temperature of  $0 \pm 1$  °C, without stirring the polymerization solution using 1.6 V at 2 mA/cm<sup>2</sup> current density. Four-point probe conductivity tests were done to determine the electrical properties of the PTp nanowires formed. PTp nanowires revealed a higher conductivity of 11.30 S/cm (0.50% RSD, n=3) compared to the bulk polymerized Al foil (1.45) S/cm). The scanning electron microscope image of PTp nanowires showed uniform cylindrical nodules with a diameter of ~150 nm. In this study, the facile AAO template synthesis method utilized for PTp nanowires growth gave higher conductivity that could find wider environmental and medical applications,

**Keywords**: nanowires, polythiophene, template synthesis, anodic alumina oxide, SEM

#### CMPS-10

# MICROWAVE-ASSISTED CADOGAN REACTION: ITS APPLICATION TO SYNTHESIS OF HETEROCYCLIC COMPOUNDS

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Microwave-assisted heating under controlled conditions is a valuable technology for chemical syntheses since it often dramatically reduces reaction times and the formation of side products.

The Cadogan method for the synthesis of *N*-containing heterocycles involves the deoxygenation of aromatic nitro compounds in the presence of triethyl phosphite which acts as the reducing agent. The reaction is carried out at high temperature under nitrogen condition for several hours. Due to the versatility of Cadogan reaction, we have investigated it using microwave irradiation as the source of energy to drive the reaction to produce the desired products at shorter reaction time.

The aromatic nitro compound was mixed with triethyl phosphite in a suitable reaction vessel and irradiated with microwave radiation at a particular power for a number of minutes. The identity of the product was determined by IR, <sup>1</sup>H and <sup>12</sup>C spectroscopic data.

For the synthesis of carbazole from 2-nitrobiphenyl, we obtained a yield of 64% carbazole by irradiating 0.5 mmol of 2-nitrobiphenyl with 3.0 mmol of triethyl phosphite in a test tube for 7.5 min at 600 W. When 0.5 mmol of 2-nitrodiphenylamine was added to 2 mmol of triethyl phosphite in a test tube and irradiated at 600 W for 5 min, 43% phenazine was obtained. When 2,2'-dinitrobiphenyl was mixed with triethyl phosphite and irradiated at 80 W for 5 min or at 200 W for 36 no reaction was observed as evidenced on the TLC plates. At higher power or longer periods of time, decomposition took place and the mixture ignited. The following products were obtained when a mixture of 0.5 mmol of 2,2'-dinitrobiphenyl and 3.0 mmol of triethyl phosphite were irradiated at 600 W for 3 min, 200 W and 17 min and 200 W and 20 min: 24% carbazole and 11% benzo[c]cinnoline, 38% carbazole, and 35% carbazole, respectively.

The results showed that microwave radiation could be conveniently used for organic synthesis as the reaction time is greatly shortened and little

or no side products are formed.

Keywords: microwave, carbazole, Cadogan reaction, 2-nitrobiphenyl, 2-

#### CMPS-11

# ANALYSIS OF ECSTASY IN URINE USING GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY

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Ecstasy or methylene-dioxymethamphetamine (MDMA) is the most popular club drug and it acts as a stimulant and psychedelic, producing euphoria and sensual effects. This study aimed to develop a definitive method to analyze this drug in urine samples.

Ecstasy was analyzed by gas chromatography with mass spectrometry (GCMS). Blank urine spiked with known concentration of ecstasy was extracted with diethyl ether, concentrated and derivatized with N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and injected into a Thermo GCQ Ion Trap. The gas chromatogram showed that the average retention time of MDMA was 7.50 min with diagnostic ions at m/z 130, 135, 250. Ephedrine was utilized as the internal standard and had an average retention time of 6.41 min with diagnostic ions m/z 130, 149 and 163.

Chromatographic results were repeatable. The coefficient of variation (CV) of the peak areas of MDMA at 250 ppb and 750 ppb, were 11% and 7% respectively. Linear response was obtained for MDMA standards over the concentration range of 50 ppb to 1000 ppb using the internal standard method. The limit of detection (LOD) was 8 ppb while the limit of quantitation (LOQ) was 27 ppb.

Percent recoveries obtained for MDMA spiked in urine samples above the cut-off concentration of 500 ppb ranged from 95.5% to 102.9%, while for below cut-off concentration, they ranged from 95.5% to 102.3%.

This procedure may be used for routine confirmation of ecstacy in urine samples.

**Keywords**: ecstasy, gas chromatography with mass spectrometry (GCMS), urine

#### CMPS-12

# SPECTROPHOTOMETRIC ANALYSIS OF α-TOCOPHEROL USING EMERALDINE FILM

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α-Tocopherol is an antioxidant, which slows down or prevents the oxidative damage to our body. This antioxidant was analyzed spectrophotometrically using emeraldine film as the sensor.

The polyaniline (PANI) film in emeraldine form (green film) was first chemically synthesized using aniline hydrochloride and ammonium persulfate onto the surface of a plastic acetate film. The polymerization was carried out for 2 hours at room temperature.

The prepared emeraldine film exhibited maximum wavelength at 700 nm in the optimum pH of 2. The changes in absorbance of the chemically polymerized emeraldine film in the presence of  $\alpha$ -tocopherol at 700 nm were studied.

α-tocopherol produced a decrease in the absorbance of the emeraldine film and the change in absorbance was proportional to its concentration. The plot of the change in absorbance versus α-tocopherol concentration was linear in the range of 0 to 150 mg/L. Repeatable responses were obtained. Values of the coefficient of variation (CV) ranged from 0.09 to 0.2 %. The method was sensitive and the limit of detection for α-tocopherol was 0.03 mg/L.

The method was applied to the analysis of a pharmaceutical preparation of  $\alpha$ -tocopherol. The amounts of  $\alpha$ -tocopherol measured were in good agreement with the label claim. This study has shown that PANI film in the emeraldine form can be an effective sensor for the detection of  $\alpha$ -tocopherol.

**Keywords**: α-tocopherol, antioxidant, emeraldine film, polyaniline (PANI), spectrophotometric analysis

#### CMPS-13

# ESSENTIAL OIL CONTENT AND CHEMICAL COMPOSITION OF PHILIPPINE Cinnamomum mercadoi VIDAL (LAURACEAE)

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The essential oil content of the leaves and bark of Cinnamomum mercadoi Vidal (Lauraceae) found in the Philippines was determined by water distillation in Clevenger type apparatus. The chemical composition of the leaf and bark oils was analyzed by gas chromatography. The compounds present in the hexane (non-polar) extract of the bark of C. mercadoi was also determined by gas chromatography-mass spectrometry.

The leaves of C. mercadoi yielded 2.0-3.22% (dry weight basis) essential oil while the bark oil yield was 1.0-4.76% (dry weight basis). The leaf essential oil was made up mainly of cinnamic aldehyde at 85.89%. Eugenol (6.28%) and caryophyllene (1.17%) were the other constituents detected. The total constituents detected represent more than 90% of the oil's composition.

Meanwhile, Philippine cinnamon bark oil's main components were methyl eugenol (57.55%), safrole (17.21%) and eugenol (6.27%). Cinnamyl alcohol (1.16%) and terpineol (0.48%) were the other components present.

Ten compounds were identified in the GC-MS analysis of the hexane extract comprising mainly the oily components of the bark. The major component identified was methyl eugenol (20.87%). Safrole (2.38%), eugenol (1.27%), ledol (3.22%); 4,4,8-trimethyltricyclododecane-2,9-diol (2.74%), tridecanoic acid (2.10%), spathulenol (1.75%), (-)Globulol (1.09%), (+)-epi-bicyclosesquiphellandrene (1.68%) and 14-methylmethylester pentadecanoic acid (1.46%) were the other constitutents detected.

Keywords: Cinnamomum mercadoi, Lauraceae, essential oil, chemical composition, cinnamic aldehyde, methyl eugenol

#### CMPS-14

# ESSENTIAL OIL CONTENT AND CHEMICAL COMPOSITION OF PHILIPPINE Zingiber officinale ROSC.

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The essential oil content of Philippine Zingiber officinale Rosc, was determined by water distillation using the Clevenger type apparatus. The chemical composition of ginger oil was analyzed by gas chromatographymass spectrometry. The physicochemical properties of the oil were also evaluated.

