Science Last Fortnight

Declining Peanut Production
Effects of salinity

Gujarat is the major producer of peanut in the country. However, it is claimed that peanut production is decreasing due to climate change. Since freshwater for irrigation is a challenge in the coastal belt, farmers use saline water to irrigate crops. Salinity affects soil health and results in yield loss. Another factor is the insufficient supply of quality seeds. Farmers sow seeds from previously harvested crops.

This further impacts yield and quality. Soil type and health as well as seed type and water quality determine yield. Though there are many studies on the relationship between these parameters and yield, there is insufficient information on the effect of the reuse of salinity grown peanut seeds on yield and quality traits such as oil content, protein and seed mass.

Recently, Meena and Yadav from the ICAR-Directorate of Groundnut Research, Gujarat reported a solution for the decrease in peanut production in the coastal belt. They chose two Spanish peanut cultivators, TG 37A and GG 2. The team cultivated both cultivars and irrigated the sample plots with normal and saline water from germination to harvest. They found salinity delayed the germination process – germination percentage and velocity as well as the real value of the seeds. Salinity resulted in an accumulation of soluble salts around peanut seeds.

Peanut seed setting and flowering were also affected. Salinity also reduced pollen viability, and photosynthesis at grain filling and seed setting stages. High salinity reduced the number of nodules, mature pods and kernels. And it decreased pod and seed mass. Using these seeds will lead to a crop with even lower productivity.

The researchers also noted that the survival rate of the GG 2 cultivar is higher than that of the TG 37A in saline conditions. Though salinity levels influenced the oil, protein and sugar content of the peanut seeds, the GG 2 cultivar seems to be more tolerant of salinity. Therefore, they suggest that farmers use quality seeds of the GG 2 cultivar from areas not affected by salt for improving peanut production. Agriculture extension agencies and the Krishi Vigyan Kendras must ensure sufficient supply of quality seeds in salt-affected areas.

J. Irrig. Drain. Eng., **144**(3): 04018002

Tale of Transmission
Plasmid adds genes in Vibrio

Vibrio species are the causative agents of the life-threatening diarrhoeal disease, cholera. To manage outbreaks effectively there has been extensive research on the *vibrio* family of bacteria, to understand the epidemiology. *Vibrio fluvialis* BD146 is a clinical isolate from Kolkata collected during the 2002 cholera-like gastroenteritis outbreak. It emerged resistant to many antibiotics and is, thus, a threat to human health.

Last fortnight, scientists from the Indian Institute of Advanced Research and the University of Baroda, Gujarat reported the role of plasmids in conferring multidrug resistance to *Vibrio fluvialis* BD146. They found two plasmids in the *Vibrio* strain – one a high copy number, and the other, a low copy number plasmid.

They carried out a detailed gene analysis of the two plasmids. The team found the presence of a class 1 integron in the low copy number plasmid, by polymerase chain reaction amplification and sequence verification in databases. Using BLAST search and DNA analysis, they annotated the genes in the high copy number plasmid and found the presence of integrase, efflux pumps and toxin–antitoxin genes – genes potentially involved in antibiotic resistance.

Then they checked antibiotic susceptibility to establish minimal inhibitory concentration. By doing a synergy test of antibiotics in the presence of an efflux pump inhibitor, they observed a decrease in the minimal inhibitory concentration of antibiotics, confirming their role of imparting antibiotic resistance.

To check whether the high copy number plasmid could transfer genes horizontally from one bacterium to another, they performed conjugation experiments with a *Vibrio fluvialis* BD146 donor and a standard strain of *E. coli* XL1-blue as recipient. In the first step, they scored for transconjugants by antibiotic screening specific to the plasmid.

They further confirmed the presence of the class 1 integron region in the transconjugants after primer specific polymerase chain reaction amplification.

The team reports that the plasmid may be conserved across *Vibrio* species: *Vibrio cholerae* O1 El Tor from Vietnam and *Vibrio parahaemolyticus* v110 from Hong Kong, suggesting a horizontal gene transfer event across South Asian countries.

Vibrio species are ubiquitous, and resistant to many antibiotics. This poses a challenge to manage outbreaks globally. It is critical to understand the mechanism and its mediators to arrive at better disease treatment strategies. The scientists from Gujarat have now adequately addressed the genetic basis of antibiotic resistance and the role of mobile genetic elements in the *V. fluvialis* involved in the Kolkata outbreak.

Indian J. Microbiol, **58**: 60–67

Gamma Irradiated Oat Glucan
Enhancing bioactivity

Oats are a good source of dietary fibres, proteins, anti-oxidants and unsaturated fats. Their beneficial properties – reducing cholesterol and blood glucose levels, stimulating the immune response and protection from cancer – are mostly due to oat soluble fibres, especially beta-glucan. Recent reports show that when these high molecular weight polysaccharides are broken down into smaller ones, their bioactivity increases. However, we lack methods to produce the desirable compounds without affecting structure and function.

