Science Last Fortnight

Geographic Mapping
Conserving Myrica esculenta

The *kaphal* tree, *Myrica esculenta*, found in the hilly regions of the Himalayas, is popular for its edible fruits. Different parts of the plant are reported to have analgesic, anxiolytic, antiallergic, antidiabetic, antimicrobial, antihypertensive, antiulcer, antioxidant and anti-inflammatory properties. Due to overexploitation for these benefits, the *kaphal* tree is now under threat.

Last fortnight, H. S. Ginwal and team from the Forest Research Institute, Dehradun mapped the species and its habitat to understand the distribution patterns of *M. esculenta* in the north-western Himalayas.

The team collected secondary data on the occurrence of the species in eleven districts of Uttarakhand, belonging to different agro-climatic zones. They used remote sensing and geographical information systems to measure parameters such as latitude, longitude, altitude, aspect, slope and bioclimatic variables. They analysed these variables using a maximum entropy model. Out of the well-distributed geo-coordinates, they used only approximately 30% of data for estimating and predicting distribution. The rest of the data was used to validate the model.

The team examined the most suitable environmental variables to model and predict the probability of species distribution. They found the species highly scattered across the region. They also observed that it prefers to grow on southern slopes and in mixed forests. The distribution of *M. esculenta* was limited to 1500 and 1800 m above sea level. It declines with increasing altitude.

The model shows that environmental variables such as temperature, seasonality and mean temperature of the wettest quarter, as well as slope and precipitation of driest month contribute in determining tree distribution. The team achieved up to 70% accuracy in predicting the distribution of *M. esculenta*.

'The model can be used to plan conservation. We need to manage this medicinally important tree', says H. S. Ginwal, FRI, Dehradun.

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Greenhouse Gas Mitigation
Improved agricultural practices

Agriculture contributes to about 18% of greenhouse gas emission in India. To mitigate such emissions, we need improved agricultural practices. However, to successfully adopt mitigation programmes at the grassroots, a cost–benefit analysis is required.

Image: Raju Karthic

Recently, T. B. Sapkota and colleagues from the CIMMYT, New Delhi collaborated with researchers from other institutes in India and abroad, to investigate agricultural practices for cost-effective climate change mitigation.

They analysed practices such as using neem-coated urea, improving water management in rice, zero-tillage, and no residue burning for cost–benefit ratio with emission savings, cost and expenses incurred.

The research team took crop production data from a household survey conducted by the Government of India, the IndiaStat website and the FAOSTAT Common Database. They gathered climatic information from the WorldClim global climate database.

The team collected state-wise details of livestock from the 19th livestock census of the Government of India as well as from data related to livestock management and emission from the National Dairy Research Institute.

These large datasets in crop and livestock management from all over the country formed the basis for quantifying total greenhouse gas emission from the agricultural sector and estimating the mitigation potential by the year 2030.

The team estimates gross national greenhouse gas emission from the agricultural sector at about five hundred metric tonnes of equivalent carbon dioxide. Livestock contributes nearly 60% and crop production, the rest. Buffalo production alone emits nearly one tonne of equivalent carbon dioxide, while one kilogram of rice gives more than three kilograms.

Maharashtra, Andhra Pradesh, Madhya Pradesh, Rajasthan, Uttar Pradesh and West Bengal are major contributors, report the researchers.

In popular perception, tree planting is a mitigation measure. However, the team finds that it is cost prohibitive due to the current price of carbon under carbon trading schemes.

They suggest that cost-saving practices, such as efficient fertilizer and water management, and zero-tillage, can annually mitigate more than 42 metric tonnes of equivalent carbon dioxide. The team recommends using appropriate policies, awareness mechanisms and incentive schemes for the widespread adoption of best practices by farmers.

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Palmarosa and Mycorrhiza
Solution for saline soils

Palmarosa, lemon grass, is an aromatic plant, grown for its essential oil. This oil is used in perfumes, cosmetics, flavours and pharmaceuticals. India exports nearly 40 tonnes per year, fetching good foreign exchange. Many farmers cultivate palmarosa. However, is it wise to grow aromatic plants at the cost of food crops? Can palmarosa grow in soils unfit for food crops?

Recently, Umesh Pankaj and team from the CICR-Central Institute of Medicinal and Aromatic Plants, Lucknow screened palmarosa varieties that thrive in saline soils. They report that two varieties, Tripta and Trishna, are promising for cultivation

in such soils. Trishna yielded 20% more essential oil when grown in saline, rather than in normal, soils.