The yield of essential oil from the sliced rhizomes of Z. officinale was 0.12% to 1.3% (v/w). Ground sample produced higher oil yield of 2.1%. By GC-MS analysis, the constituents identified in the oil were cineole (5.49%), -linalool (3.5%), D-limonene (3.02%), camphene (2.65%), -citral (6.69%), -citral (9.12%), trans-geraniol (10.1%), borneol (1.55%), p-menth-1-en-8-ol (2.09%), -citronellol (1.43%), citronellol acetate (1.25%), zingiberene (6.06%), -farnesene (3.45%), myrcene (1.33%), -sesquiphellandrene (2.54%), hedycaryol (1.16%), 2methyl-6-p-tolyl-2-heptene (2.75%) and eudesm-4(14)-en-11-ol (1.17%). The constituents identified represent 74.8% of the oil's total composition.

**Keywords**: ginger, Z. officinale, essential oil, chemical composition, GC-MS

# ENGINEERING SCIENCES AND TECHNOLOGY

#### EST-1

# STRUCTURE AND MECHANICAL PROPERTY OF MgO-ZrO, CERAMIC DOPED WITH CeO,

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Zirconia-based ceramics are examples of advanced ceramic materials with superior mechanical properties such as hardness and fracture toughness. The structure and properties of pure zirconia can be modified by addition of certain dopants such as MgO and CeO<sub>2</sub>. This study, which was intended to lower the fabrication temperature of zirconia-based ceramic, investigated the effect on the structure and hardness of MgO-ZrO, ceramic by doping with CeO, and sintering at relatively lower temperatures.

MgO-ZrO, ceramic containing 90.3% mole ZrO, and 9.7% mole MgO was doped with CeO, ranging from 0 % to 24% mole and sintered at 1300°C, 1400°C and 1500°C for varying times of 1 hour to 6 hours following a factorial experimental design. Four levels of composition and three levels of sintering time were used in the experiment.

Results of X-ray diffraction (XRD) analysis of sintered samples showed that the structure of ZrO<sub>2</sub> phases formed were dependent on composition and temperature but independent of time. Cubic zirconia was observed to be the dominant phase at 1500°C for all compositions while the monoclinic phase was present in compositions containing less than 12% mole CeO, at all temperatures. The amount of tetragonal phase was highest in samples containing 12% mole CeO, sintered at 1300°C. The hardness measured by Vickers indentation technique was found to increase linearly with the square root of CeO, concentration at compositions less than 12% mole CeO<sub>2</sub>. This same property decreased with CeO, content at compositions above 12% mole CeO,. The highest hardness value was obtained in single-phase cubic zirconia containing 12% mole CeO, sintered at 1500°C. Results obtained in this study were comparable with reports in the literature sintered at much high temperatures, indicating the possibility of reducing the fabrication temperature of MgO-ZrO, binary ceramic by doping with CeO,.

ceramic, doping, zirconia, magnesia, ceria, mechanical, hardness, properties, structure, phase, sintering

#### EST-2

# SYNTHESIS AND CHARACTERIZATION OF CORDIERITE PREPARED FROM MIXTURES OF PHILIPPINE KAOLINITE CLAY, TALC, AND OTHER ADDITIVES

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Imported commercial kaolin clay, tale powder and light magnesium carbonate were used to synthesize cordierite body, based from the stoichiometric composition of 2MgO.2Al,O<sub>3</sub>.5SiO<sub>3</sub>, as control mixture by solid state sintering process. Philippine kaolinite clay and tale mineral rock, both from the province of Hocos Norte were used to substitute the imported commercial materials in the following mixture ratios of 90:10, 80:20, and 70:30. Test specimens in the form of pelletized circular disks of 20 mm diameter by 2.5 mm thickness were prepared from the mixtures by powder pressing in a stainless metal mold using hydraulic press with a pressing load of 80-100 MPa. The pellets were sintered at the temperature range of 1000°C-1200°C for two and three hours in an electric furnace. The effects of mixture ratio, sintering temperature and sintering time to the bulk density, apparent porosity, linear shrinkage and compressive strength of the synthetic cordierite were determined by conducting full factorial experiment. Results in the characterization of synthesized cordierite body using x-ray diffraction (XRD) analysis revealed the formation of  $\mu$ -cordierite and  $\alpha$ -cordierite in all test specimens of the three mixture ratios sintered at 1000°C within the sintering time of two and three hours. At 1100°C, the diffraction peaks detected were mostly formation of a-cordierite as the major crystalline phase with the presence of small amount of u-cordiente and spinel. SEM characterization analyses show that the microstructures were transformed from uniformly euhedral particles (size range of 1-10 µm) to spherical particles as the sintering temperature increases from 1000°C to 1200°C. Open pores between spherical particles can also be distinguished.

Results of the full factorial experiment indicate that as the amount of substitution of local materials increased in the stoichiometric composition of cordierite using 100% imported materials, bulk density and linear shrinkage

increased with a linear relationship having a positive slope, while apparent porosity decreased having a negative slope in all test specimens within the sintering temperature range of 1000°C-1200°C and sintering time of 2 and 3 hours. Compressive strength increased as the bulk density of the test specimen of synthetic cordierite increased. The maximum compressive strength of the synthetic cordierite test specimen was determined to more than 2,000 psi.

Keywords: synthesis, cordierite, solid state sintering

#### EST-3

#### CHARACTERISTICS OF PANGASINAN ZEOLITE AS A MOLECULAR SIEVE FOR BIOETHANOL

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This study was conducted to determine the physical and chemical characteristics of Pangasinan zeolite clay. The physical property includes moisture content, specific gravity, raw streak color and fired streak color, particle size distribution (PSD) and water of plasticity. The moisture content of zeolite clay sample had an average of 10.53%, specific gravity of raw powder zeolite clay had a value of 1,92 while the fired material at 1000°C has 1.84 which was lower compared to the natural zeolite clay which had a range of 2.1-2,4,

The powder color was determined by the munsell color chart and the uncalcined raw color was pale yellow. The calcined material at 1000°C was red. The Pangasinan zeolite clay had at most an approximately 0.149mm particles, and the PSD analysis exhibits the highest percent retained in sieve No.100 (150µm) which has a nominal sieve opening of 0.149 mm (149µm) and it has a water of plasticity of 57.05%.

Chemical and mineral structural characterization of local zeolite clay was investigated through wet method and X-ray diffraction (Siemens X-ray Gen with Phillips Goniometer). Chemical analysis of local zeolite clay compared to the analysis of natural zeolite was found out that some of the weighted percentage compositions were different. The alumina (Al<sub>2</sub>O<sub>3</sub>) with

12.26% and silica (SiO<sub>3</sub>) with 73% were almost the same to the commercial zeolite. The difference was due to the nature of deposit and preparation of the sample.

In the XRD patterns of Pangasinan zeolite clay, the beneficiated raw beneficiated calcined at 600°C, and raw calcined 1000°C revealed the presence of clinoptilolite (Na,K,Ca)<sub>2.1</sub>(A1,Si)<sub>2</sub>Si<sub>13</sub>O<sub>36</sub>,12(H<sub>2</sub>O) as major components. The zeolite also contained tridymite, alpha cristobalite, and alpha guartz.

The material was formed as a molecular sieve to dehydrate hydrous bioethanol from sweet sorghum and the result showed that anhydrous ethanol was obtained with a purity of 99.4%

**Keywords**: alpha cristobalite, alpha quartz, bioethanol, clinoptilolite, molecular sieve, tridymite, zeolite

#### EST-4

#### ETHANOL FERMENTATION UNDER VACUUM PRESSURE

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A kinetic model for ethanol fermentation process was developed to investigate the effects of high glucose concentration on the growth rate and production rate of a baker's yeast, Saccharomyces cerevisiae. The model was built from the batch experimental data and validated by a continuous fermentation experiment. A high glucose concentration led to a decrease in the growth and production rates. The maximum ethanol concentration obtained from batch fermentation was 15.8% (v/v) within 72 hours. All parameters were determined as function of merely initial glucose concentration, when fermentation temperature was kept constant. The results indicated that the fermentation process can be generally described by reducing sugar concentration variable.