Last fortnight, a team led by R. Hussain, at the Bhabha Atomic Research Centre, Srinagar, reported an undisruptive solution for degrading oat beta-glucan using gamma irradiation. They were inspired by earlier studies using this method for degrading complex polysaccharides.

The team extracted oat beta-glucan by solvent precipitation. They found that the purity of the beta-glucan in such extracts was 91.2%. The scientists irradiated dried beta-glucan at different gamma radiation doses such as 3, 6, 9, 12 and 15 kGy. The resultant molecular weight of the beta-glucan reduced from 200 to 45 kDa – a unit of molecular weight – at a dose of 15 kGy.

The scientists analysed the structure of the irradiated samples with Fourier Transform Infrared Spectroscopy and X-ray diffraction and found that, compared to the control, it was unchanged. They also found enhanced solubility and water absorption capacity as the dose increased.

The researchers then studied the toxicity of the degraded beta-glucan in colon and breast cancer cells. They found arrested growth of cancer cells and no such effect on normal cells. The team also proved the increased antioxidant activity of the degraded beta-glucan.

Interestingly, the irradiated samples enhanced hypoglycaemic activity, a condition of lower blood glucose level. They confirmed this by studying the inhibition of the activities of alpha-glucosidase and alpha-amylase – enzymes responsible for carbohydrate digestion.

The scientists say that consuming this irradiated beta-glucan is safe and provides improved biological properties. Further studies in animals and humans are needed to authenticate edibility and acceptability.

Radiat. Phys. Chem., **144**: 218–230

A Cocktail for Health

Bacteria produce antioxidants

Carotenoids are fat soluble pigments associated with the 'redness' of fruits and vegetables such as carrots, cantaloupe and orange. Humans cannot synthesise them. Dietary carotenoids have been proved to alleviate risks of cataract, cancer, cardiovascular disease and osteoporosis. Biofortification of edibles and dietary supplements have enabled the prevention of vitamin malnutrition in poorer countries. Additionally, they are used as alternatives for toxic colorants. The increasing demand for producing carotenoids calls for novel strategies.

Carotenoids are also found in the cell membrane of non-photosynthetic bacteria. Saroj Mishra and her team from the IIT Delhi tested a salt loving bacterium, *Microbacterium paraoxydans* for the production of carotenoids. The scientists found that a methanolic extract of 100 ml culture gave a yield of 7.5 mg per g of wet weight biomass.

The team established the presence of carotenoids with silica based chromatographic analysis. They did spectroscopic studies to identify the carotenoids. And identified three peaks, characteristic of the carotenoid profile, in Raman spectroscopic analysis for the whole cell. Using an absorption analysis in the range of 400–460 nm, they additionally confirmed the presence of pigments.

$C_{40}H_{56}$ neurospene family members were identified on the basis of their mass/charge ratio by mass spectroscopy. Antioxidant efficacy of the neurospene 'cocktail' was assessed by TLC autobiography using 1,1-diphenyl 2-picrylhydrazyl.

Neurospene exposed to breast cancer cell line was evaluated in both time and concentration dependent manner for the anticancer effect. The researchers modulated bacterial growth parameters in solid and liquid media to assess changes in production levels. They observed that adding sucrose enhanced production 3.1 fold. And NaCl increased carotenoid yield to 0.051 g (g wet wt cells)$^{-1}$.

With changing growth parameters, the neurospene production pathway in *Microbacteria* was charted based on the accumulation of different intermediates. They found that Crt-I type phytoene desaturase catalysed the conversion of geranylgeranyl pyrophosphate to 'phytoenes' like lycopene.

The method presents a clean, non-toxic sustainable mechanism for producing carotenoids. Using non-photosynthetic bacteria enables the manufacture of neurospene at submerged conditions and, hence, may be scaled up, without much variation, to industrial levels.

Indian J. Microbiol., **58**(1): 118–122

Reversing Cervical Cancer

MicroRNA epigenetic regulation

Cervical cancer ranks second as most commonly diagnosed cancer in women. The American Cancer Society's Global Cancer Statistics state that nearly 67,500 women died in India due to this cancer. Cervical cancer is caused by multiple factors, including infection with human papillomavirus, multiple sexual partners, use of tobacco and oral contraceptives. These factors alter the genome expression epigenetically by methylating the promoters of some important genes and this leads to the conversion of healthy cells to cancerous cells.