The team investigated whether arbuscular mycorrhiza, a soil fungus, can improve essential oil productivity in degraded soils. They found that Tripta yielded 46% more essential oil in saline soils inoculated with *Funneliformis mosseae* than it did in normal soils. However, Trishna did not show any positive impact when inoculated with any of the four arbuscular mycorrhiza tested.

The team used gas chromatography-mass spectrometry, and found 28 different compounds in the essential oil. Geraniol was the principal compound at 80%. No significant effect was observed in the essential oil composition of varieties grown in normal or saline soils or with mycorrhizal inoculants.

It is estimated that 20% of agricultural land is affected by salinity. So, palmarosa, especially the Tripta and Trishna varieties, are a boon to farmers in salt-affected areas. Palmarosa, in other words, can be used to remediate sodic soils while providing economic benefits.

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Crab Hemolymph Microbiota

Probiotics for aquaculture?

Crab aquaculture is popular in coastal India. The Indian coast has around twelve crabs of local and export trade value. Crab farmers, however, face a problem – bacterial infections. Is there a way to avoid these infections?

Crab body fluid, hemolymph, reportedly contains many antimicrobial factors. Healthy microflora in crabs helps develop and maintain immunity, just as it does in us.

So, T. G. Sumithra and team from the ICAR-CMFRI, Cochin, recently investigated indigenous microorganisms in crab hemolymph. They collected hemolymph after surface sterilizing crab legs. Then, they cultured the hemolymph to analyse the microflora.

The researchers identified bacteria using microbiological tests and compared 16S rRNA sequences with sequences in databases. They identified 33 species from 14 genera.

The team found that Gram negative isolates predominated. *Vibrio* was abundant, followed by *Photobacterium*, *Staphylococcus* and *Shewanella*. They also report, for the first time, the presence of *Enterovibrio*, *Pantoea*, *Kluyvera* and *Enterobacter* in crustacean hemolymph.

The team then tested whether the bacterial isolates could inhibit the growth of aquatic pathogens. Most of the isolates inhibited marine pathogen growth. *Vibrio* species were the most commonly inhibited.

Is hemolymph microflora nature's way of priming the crab immune system against infections? Can they be used as probiotics in crab culture?

'In hemolymph, bacterial isolates and their numbers depend on the species. But the composition of microorganisms and their abundance in each species is yet to be documented', says T. G. Kishor, CMFRI.

'Using these strains as probiotics is another area to be explored', adds T. G. Sumithra, CMFRI.

This discovery of bacterial strains in marine crab hemolymph, even in apparently healthy conditions, might help predict, diagnose and control disease in commercial aquaculture.

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Fingerprinting Gut Microbiome

A profile of the healthy Indian

In recent years, scientists have found a correlation between gut microbiome and many medical conditions such as diabetes, obesity and even depression. Gut microbiome depends on what we eat, our lifestyle, hormonal conditions and other physiological and environmental factors. So, does unhealthy gut microbiome cause these illnesses or is it a consequence? To answer, we need to understand healthy gut microbiome. However, we lack a baseline microbial profile of the healthy gut, especially of Indian populations living in different geographies and eating diverse regional cuisines.

Last fortnight, Yogesh Shouche and team from the National Center for Cell Sciences, Pune reported tackling the issue. They profiled the healthy gut microbiota of people in Pune and Kolhapur, using a clone library sequence. For this, they extracted 16S rRNA from the faecal samples of 43 healthy individuals and sequenced them. The data consisted of about 9000 16S rRNA sequences.

Matching the data with available reference sequences, they found that healthy guts in the region harbour distinct species of the bacteria, *Prevotella* and *Megasphaera*. These bacteria were closely related, but differ from European and American strains.

Using bioinformatic tools, the team also found that about 30% of the genes were responsible for carbohydrate metabolism. They suspect this might be due to the sampled population's carbohydrate rich diet.

Even though using clone library sequences is reliable, it does not give a complete and accurate picture of bacterial communities. The presence of a large number of clones of the dominant bacterial species leads to overrepresentation. So the team plans to use next generation sequencing methods along with culture-based studies.

'Diversity in ethnicities, geographies and diets influences gut microflora. A pan-India microbiome mapping project would provide insights into features unique to Indians', says Yogesh Shouche, NCCS.

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Detecting Lung Cancer

Optimal deep learning model

Early detection improves chances for survival in cancer patients. But detecting lung cancer in early stages is difficult. Lung cancer is examined with CT scan images. Classification using CT scans is expensive and detects cancer only in advanced stages.