Keywords: Kinetic model, Ethanol fermentation, High glucose concentration, Baker's yeast

# OPTIMIZATION OF Jathropha curcas (TUBANG BAKOD) AND Calophyllum inophyllum (BITAOG) AS A VIABLE SOURCE OF ACTIVATED CARBON FOR METHYLENE BLUE ADSORPTION

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Activated carbon adsorption techniques were proven successful in removing colored organics. Methylene blue is a thiazine (cationic) coloring dye. The dye causes hazards such as permanent injury to the eyes, irritation to the gastrointestinal tract with and skin and abnormalities in respiration. Furthermore, Jatropha curcas (Tubang Bakod) and Calophyllum inophyllum (Bitaog) seed kernels have been evaluated as potential alternative sources for biodiesel production. Their nutshells and seed husks are considered agricultural wastes.

In this study, locally produced activated carbon derived separately from Jatropha curcas nutshells and Bitaog seed husks were developed. The production involved chemical activation with Phosphoric Acid and Zinc Chloride, respectively. The optimization of carbonization conditions involved the determination of the appropriate temperature and burn off time, for effective pore size and structure. Each developed activated carbon was contacted with Methylene Blue dye for the adsorption test and then characterized by Scanning Electron Micrograph (SEM) analysis. Kinetic Model, the Langmuir Isotherm and the Freundlich Isotherm were used to analyze the adsorption capacity of the activated carbon.

The best conditions for activated carbon from *Jatropha curcas* nutshells based on the linear correlation (R') were: 500°C, 240 min, and 1:1 chemical ratio. The carbon showed pore diameter of 13.3-26.6 µm which is accessible to Methylene blue pores. The isotherm equilibrium data were well-fitted by the Langmuir model and gave a maximum adsorption capacity of 153.8 mg/g. The best parameters identified for the Bitaog activated carbon were 850°C and 6 hrs.

The resulting activated carbon from Jatropha curcas and Bitaog showed substantial capabilities to adsorb Methylene blue from wastewater as compared to the standard activated carbon. This could definitely aid in the removal of wastewater pollutants as well as reduce the solid wastes that could be generated from biodiesel production.

Keywords: Activated carbon, Jatropha curcas, Tubang Bakod, Calophyllum inophyllum, Bitaog

EST-6

## PRELIMINARY INVESTIGATION OF Calophyllum inophyllum (BITAOG) AS A POTENTIAL SOURCE OF BIOFUEL

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In support to the Philippine's thrust of sustaining the supply of fuel while maintaining a steady food supply, a non-edible forest crop was examined for biodiesel production. Our study focused on the investigation of Calophyllum Inophyllum (Bitaog) oil as a potential biodiesel source. It aimed at determining the optimum transesterification time and methoxide concentration, the chemical composition of the Bitaog Methyl Ester and the fuel characteristics compared to the petroleum-based diesel and accepted biodiesel in the Philippines based on national standards.

Bitago is a tropical tree that is abundant in the Philippines. Mature Bitag fruits from Zambales were dried, decorticated, grinded, immersed in n-Hexane and percolated for oil extraction. Transesterification proceeded by preheating the oil at 70°C. Varying concentrations of methoxide solution composed of methanol and sodium hydroxide, as catalyst, was added to the oil in each transesterification with 10% excess methanol The solution was allowed to settle followed by separation of the Bitaog Methyl Ester (BME) and subsequent washings.

About 40% of oil yield was obtained from the Bitaog oil extraction. Transesterification proceeded for two hours at 65 - 75°C with optimum 0.85M methoxide concentration. The GCMS analysis of the transesterified oil indicated that Methyl Oleate and Methyl Linolelaidate are the most dominant fatty acids with 24.20% and 44.98% abundances, respectively. BME slightly exceeded the PNS limit by 0.11 cS for kinematic viscosity. Subsequent analyses may be carried out on BME in blended forms. The 220C flash point indicated that BME has less tendency to forming flammable mixtures at low temperature which proves to be safer to handle as compared to diesel fuel and CME. BME presents a source of biodiesel that assures compatibility with the emission standards preventing the wear of the fuel system and internal engine components, a cleaner biodiesel, with lesser corrosion on metallic alloys.

Keywords: Bitaog, Fatty Acid Methyl Ester (FAME), Biofuel, Transesterification

#### EST-7

# PRODUCTION OF PARTICLEBOARD FROM Nymphaea stellata willd (WATER LILY) FIBER WITH HIGH-DENSITY POLYETHYLENE (HDPE) AND POLYSTYRENE (PS) AS BINDERS

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Water-lily, best describe as queen of the water, is a floating-leaved perennial herb that grows rooted in shallow lakes and swamps. When left unmanaged, it tends to form hence mono specific strands that can cover hundred of hectares. This study was undertaken to produce particleboard made of waste materials plastics and water lily plant.

Three sacks of water lily plants were collected from the Pasig Rivera and oven-dried. The plastics used were high density polyethylene (HDPE) and polystyrene (PS). The particleboards that were made from water lily fiber and HDPE and PS had the following weight- weight ratio of 20:80, 25:25 and 40:60. The fiber and the polymer were mixed on the roll milling machine to form a homogeneous mot. The tests made were: chemical

resistance analysis, tensile strength, breaking strength, density, moisture and scanning electron microscope. The 40:60 water lily/HDPE and water lily/PS became slightly rough upon exposure to 30% NAOH. The 40:60 ratio also gave the best for tensile strength with 13.33 MPa for water lily/PS and 14:00 MPa for water lily HDPE. The results showed that the 40:60 on both PS and HDPE gave the highest value for the ultimate load. Both particleboards of the same ratio gave 28MPa Flexural Strength. As the ratio of the fiber against the polymer increases, the denser the particleboard becomes. The density ranged from 2.31 to 2.37 g/cm<sup>3</sup> for the 40:60 water lily/HDPE and 40:60 water lily/PS. The moisture content ranged for 0.16 to 0.47 for all the different ratios of the particleboards. The scanning electron microscope was used to characterize the surface morphology of the specimen and to check the distribution of the fibers on the particleboard. The magnifications used were; 150, 750, 1500 and 3500.

Particleboard, High Density Polyethylene Polystyrene, Keywords: Scanning Electron Microscope

#### EST-8

## REDUCING FOSSIL FUEL EMISSION USING TREADLE PUMP TECHNOLOGY

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The impact of treadle pump technology in reducing the fossil fuel emission coming from agricultural sources was assessed. Specifically, the study estimates the amount of carbon dioxide (CO,) emission that can be reduced by using treadle pump technology and determines the appropriateness of the technology.

Estimating the fossil fuel emission followed the "bottom up" approach as recommended by the Intergovernmental Panel on Climate Change.

The appropriateness of the technology was determined by evaluating the pump's capacity to irrigate farms. Acceptability to farmers was assessed and farmer perceptions on the impact of treadle pump on vegetable production were gathered using a questionnaire.

Estimating the fossil fuel emission from vegetable production in Nueva

Ecija revealed that it could contribute from 327 to 654 metric tons of CO<sub>2</sub>. This can be reduced by a treadle pump by 81.82 to 490.89 metric tons per year.

The treadle pump is appropriate for small plots of lands. Water source can be creeks, ponds, etc. and in open wells with depth up to 5 meters. Marginalized farmers can use the pump because of its low initial, operational and maintenance cost. Treadle pump can be used in areas where grid electricity and source of fuel are difficult to find.

**Keywords**: Treadle pump, Pressurized treadle pump, human powered pump, Alternative pump technology, Fossil fuel emission, CO, emission

#### EST-9

# PHYSICAL QUALITY CHARACTERIZATION OF MILLED RICE USING COMPUTER VISION TECHNIQUE

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The use of a low cost computer vision technique in physical quality characterization of milled rice was explored and developed. The technique extended the application of freeware image processing software and ordinary desktop scanner. Classification of milled rice kernel was aided by neural network (NN) and discriminant analysis (DA).