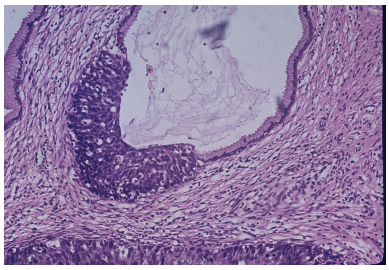


Image: John Hayman via Wikimedia Commons

Last fortnight, researchers from the Manipal University, Karnataka, found a way to turn cancerous cells back into healthy cells. They identified key master regulators such as microRNAs that play a key role in the development of cervical cancer. MicroRNAs are small non-coding RNAs that regulate the expression of a set of genes necessary for maintaining a healthy state in cells. The researchers collected tissue biopsy samples from 30 participants aged 25 to 75. They studied the methylation of microRNA promoters in these samples using microarray and sequencing technologies.

The researchers found changes in the methylation pattern of microRNA-200b and microRNA-424 leading to the development of cervical cancer. The increase of microRNA-200b expression and decreasing of microRNA-424 expression due to methylation leads to cancer development.

Moreover, the researchers over-expressed microRNA-424 and suppressed microRNA-200b in the cervical cancer cell lines and that resulted in the conversion of cancerous cells to healthy cells.

Cervical cancer is one of the most widespread gynaecological malignancies in women. It remains a national and global health challenge. Death from cervical cancer is preventable with early identification and treatment. In this regard, early identification of changes in DNA methylation is useful for reversing the cancer state. The authors' demonstrated findings can be helpful for early cancer diagnosis and for the development of better therapies.

Mol. Carcinog., **57**(3): 370–382

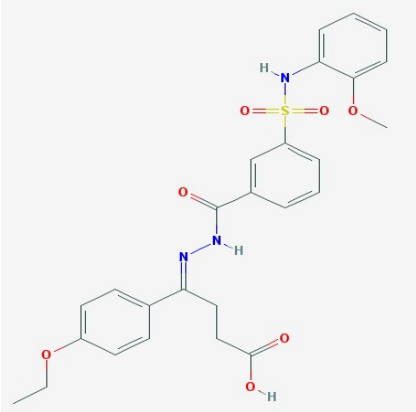
Leishmania Therapeutics
New drug for old disease

Leishmaniasis, a vector-borne disease, transmitted by female blood-sucking sand flies, is spreading worldwide. Caused by the *Leishmania* parasite, this neglected tropical disease leads to skin lesions, *kala-azar*, which attract social stigma and visceral symptoms, fatal if untreated. Though WHO efforts have reduced fatality, around one billion people are still at risk.

The existing Amphotericin B and Miltefosine therapies have low efficacy, adverse side-effects and are costly. Moreover, they tend to generate drug resistance. Thus, there is urgent need for new, effective medicines for leishmaniasis.

Abdur Rub from Jamia Millia Islamia, Yusuf Akhtar from the Central University of Himachal Pradesh with collaborators from BHU, JNU and the Majmaah University, Saudi Arabia focused on an enzyme, UDP-galactopyranose mutase, absent in humans. But, in the *Leishmania* pathogen, it is responsible for a very important sugar, β-galactofuranose, a precursor for the synthesis of the complex lipids on the cell surface. These lipids play an essential role in the survival and virulence of the pathogen. So the researchers decided to search for small molecules to stop the synthesis of β-galactofuranose by inhibiting the enzyme, UDP-galactopyranose mutase, involved in the synthesis of this sugar.

As the structure of the enzyme is not yet known, they predicted its structure to facilitate computer-aided drug discovery. They carried out molecular docking and discovered molecules that could inhibit the enzyme. Then, they validated their prediction via wet-lab experiments.



The molecule showed lesser toxicity on the same concentrations than the existing drug, miltefosine hydrate, when tested on human macrophage cells. The researchers claim that the molecule could be further optimised. This report brings hope in the fight against leishmaniasis.

J. Cell. Biochem., **119**: 2653–2665

Tuberculosis in Wild Animals
Rapid, sensitive diagnosis

Tuberculosis in wild animals is of global concern. They form a reservoir for tuberculosis bacteria that can be transferred to humans. This impacts public health. Diagnostic kits for testing wild animals are based on antibodies of mycobacteria. Since wild animals are exposed to both pathogenic and non-pathogenic mycobacterium species, the antibodies tend to cross-react. Hence, it is a challenge to detect pathogenic species in infected animals. Moreover, in the case of wild animals, there are practical difficulties in implementing existing diagnostic methods, such as the culture method, Interferon Gamma Release Assays, and skin testing.

Recently, B. Mohana Subramanian from the Veterinary and Animal Sciences University, Chennai and researchers from Wildlife SOS, an NGO based in New Delhi, developed a cheap, rapid point-of-care serological kit that can be used on a variety of wild animals. This kit uses special conjugate proteins unlike the species-specific antibodies used in diagnosis. The diagnostic kit can differentiate pathogenic from non-pathogenic bacteria.