Physical quality parameters evaluated were chalky grains (%CG), head rice (%HR), broken rice (%BR), and grain shape. Characteristic dimension ratio (CDR) of the area, length and volume as determined by computer vision technique were compared using mean squared error (MSE) to the results of standard laboratory method (SLM).

Computer vision technique using CDR based on length was found effective in evaluating the %HR and %BR of both medium and long grain samples. Analysis revealed a strong relationship between the results of CDR and SLM as reflected by low MSE and high R<sup>2</sup> values (> 0.95), defined by 3<sup>rd</sup> order polynomial functions. Effective classification were found using DA in medium grains and NN on long grains samples.

Chalkiness (%CG) by CDR based on length in NN resulted in the

lowest MSE value of 1.95 compared to other CDR features and of higher R<sup>2</sup> value of 0.91 as defined by an exponential function. Results on grain shape shows medium grains having length 4.76 to 5.99 mm and circularity of 0.71 to 0.82 indicating medium to bold shape. Long grains have length 5.36-7.43 mm and circularity of 0.44-0.67 indicating slender shape.

Low cost semi-automated computer vision technique for milled rice physical quality characterization can be implemented with good accuracy (> 90%) in reference to SLM. The applications and potential use may be extended to agencies and people working on grain quality inspection, milling and retailing.

Keywords: rice milling, neural network, computer vision, discriminant analysis, characteristic dimension ratio



HS-1

# BEING DIABETIC: SYMPTOM DISTRESS AND QUALITY OF LIFE

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Diabetes mellitus is an increasingly important public health concern in the Philippines. It is a disease that must be controlled over the lifetime of a In addition, there is a high prevalence of chronic medical complications among subjects with diabetes mellitus. It is especially prevalent in the elderly and is one of the leading causes of disability in adults over 45 years of age. In order to provide information to health care providers and professionals on the ways to facilitate self-management of patients with diabetes, this study was therefore conducted. In this study, we relied on the client's own perception of their quality of life and distress experiences with their illness. The General Health Questionnaire developed by Goldberg in the 1970's was used to measure minor psychological distress, the Diabetes Empowerment Scale was used to measure the psychosocial self efficacy of people with diabetes, the Quality of Life Index developed by Ferrans and Powers was used to to measure the quality of life in terms of satisfaction and importance of the various aspect of life and the Beck Depression Inventory created by Aaron T. Beek was used to measure characteristic attitudes and symptoms of depression in the patients. The results of the study showed that 65% of the patients do not experience any significant changes with regards to their general health status. The Quality of Life Index results showed that 73% of the respondents are very satisfied with their quality of life and 92% consider the different aspects of life as very important. The results from the Beck Depression Inventory showed that 91% of the diabetic respondents do not have thoughts of killing themselves because of the disease. In the Diabetes Empowerment Subscale it is shown that most of the respondents know what helps them stay motivated to take care of their diabetes. Comparing the different demographic predictors of the respondents, age was shown to have a bigger impact on the Quality of Life of patients with diabetes, which implies that health care management of these patients should

be highly individualized.

**Keywords**: Diabetic, depression, quality of life

HS-2

# TREATMENT OUTCOMES OF PULMONARY TB PATIENTS ENROLLED AT THE UNILAB-DOTS CENTER 2004-2007

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UNILAB-DOTS Center, the first and only DOTS Center established and operated by a pharmaceutical company offering actual supervision of tuberculosis (TB) therapy to patients in the Philippines, was chosen as one of the five private-public mixed DOTS (PPMD) models in the country through a three-year project in support of PPMD funded by the USAID through the Centers for Disease Control (CDC), Atlanta, Georgia and the Philippine Coalition Against Tuberculosis (PhilCAT).

This study evaluated the treatment outcomes and success rate among sputum smear-positive patients enrolled at UNILAB-DOTS Clinic from 2004-2007. Screening of subjects entailed a positive chest x-ray and new positive smears. A total of 153 TB patients were enrolled over four years: 35 in 2004; 28 in 2005; 38 in 2006; and for 2007, 52.

Following the guidelines set by the World Health Organization (WHO), CDC, and the National TB Program of the Philippines for DOTS Centers, screening procedures, laboratory diagnosis, and treatment protocols were performed: management of patients at the UNILAB-DOTS Center and the Acid Fast Ziehl Neelsen Method at the Health First Clinic of UNILAB. Treatment success rates were: 97.2%, 92.9%, 84.2%, and 86.5% for 2004-2007, respectively. The following factors contributed to the low rates in 2006-2007: default, failure, and transferred out. Using sensitivity analysis and assuming that those who transferred out were able to continue their treatment and achieved success, results suggest that for year 2006 treatment success rate would be 94.74% and for year 2007, 92.31%.

UNILAB-DOTS Center has met the WHO global target of 85% treatment success rate for new TB smear positives across the years. Though there were some transferred-out patients we are hopeful that with the implementation of this DOTS program in the Barangay Health Centers, success rates would go on escalating.

**Keywords**: Tuberculosis, Directly Observed Treatment Short Course, Treatment Success

HS-3

# COMPARATIVE ANTIDIABETIC ACTIVITY DETERMINATION AND CHARACTERIZATION OF POTENTIALLY ACTIVE METABOLITES FROM THE LEAVES OF Syzygium malacunse (MAKOPA) AND Momordica charantia (AMPALAYA)

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Diabetes is a disease characterized by persistent hyperglycemia due to a disorder of carbohydrate metabolism. This study was undertaken to compare the hypoglycemic activity of the bioactive components extracted from the leaves of Syzigium malacunse (makopa) and Momordica charantia (ampalaya).

The dried leaves of makopa and ampalaya were soaked in ethanol for 5 days, filtered and concentrated. These extracts were subjected to polarity based partitioning and yielded: hexane extract as makopa hexane and ampalaya hexane, dichloroethane extract as makopa DCM and ampalaya DCM and water extract as makopa aqueous and ampalaya aqueous.

Ascentia Entrust Glucometer results of the hexane extract (makopa, ampalaya, dichloromethane extract (makopa, ampalaya), aqueous extract (makopa, ampalava) yielded 50% and 66%, 64% and 45%, 51% and 22% decrease in blood glucose level respectively collected from the serum of Swiss Webster Albino mice. The extract showed significant decrease in glucose level compared to the control. The ethanol extract of each plant was subjected to chromatographic separation.

Chromatographic separation of the extract of makopa yielded five fractions as M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub> and M<sub>3</sub>. The ampalaya extract gave five fractions as A<sub>1</sub>, A<sub>2</sub> A<sub>3</sub> A<sub>4</sub> and A<sub>5</sub>. Ascentia Entrust Glucometer reading revealed that  $M_1$ ,  $M_2$ ,  $M_3$ ,  $M_4$  and  $M_5$  gave 39.3%, 22.5%, 78.8%, 24.7% and 2.7% decrease

in blood glucose level respectively. While the ampalaya fractions A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> A, and A, gave 27%, 42%, 2.3%, 61.6%, and 42% decrease in blood glucose, respectively. The results suggested that M, contained the most active metabolite.

Instrumental analysis by IR, UV-VIS and gc-mass spectra was made on M<sub>a</sub>. Structural analysis and comparison with existing drugs showed that phosporothoic acid, 0.0 diethyl -(3.5.6-trichloro-2-pyridine) ester was the possible structure that accounted for the large decrease in blood glucose level of fraction M. The results of the study showed that makopa is more hypoglycemic than ampalaya leaves.

**Keywords:** Hypoglycemia, Polar based Partitioning, Chromatography, Diabetes

#### HS-4

# HYPOGLYCEMIC ACTIVITY DETERMINATION AND CHARACTERIZATION OF POTENTIALLY ACTIVE METABOLITE(S) FROM THE ROOT CROP

Smallantus sonchifolius (YACON)

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Smallantus Sonchifolius (yacon) is a perennial herb, classified under the Asteraceae family. Each plant forms underground clump of 4 to 20 fleshy large tuberous roots. This study was undertaken to determine the hypoglycemic property of yacon tubers.

One thousand five hundred grams of yacon tubers were air-dried, homogenized, and percolated with 1.5 litters of methanol for one week and rota-evaporated. Forty ml of the extract was dissolved in 20ml distilled water and subjected to polarity-based partioning with hexane and ethyl acetate. The extracts were concentrated and subjected to bioassay to test for their hypoglycemic activity. The semi-crude ethyl acetate extract was

purified by column chromatography, characterized by infrared spectra. ultraviolet and gas chromatography-mass spectra analysis.

The hypoglycemic property was confirmed using an Ascentia Entrust glucometer. The experimental design of the 4 groups of mice consisted of the (+) control group, (-) control group, ethyl acetate extract group, hexane and pure extract juice of yacon. The mice were orally fed with 0.4 ml of 35% glucose solution and the blood glucose levels were read at 0, 30, 60 and 90 minutes. The ethyl acetate extract had an initial reading of 20.27, 11.9, 10.23 and 5.92, respectively. While the hexane extract had an initial reading of 20.5, 21.06, 17.9 and 13.23, respectively. The pure extract had an initial reading of 21.3, 14.9, 12.1 and 10.65, respectively.

The infrared spectra revealed the presence of carboxylic acid, aromatic and aliphatic groups. Ultraviolet spectroscopy analysis of the semi-pure isolate had a maximum wavelength at 316 nm with an absorbance of 2.042 and the minimum wavelength at 202nm at 6.045. Gas chromatographymass spectra analysis revealed the presence of 7,10-octadecadienoic acid, methyl ester, 9,10-secoholesta-5,7,10(19)- triene, 3,24,25,-triol (3a, 5z, 7E) and 1,2 benzenedicarboxylic acid, diisooctyl ester.

Yacon had a hypoglycemic action which can be used as a food for diabetics and can be used as a substitute for expensive drugs.

**Keywords**: Hypoglycemic, Diabetes, Infrared Spectra, Ultraviolet Spectra, GC-Mass Spectra

#### HS-5

#### EFFECT OF HOUSEHOLD LEVEL COCONUT OIL USAGE ON THE LIPID PROFILES OF FILIPINO WOMEN

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There is a debate whether coconut intake adversely affects cardiovascular disease risk. This is a public health concern as well as a concern for the vegetable oil industry.

We aimed to contribute to the resolution of the existing debate on the health effects of coconut oil by examining the association between coconut oil intake and lipid profiles as indicated by total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and the total cholesterol to high density lipoprotein ratio (TC/HDL) of adult Filipino women. Data were collected in 2005 from a cohort of 1,732 women aged 35-69, participating in the Cebu Longitudinal Health and Nutrition Survey, a community based study in Metropolitan Cebu. Coconut oil intake was measured as per capita daily intake based on the household's weekly consumption while cholesterol and lipoprotein levels were measured in plasma samples collected after an overnight fast,

Linear regression models were used to estimate the association between individual coconut oil intake and each plasma lipid outcome, after adjusting for total energy intake, age, body mass index, fish consumption and socioeconomic status (education, assets and urban residency). Women who were older, were college educated, had larger body mass indices and were from richer and urban households had high TC levels. Coconut oil consumption was positively related to TC and HDL but unrelated to LDL and the TC/HDL ratio. These findings provide modest support for the proposal that coconut intake could yield positive health effects among Filipinos by enhancing good cholesterol (HDL). A randomized study that supplements the diet with coconut oil will be necessary to confirm that these relationships are causal. The rising burden of cardiovascular disease in the Philippines warrants such an intervention.

**Keywords**: cholesterol, diet, high density lipoprotein, low density lipoprotein, cardiovascular disease.

#### HS-6

# FUNCTIONAL ACTIVITIES OF Schizophyllum commune

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Schizophyllum commune is a newly domesticated species of medicinal mushroom in the Philippines. Production technology for the cultivation of this medicinal mushroom has been developed at the Center for Tropical

Mushroom Research and Development of the Central Luzon State University. Bioassay on the functional activities of S. commune was done primarily to evaluate the potential of basidiocarp and mycelia as possible sources of anti-inflammatory and anti-spasmodic medications. Fifty-four mice were assigned randomly into six treatment groups namely; hot water extract of the basidiocarp of ATCC 38548 (T1), hot water extract of the basidiocarp of wild strain (T2), mycelial hot water extract of ATCC 38548 (T3), mycelial hot water extract of wild strain (T4), commercial drug as positive control (T5) and distilled water as negative control (16).

S. commune regardless of strain exhibited functional activities. Carageenan induced edema test for anti-inflammatory effects revealed that basidiocarp extract of ATCC 38548 is comparable with commercial drug. Charcoal tracing assay for anti-spasmodic activity showed that the mycelial hot water extract of ATCC 38548 is significantly better than the commercial drug in reducing the spasm of the intestine of mice.

**Keywords**: Medicinal mushroom, Mushroom nutriceutical, Schizophyllum commune

#### HS-7

# GLYCEMIC EFFECT OF BETEL NUT (Areca catechu Linn.) FRUIT

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The acceptance of unconventional medicine or the so called alternative medicine is favorable at this time because pharmaceutical drugs are increasingly becoming expensive due to worldwide economic crisis. This study attempted to assess some alternative medicines, for diabetes, from plants that are most abundant to our native places here in the Philippines using phytochemical analysis and bioassay.

Phytochemical analysis of the extract of the fruit of the betel nut showed that alkaloids, flavonoids, triterpenes, saponins and glycosides are the constituents that are present in the extract.

Preparations of the extract of the fruit of the betel nut were done through decoction, infusion and alcohol extraction. The bioassay of the extracts showed that there is hypoglycemic effect of the different preparations of the betel nut fruit extract on the test animals (guinea pigs). Among the preparations of the extract, betel nut alcohol extract showed a higher significant hypoglycemic effect.

Phytochemical analysis and bioassay of the fruit of the betel nut have led to the detection of hypoglycemic effect of the fruit. This study is one way to alleviate the sufferings of people who cannot afford to buy expensive medicines in the Philippines.

Keywords: Hypoglycemic effect, bioassay, decoction, infusion, phytochemical analysis

HS-8

# MARINE COPEPODS: A POTENTIAL RICH SOURCE OF OMEGA-3 POLYUNSATURATED FATTY ACIDS, A DIETARY SUPPLEMENT FOR HEALTHY AGING

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Aging gracefully may be traced back to a healthy youth. Omega 3 polyunsaturated fatty acids (PUFA) play a major role in healthy functioning of immune, central nervous and cardiovascular systems from fetal age to older years. Omega 3 PUFAs are now regularly prescribed to pregnant women for normal fetal central nervous functioning, and to the elderly for healthy cardiovascular and nervous systems. This study was aimed to screen for PUFA and culture marine copepods from northern Mindanao coastal waters. Screening microscopic (0.1 to 1.5mm average total length), shrimplike animals, called copepods from the northern coastal waters revealed very promising amounts of PUFA. Out of the 43 identified species, a preliminary PUFA analysis of a very common species, Acartia erythraea, revealed relatively large amounts of the three PUFA of interest. The concentrations of arachidonic acid (ARA), eicosapentaenoic acid EPA, and docosahexaenoic acid (DHA) are 0.9%, 1.4%, and 13.4%, respectively. Optimization of culture conditions of copepods is currently being developed with the goal of mass producing omega 3-rich copepods not only for the food supplement but for the aquaculture industry as well.