To create the kit, they used a recombinant fusion protein of *Mycobacterium tuberculosis* with purified protein derivatives of *Mycobacterium bovis* and *Mycobacterium avium*.

The team validated the specificity of the kit on a wide range of wild animals – elephants, cape buffaloes, wild bear and wild dogs as well as animals of the cat and deer family. Alarmingly, the tests revealed that most wild animals in India are seropositive for tuberculosis!

Given this high prevalence of tuberculosis in Indian wildlife, the serological kit will surely help zoo caretakers and wildlife authorities in systematic tuberculosis surveillance and for control programmes to prevent TB in wild animals.

Indian J. Microbiol., **58**(1): 81–92

Artificial Neural Network
For cancer diagnosis

Thyroid swelling is a commonly occurring symptom in clinical practice. Most benign and malignant thyroid lesions are successfully diagnosed by fine needle aspiration cytology. It is a rapid, easy, reliable and well-recognised technique to diagnose various thyroid enlargement symptoms. However, the efficacy of the method is limited because it fails to differentiate between follicular adenoma and follicular carcinoma of the thyroid.

Immunohistochemical and molecular markers, miRNA analysis and specific mRNA have also been used to distinguish malignant follicular lesions from those which are benign. These methods have a specificity of nearly 90% and show overlapping features but are not successful in all cases.

Recently, scientists from the Postgraduate Institute of Medical Education and Research, Chandigarh, examined the cytological features and morphometric data of follicular

cells. Based on the data, they developed an Artificial Neural Network model to differentiate between follicular adenoma and follicular carcinoma using fine needle aspiration cytology.

The model was made with the help of neuro-intelligence software, Alyuda Neurointelligence 2.2, and trained by online back propagation. The efficiency of the model was verified in a test set. The model successfully distinguished all cases of follicular adenoma and follicular carcinoma with 100% accuracy. This is an open-ended Artificial Neural Network model and more parameters and cases can be included to make the model more reliable. The study provides a promising technique that may be extended to include other types of cancers.

Presently, the Artificial Neural Network model is efficient enough to diagnose follicular adenoma and carcinoma cases in cytology smears without error and can be used in clinical practice.

Diag. Cyt., **2017**: 1–6

Coconut Shell Oil

Eco-friendly wood protectant

Throw coconut shells, along with your kitchen waste, into the garden. The kitchen waste disappears in a few weeks. The coconut shell will remain for years. It seems to be impervious to the attacks of fungi and termites that feed on dead plant materials.

When coconut shells are burnt under anaerobic conditions, pyrolytic oil is produced. This oil contains active principles with antifungal and termiticidal properties. So, pyrolytic oil is used as an eco-friendly wood protectant. However, the dark colour of the oil restricts its use to outdoor purposes.

Image: Uzhavan, via Wikimedia Commons

Last fortnight, K. S. Shiny and team from the Institute of Wood Science and Technology, Bengaluru reported the development of an ecofriendly wood protectant from coconut shell pyrolytic oil. They distilled dark coloured coconut shell pyrolytic oil into one that was colourless and analysed the composition of this colourless distillate using gas chromatography–mass spectroscopy. The team found that it essentially comprises phenol and phenolic compounds.

To test the efficiency of the coconut shell pyrolytic oil distillate, they performed a decay test as per BIS 4873: Part I: 2008 using rubber wood blocks. They adopted two methods of treatment: surface application and dipping. They inoculated the treated wooden blocks with a pure culture of white rot and brown rot fungus. After 16 weeks of inoculation, the scientists estimated weight loss in the blocks and compared it with that of the control blocks to assess the improvement in durability.

As the weight loss in the wooden block treated by the dipping method was significantly less than that of the brush coated and control blocks, the team concluded that the dipping treatment was effective against both white rot and brown rot fungus. Coconut shell pyrolytic oil distillate increased the resistance of rubberwood to wood decay fungi and consequently improved its durability. They also confirmed the decay resistant property of coconut shell pyrolytic oil distillate using FTIR spectroscopy.

The results indicate the potential of coconut shell pyrolytic oil distillate, a colourless liquid, as an effective wood protectant. Coconut shell is an abundant raw material. It is now up to entrepreneurs and industries to produce and market this technology for use.

Eur. J. Wood Wood Prod., **76**(2): 767–773

Reports by: **K. Siranjothi, S. Balaji, K. V. Srividhya, S. Suresh Ramanan, Manali Datta, Jinsu Varghese, Sileesh Mullasseri, G. Sharath Chandra** and **Saravanan Parameswaran.**

ACKNOWLEDGEMENT: IISER Pune, for access to scientific databases.

scienceandmediaworkshops@gmail.com