Keywords: Crustacea, Marine Copepoda, PUFA, Omega 3, Healthy Aging

HS-9

# PHYSICAL ACTIVITY, ENERGY REQUIREMENTS AND ADEQUACY OF DIETARY MACRO- AND MICRONUTRIENT INTAKES OF OLDER PERSONS IN A RURAL FILIPINO COMMUNITY

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Background: Aging is a process associated with physiological changes such as in body composition, energy expenditure and physical activity. Data on energy and nutrient intake adequacy among older individuals is important for disease prevention, health maintenance and program development. Objective: To determine the energy requirements and the adequacy of energy and nutrient intakes of older persons living in private households in a rural Filipino community. Design: Descriptive cross-sectional study. **Subjects**: Generally-healthy, ambulatory, coherent and free-living elderly aged 60-100 y (n=98), 88 of whom provided dietary information in three non-consecutive 24-hour food recall interviews. Results: There was a decrease in both physical activity and food intake with increasing years. Based on total energy expenditure and controlling for age, gender and socioeconomic status, the average energy requirement for near-old (>60 to <65 y) males was 2074 kcal/d, with lower requirements, 1919 and 1699 kcal/d for the young-old (>65 to <75 y) and the old-old (>75 y), respectively. Among females, the average energy requirements for the 3 age categories were 1712, 1662, and 1398 kcal/d, respectively. Actual energy intakes, however, were only ~65% adequate for all older persons. The intakes of protein, fat, and

micronutrients (vitamins A and C, thiamin, riboflavin, iron and calcium) were only ~24-51% adequate. Among this population, there was a weight decrease of 100 g (P=0.012) and a BMI decrease of 0.04 kg/m2 (P=0.003) for every 1% decrease in total caloric intake adequacy. Conclusion: These free-living elderly participants suffer from both macro- and micronutrient malnutrition. Their intakes of energy are ~65% of the amounts required based on their total energy expenditures, and their intakes of selected vitamin and mineral nutrients do not meet the Philippine dietary recommendations.

**Keywords**: elderly, energy expenditure, energy requirements, dietary intake, physical activity

#### HS-10

# INTERLEUKIN 4 -590 C/T GENE POLYMORPHISM AS A GENETIC MARKER FOR ATOPIC ASTHMA IN A SELECTED FILIPINO PEDIATRIC POPULATION

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Allergic asthma is the most common chronic disease in childhood worldwide, characterized by airway inflammation associated with hypersensitivity and is brought about by the interaction of well-recognized environmental and largely uncharacterized genetic factors. Interleukin-4 (IL-4) cytokine modulates the pathogenesis of atopy, immunoglobulin-E (IgE) secretion, and allergic inflammatory response. The IL-4 cytokine is regulated by the IL-4 gene located in human chromosome 5q31-33. This study aimed to determine the association of -590 C/T IL-4 gene polymorphism and the risk of atopic asthma in a selected Filipino pediatric population. Fifty one gender- and age-matched pairs of allergic and nonallergic individuals were phenotyped for total serum IgE using enzymelinked immunosorbent assay (ELISA). Atopic status was defined by serum IgE concentration ≥100 IU/mL. DNA was extracted from peripheral blood and genotyped for the -590 C/T polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis with Ava II endonuclease. The study population conformed to the Hardy-Weinberg Equilibrium proportion  $(y^2 p > 0.05)$  based on the gathered genotype frequencies. An association between the -590 C/T IL-4 polymorphism and IgE levels was confirmed in the study population (ANOVA p=0.0157). The IL-4 T allele frequency was significantly higher in allergic (0.392) than in non-allergic (0.235) subjects (OR=2.10, 95% CI=1.144-3.844, y'p=0.0232) was also established. Thus, the -590 C/T IL-4 gene polymorphism is a risk factor to Filipino pediatric atopic asthma. The -590 C/T IL-4 gene polymorphism can be used as a genetic marker for the diagnosis of allergic asthma among Filipinos.

**Keywords**: interleukin 4, single nucleotide polymorphism, allergy, atopic asthma

#### **HS-11**

# MULTIDRUG-RESISTANT ESBLS IN COMMON CLINICAL ISOLATES: FIRST DOCUMENTATION OF $BLA_{CIVM}$ GENOTYPE IN THE PHILIPPINES

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Extended-Spectrum \( \text{B-Lactamases} \) (ESBLs) are enzymes like TEM, SHV, and CTX-M produced by bacteria that renders the bacteria resistant to antibiotics commonly used for treatment. Laboratory tests may indicate that cephalosporins and monobactam are effective against ESBL-producing bacteria, but are actually ineffective clinically. This susceptibility/treatmentmismatched pattern leads to treatment failure, serious complications, and even death. This study determined the prevalence of ESBL-producing Enteropacteriaceae from in-patients of a tertiary hospital in Cavite. Isolates were collected from November 2007 to April 2008, screened and confirmed for ESBL-production using the Clinical Laboratory Standards Institute method. Molecular genotyping for TEM, SHV and CTX-M enzymes and DNA sequencing were performed on confirmed ESBL-producers. The patients' demographic profile was analyzed in correlation with presence of ESBLs. Extended-spectrum \( \beta \)-lactamase production was confirmed in 15 out of 53 (28.3%) isolates that included 6 out of 15 Enterobacter aerogenes, 5

out of 25 E.coli, 3 out of 4 Proteus mirabilis, and 1 out of 1 Citrobacter diversus. Seven out of the fifteen of the ESBL-confirmed isolates expressed  $bla_{\text{TEM-L}}bla_{\text{CEX-M-13}}bla_{\text{SHV-1}}$  and  $bla_{\text{SHV-5}}$  genotypes. Following earlier reports on TEM and SHV types of ESBL, infection with bacteria producing blacteria now being reported worldwide. Demographic profile of 15 patients infected with ESBL-producing bacteria includes the following: 14/15 females; 9/15 belonged to the elderly group (mean 72 yrs); 12/15 of the patients acquired the ESBLs from the community, and 3/15 from the hospital. Furthermore, these ESBL-producing bacteria were isolated from patients who have previously taken multiple antibiotics, were attached to invasive devices, and with underlying conditions like diabetes mellitus, hypertension, and other infections. Antimicrobial susceptibility patterns indicate high-level resistance to both β-lactam and non-β-lactam antibacterial agents, and were found to be susceptible only to meropenem (7/7) and imipenem (12/15), followed by amikacin (12/15). This study documents the first report in Cavite of isolates harboring  $bla_{red}$  and  $bla_{sev}$ , and of  $bla_{cross}$  genotype in the Philippines.

**Keywords**: extended-spectrum β-Lactamase (ESBL), *Enterobacteriaceae*, cephalosporins, monobactam, carbapenem

#### **HS-12**

# CLONING AND EXPRESSION OF NON-STRUCTURAL PROTEIN 1 (NS1) OF DENGUE SEROTYPES 1 AND 2

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Dengue remains an endemic viral disease affecting the tropical and subtropical regions of the world. While the pathophysiology of dengue infection remains to be fully understood, diagnosis of dengue based on clinical symptoms is oftentimes unreliable, requiring a rapid laboratorybased diagnostic test.

The role of the dengue non-structural protein 1 (NSI) in viral pathophysiology has not yet been fully understood. In this regard, the NS1 protein of dengue virus opens up a lot of opportunities for research on how the virus multiplies in the host and on development of diagnostic tests. The Biorad<sup>TM</sup> Dengue NS1 Ag STRIP is based on the qualitative detection of the NS1 antigen. Here we describe the detection, cloning and expression of dengue NS1 protein aiming to develop a rapid diagnostic kit that would detect and quantitate anti-NS1 antibodies.

Briefly, primers for NS1 were designed from consensus sequences of dengue serotypes 1 and 2. After PCR amplification, the 1056 bp NS1 amplicon was inserted into the pCR®8/GW/TOPO® TA (Invitrogen) vector and transfected into the One Shot® Chemically Competent E. coli (Invitrogen). For the expression of the recombinant protein, PCR amplicons were ligated into the pET SUMO (Invitrogen) expression vector and transfected into the Mach 1<sup>TM</sup>-T1® cells for storage or BL21 (DE3) cells for expression using IPTG. Gene inserts and orientation were checked by PCR.

We have successfully expressed a 53 kDa recombinant histidine-tagged recombinant NS1 protein. This was further purified using the TALONTM Superflow<sup>TM</sup> Metal Affinity Resin (BD Biosciences). Immunoreactivity of the dengue rNS1 protein is currently being evaluated by using serum samples from the dengue serum bank archived at St. Luke's Medical Center.

**Keywords:** dengue, non-structural protein 1, recombinant protein, molecular diagnostics

#### HS-13

# MULTIPLEX PCR DETECTION OF Mycobacterium tuberculosis AND CHARACTERIZATION OF MUTATIONS IN katG and rpoB GENES OF RESISTANT STRAINS IN METRO MANILA

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Tuberculosis (TB) remains to have the highest morbidity among infectious diseases. On the recent WHO survey, the Philippines ranks as the 9th high TB burden country, possibly due to poor sanitation, malnutrition, and lack of efficient and cost-effective means of detection and characterization of TB. This study tackles the problem of TB detection and characterization by developing a protocol that is able to detect and qualify resistance faster and cheaper than current gold standards. Eight (8) respiratory aspirates were

assayed by subjecting them to culture and drug susceptibility testing (DST), the gold standards for determination and qualification of TB strains. Four tested negative with culture and was automatically used as negative controls. Of the four positive controls, two were multi-drug resistant (MDR) and the other two were sensitive. The genome sequence of H37Rv strain (Genbank Accession NC 000962) was then used to design primers for polymerase chain reaction (PCR) amplification. For multiplex detection, the primer pairs flank the insertion sequence IS6110 which is ubiquitously found in several copies in the genome, and the hupB gene that is able to differentiate the closely related strains of M. bovis and M. tuberculosis. The two primer sets were able simultaneously determine if the sample contained M. tuberculosis genes. Of major relevance to the management of community TB is the enrolment od a patient into the correct treatment program based on drug susceptibility. For this, distinction between MDR from non-MDR strains was done using newly designed four in-house primers for katG and rpoB genes which have been documented to contain the most common single nucleotide polymorphisms (SNPs) highly correlated with resistance to the first-line drugs rifampicin and isoniazid. These primers were used to characterize mutations in 15 MDR and five sensitive samples, and our results corroborate documented data that there is no one SNP that can universally predict resistance or susceptibility to the two MDR strains. However, the presence of some of these SNPs can initially direct treatment after rapid detection.

**Keywords**: multiplex PCR, *M. tuberculosis*, multi-drug resistance, IS6110, hupB, rpoB, katG

#### **HS-14**

# OCCURRENCE OF SHV AND TEM GENES IN PHENOTIPICALLY SCREENED-POSITIVE EXTENDED SPECTRUM B-LACTAMASES (ESBLS) PRODUCING ORGANISMS ISOLATES FROM SELECTED TERTIARY HOSPITALS (2008)

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Extended Spectrum beta-lactamases (ESBLs) are a large, rapidly evolving group of plasmid-mediated enzymes that confer resistance to third and fourth generation cephalosporins, in addition to the earlier generation of cephalosporins. These enzymes have been derived from TEM and SHV genes by mutations and are produced by gram-negative bacilli. The presence of ESBLs in these organisms can result in treatment failure if the above mentioned antibiotics are used. There is still paucity of surveillance data regarding ESBLs locally. Laboratory diagnosis is a problem for ESBL Positive screening results must still be verified with a detection. confirmatory test. Molecular methods will identify and characterize the gene type rapidly. The objective of the study is to detect the occurrence of TEM and SHV genes among ESBLs producing organisms isolated from selected tertiary hospitals in Metro Manila (2006-2008). PCR was used to detect the expression of TEM and SHV genes. Klebsiella pneumoniae isolates were mostly positive for SHV gene whereas Escherichia coli isolates were mostly positive for TEM gene. Some organisms were found to be positive for both ESBLs have become as serious problem and therefore genes. implementation of appropriate ESBL detection method is recommended. The information will guide the infection control community in determining how to focus its efforts in reducing the emergence and spread of bacterial resistance.

Keywords: ESBL, TEM, SHV

#### **HS-15**

# DOSIMETRY APPLICATION OF GATE IN PROSTATE BRACHYTHERAPY

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Radiation therapy is a common practice in eliminating cancerous cells. However, nontargeted neighboring tissues will also be exposed to these radionuclides which cause probable risks. Thus, there is a need for a reliable estimation of the absorbed dose from the use of radioactive therapeutic agents. Simulation tools for dosimetry applications are routinely used to assess the success of the therapy. This study evaluated the features of GATE (Geant4 Application for Tomographic Emission) specifically for use of <sup>125</sup>I (Iodine-125) radiation seeds for prostate cancer therapy. A voxelized Zubal medical torso phantom was utilized to model all major internal organs of the human body including the prostate. Sixty four radiation seeds of <sup>125</sup>I, with radioactivity set to 10 kbeq, were implanted with an even distribution covering the whole volume of the prostate.

Three post-implant dosimetry assessments were calculated to determine the efficacy of the therapy. Calculations for the time dose pattern after 10 minutes of irradiation yielded average absorbed dose rate values of 1.532898 x 10<sup>-4</sup> Gy/s and 2.279397 x 10<sup>-5</sup> Gy/s, for the whole body and prostate gland, respectively. It therefore infers that approximately 14.8% of the absorbed radiation was in the target organ prostate and the remaining 85.2% were absorbed by surrounding nontarget tissues. The results also show that the uniform seeding distribution pattern of the <sup>125</sup>I throughout the prostate gland resulted to a nonuniform dose distribution as depicted in an isodose contour plot which made use of a cross-sectional view of the target organ. A cumulative dose-volume histogram generated a summary of the dose received by the prostate after ten minutes of irradiation.

GATE simulation toolkit illustrated its capacity for dosimetry applications to prostate brachytherapy and possibly for any kind of brachytherapy with arbitrary source geometry and strength.

Keywords: GATE, brachytherapy, radiation dosage, Iodine-125,

# HISTOMORPHOMETRY AND OSTEOINDUCTIVE GROWTH FACTOR LEVELS OF FRACTURE CALLUS VERSUS ILIAC CREST BONE GRAFTS

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Autologous iliac crest bone grafting (ICBG) has been the standard bone graft for fusion and nonunions. Alternatives have been sought to address the limited quantity available and avoid the morbidities of the harvesting procedure. Fracture callus has proven osteoinductive properties but is not an advocated bone graft substitute due to its unquantified osteoinductive properties. Our study aimed to determine the histomorphology and levels of osteoinductive growth factor levels of fracture callus versus ICBG. Ten patients with long bone fractures undergoing open reduction, fracture fixation, and iliac crest bone grafting were recruited. Samples of fracture callus and ICBG from each patient were sent for histomorphologic analysis and determination of levels of the following cytokines: BMP-2, IGF-1. and TGF-B by RT-PCR. Paired t-test was used to determine any significant difference between callus and bone grafts. Preliminary results showed comparable numbers of osteoblasts and osteocytes for fracture callus and ICBG. Both contained areas of woven bone. PCR products were successfully amplified using primers for BMP and IGF. Comparable levels of BMP and IGF were found in callus and ICBG. Upon stratification, more BMP was expressed in callus than ICBG in callus <10 weeks of age. Our study demonstrates the presence in callus of cells and growth factors essential in bone formation, in quantities comparable to iliac crest bone graft, especially in callus from fractures less than 12 weeks old. This sets the stage for future

animal and clinical trials to determine the viability of callus as a bone graft substitute.

Keywords: Bone Graft Substitute; Histomorphometry; RT-PCR; Gene Expression; Growth Factors



## ELICITING INDIGENOUS KNOWLEDGE SYSTEM ON NATURAL RESOURCE MANAGEMENT STRATEGIES: A CASE STUDY IN THE CORDILLERA REGION, PHILIPPINES

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Natural resource management in the Cordillera Region has been practiced for several decades. This Indigenous Knowledge System (IKS) of the people in the region has sustained their lives and maintained the biodiversity of the region's natural resources. This paper aims to elicit the IKS for indigenous plants and its sustainable application to natural resource management (NRM) in the region and the role of information and communication technologies (ICTs) in information organization, management, and dissemination.

IKS documentation was done through a literature survey and Participatory Rural Appraisal (PRA) activities including key informant interviews, group discussions, and field visits. A Database Information System (DIS) was developed to organize the gathered data. Many indigenous plants are used in NRM in the region. The muyong of the Ifugaos is an internationally recognized and ideal NRM system. Indigenous plants are mainly found in muyongs, which are sources of food, medicinal herbs, wood and other construction materials. The batangan system in Mt. Province is mainly used as source of wood. Contrary to the existing knowledge on shifting cultivation, uma system or patch farming in the region is practiced to rejuvenate soil fertility using indigenous plants such as Tithonia diversifolia (sunflower). In payew, cuttings of sunflower are applied as organic fertilizer for growing rice and sweet potato. ICTs (e.g., Internet, radio, computer) can be used as effective tools to organize, promote, conserve, and sustain the IKS. The availability of a database provides a better understanding of the IKS and a window for IKS analysis. ICT tools can be used to organize, analyze, disseminate, and sustain such knowledge for future generations and as a learning tool for farmers, researchers, academe, and policymakers.

**Keywords**: Indigenous Knowledge System (IKS), Natural Resource Management Strategies (NRMS), Information Communication Technologies (ICTs)

**SS-2** 

## THE YOUNG, THE ELDERLY AND HOUSEHOLD SAVING: EVIDENCE FROM THE PHILIPPINES

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The economic growth implications of the nation's changing age structure have been substantial. In the course of the demographic transition, countries experience an increasing share of the working age population relative to the total population and this creates favorable effects on economic growth via higher household savings. This paper shows a link between population dynamics (young dependents and elderly share) and saving rate in the Philippines using aggregate (regional) household panel data from the Family Income and Expenditure Survey (FIES) (1985-2003).

An econometric model based on the augmented life cycle model is used. The results suggest that the country's population dynamics plays an important role in its household saving rate. Furthermore, the proportion of the elderly has a positive and significant impact on saving rate while the percentage of young dependents has a negative and significant impact on household saving rate. In particular, a one-percentage point reduction in the proportion of young dependents results in an increase in the average saving rate by 0.34 percentage point, while a one percentage point increase in the proportion of the elderly results in an estimated increase of 2.03 percentage points in the average saving rate. The other factors that positively affect saving rate are: household income, education, and remittances.

The results of the study show that the elderly population still saves, contrary to the expectation of the life-cycle model. The elderly accumulate savings perhaps to serve as buffer during the long retirement years or as bequest motive. The accumulation of savings is good for the economy for this influences economic growth. However, the Philippines' rapid population growth results in a high percentage of young dependents. This suggests that the country is paying a high price for its high population growth, resulting in a low saving rate and consequently, low economic growth.

**Keywords**: Demographic Transition, Young Dependents, Elderly, Saving Rate, Life cycle Model

## **QUALITY OF LIFE OF ELDERS**

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Many old people require more care than they are being provided. The lack of caretakers for older people is a serious problem, especially those who have chronic illnesses that limit their ability to care of themselves and their homes. Unfortunately, those who are most vulnerable are also most likely to live alone and have limited incomes. This study was conducted to acquire information regarding the quality of life of the elderly, especially those in the twilight of their lives.

One hundred elderly (50 married and 50 unmarried, 60 years old and above) were recruited to participate in this study. The guided questionnaire developed by Ferrans and Powers (1984) was used to measure quality of life in terms of satisfaction with life by the elderly. Important ratings were used to weigh satisfaction responses, so that scores reflected satisfaction with the aspects of life that is valued by the individual. The quality of life index (QLI) produced five scores: overall quality of life and in four domains -- health and functioning, psychological/spiritual domain, social and economic domain, and family domain. Results of the study show that most respondents were concerned with their physical well-being, especially losing physical strength due to aging. They were very much concerned about losing their ability to take care of self without help. They were worried about not living a better quality of life as many have lost their ability to take care of the family responsibilities. While the single elders staying at the Home for the Aged are provided with quality care, they were not satisfied emotionally as they felt that they have been forgotten by their family members and relatives.

**Keywords**: Aging, elders, quality of life index

**SS-4** 

# HYPERTENSIVE PATIENTS' SYMPTOMS DISTRESS AND QUALITY OF LIFE

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Patients with hypertension frequently report symptoms that are similar to those reported by patients without the diagnosis. Although hypertension is often thought to be asymptomatic, cognitive changes, mood alterations, and general symptoms such as dizziness and headaches have been described. Health-related quality of life (HRQOL) may be influenced by these symptoms, whether these are derived from disease or treatment. This study was conducted to know the HRQOL, specifically the physical, emotional, and social impact of hypertension.

A structured questionnaire was used to determine the health, wellness, and quality of life of hypertensive patients recruited to participate in this study. The questionnaire has six areas of concern: physical state, emotional/mental state, stress evaluation, life enjoyment, overall quality of life, and overall impressions of their quality of life.

Results show that the majority of patients diagnosed with hypertension occasionally or rarely experience physical pain, occasionally or constantly experience emotional/mental distress, moderately suffer from stresses in life, enjoy life considerably, are pleased with their overall quality of life, and have an overall impression that the quality of their lives are the same even before the diagnosis of the disease. They however anticipate that things may change if nothing will be done about their condition; their quality of life may change by then.

Keywords: hypertension, symptom distress, quality of life

## IMPACTS OF A PATIENT'S DEATH UNDER A HEALTH PROVIDER'S CARE

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When someone dies, the impact of the death on his/her family and friends are often thought about. What about the impacts on those who were caring for the patient? As health care providers are often expected to put on a brave front in public when a patient dies, it was the goal of this study to determine the effects of a patient's death on a health care provider. Data were gathered from three hospitals: 2 base hospitals of MSU-IIT Department of Nursing (Don Gregorio T. Lluch Memorial Hospital and Dr. Uy Hospital) and 1 private hospital (Mindanao Sanitarium and Hospital). The respondents were registered nurses, student nurses, and medical assistants who had experienced death and dying of patients under their care. They were recruited after having asked permission from the chief of the hospital or the chief nurse. Structured questionnaires were used.

The results showed that health care professionals, especially new and student nurses, grieved over the death of patients. Sadness, guilt, helplessness, shock, and depression were the most frequently cited reactions experienced by a health care provider over the loss of his/her patient. These emotions show that health care providers go through a uniquely human grieving process. It was however observed that as the length of experience and number of patient deaths under the health provider's care increase, grieving and depression are reduced. Health care providers usually suppress their emotions so that their efficiency and competence in work do not get affected. While a patient's death may create a general change in attitude and perceptions of the health care provider towards their work, the study revealed that health care providers do not allow their emotions to affect their ability to provide competent health care. This indicates a high degree of professionalism among the respondents.

The results of the study highlight the need to include death education courses in the nursing program. These courses must become an integral part of the nursing general knowledge curriculum before nurses take their clinical practice courses. In the clinical practice setting, there is a need for the more experienced nurses to discuss death experiences and perceptions among the health care providers to help the student nurses cope with their grief and depression over the deaths of patients under their care. Understanding the nature of death and dying will help the health care provider cope when faced by the dying and death of the patient.

**Keywords:** Death, Health care provider, impacts

**SS-6** 

## RELIGIOSITY AND WELL-BEING OF THE FILIPINO ELDERLY

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Numerous studies have found a positive association between religiosity and physical and mental health and well-being among older persons. The link between religion and health has gained acceptance over the years, with the World Health Organization including religion in its WHO Quality of Life Assessment tool.

Data from the 2007 Philippine Longitudinal Study of Aging (PLSOA), a nationally representative survey of 3,105 older Filipinos, show that the majority of the elderly engage in religious activities more than any other type of activity. This paper classifies religious activities as non-organizational (prayer) and organizational (attendance at religious activities and membership in religious organizations) due to differences in the nature of each and their effect on well-being. Eighty percent pray at least daily and 60 percent attend religious services at least weekly. Differences in religiosity by gender, marital status, education, work status, and type of residence were also analyzed. Frequency of prayer increases with age; both prayer and church attendance are higher among females than males.

Results of logistic regression models show that organizational religiosity has a positive effect on self-assessed health and a negative effect on depression. Non-organizational religious activity, meanwhile, is associated with better mental health. Although causality cannot be determined, these findings imply that religion can play an important role in

maintaining the physical and mental well-being of older Filipinos. Religion and its many functions, particularly the provision of social support and a means of coping, can be an important resource for the elderly.

Keywords: Religiosity, Prayer, Aging, Well-being, Depression

### **ADDENDUM**

As requested by the author, we are printing this addendum to reflect the addition of the co-author to the following abstract:

Transactions of the National Academy of Science and Technology, Philippines, 2008, Volume 30, Issue no. 1, page 48.

# ACID PROTEASE PRODUCTION OF THERMOPHILIC BACTERIA ISOLATED FROM MUD AND SOIL OF MUDSPRING, MT. MAKILING

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