Recently, S. K. Lakshmanaprabu, from the B. S. Abdur Rahman University, Chennai, along with researchers from other Indian institutes and the University of Cauca, Colombia, developed an automated diagnosis method for lung cancer classification. Their model can detect

lung cancer in the early stages in CT scan images.

They used the International Early Lung Cancer Action Program database to develop the model. And analysed fifty low-dose whole lung CT scans, obtained in a single breath hold with a 1.25 mm slice thickness. These images were processed to remove noise and enhance contrast.

Then, the researchers extracted histograms, and texture and wavelet features with the different bands to reduce the volume of data by estimating positive properties. These minimal features were taken for classifying lung cancer.

The researchers used an optimal deep neural network classifier and dimensionally reduced extracted features using linear discriminant analysis reduction techniques to eliminate irrelevant features.

They compared the results with those from other existing classifiers. In terms of accuracy, sensitivity and specificity, the team found this model more than 94% proficient in recognizing cancer-affected parts in CT images. Automatic lung cancer classification reduces time and human mistakes in predicting the medical condition of lung cancer in new CT images. Detecting lung cancer early and with higher proficiency will be a boon to medical practitioners and patients.

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Jamun Jams Biofilm Formation

Identifying active ingredients

Pseudomonas aeruginosa and *Staphylococcus aureus* belong to a group of antibiotic-resistant pathogens. These bacteria produce biofilms that protect them from antibiotics and make them resistant to treatments. Scientists have been seeking biofilm inhibitors among plant extracts.

Last fortnight, Manabendra Mandal and team from the Tezpur University, Assam decided to look into the anti-biofilm activity of leaves of the Jamun tree, *Syzygium cumini*. They extracted phytochemicals from the leaves and found that the active principle is extracted efficiently from jamun leaves when ethyl acetate is used as solvent.

The team found that the extract did not directly affect pathogen growth. But there was significant reduction in biofilm formation, swarming motility and pigment production – attributes signalling pathogen virulence.

Using gas chromatography-mass spectrometry they identified the compounds in the leaf extract. The most abundant phytochemicals were 3-*n*-hexylthiolane s,s-dioxide, heptacosanoic acid and 3-methyl-2-(2-oxopropyl) furan. These they note, have bioactivities as per earlier studies.

The team used docking studies with these molecules and found that they dock well with biofilm-associated proteins, jamming their activities.

To rule out adverse effects, they performed cytotoxic assays with the extracts and found that cell morphology and viability were not affected.

These findings suggest that the *S. cumini* extract is a safe anti-biofilm agent. It can effectively reduce the virulence of *P. aeruginosa* and *S. aureus*, difficult to treat hospital-acquired infections.

The extract sounds promising for managing the biofilm menace in clinical and food industry settings. However, further tests are needed before it is accepted by regulatory bodies.

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Multiple Instance Learning

For breast cancer detection

Since the advent of histology in the 19th century, physicians and pathologists have peered down microscopes to look at tissue structures. Histopathology, microscopically examining diseased tissue, soon became a sought after technique in medicine.

Gaining traction, it became the gold standard in diagnosis. Almost two centuries later, with a few incremental upgrades, medical professionals still use it to stain and study histopathological samples.

One big hurdle that plagues histopathology is the sheer magnitude of images from a single patient. Analysing and labelling each is highly time-consuming and specialized, vulnerable to errors caused by fatigue and decreased attention.

Last fortnight, P. J. Sudharshan, from the Indian Institute of Information Technology, Jabalpur, in collaboration with scientists from France and Brazil, published a study which might help alleviate this problem.

Conventionally, single instance labelling is done at individual level, one image at a time. The team used a multiple instance learning framework that works on the principle of organizing instances or images into bags or patients, each bag containing a set of partially labelled instances.

The algorithm then infers a prediction for test images, based on complementarity to the images, from the training dataset.

The researchers used a two-fold approach. They checked whether multiple instance learning is better than conventional labelling. Then they worked out which method works best for breast cancer diagnosis.

They analysed 12 state-of-the-art multiple instance learning methods on BreaKHis, a publicly available dataset of around 8000 microscopic biopsies from 82 patients. Out of the 12 methods, most surpassed the rates of conventional single instance classification. Non-parametric multiple instance learning and multiple instance learning-convolutional neural networks were highly efficient, with classification rates of up to 92% – an unprecedented feat.

The report shows how machine learning can overcome a major bottleneck in dealing with huge datasets in histopathology and can relieve the workload on pathologists.

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Re-using Natural Dyes

A boon for textile industries

Synthetic dyes are carcinogenic as well as a threat to the environment and are, therefore, banned by several countries. Hence, using natural dyes has gained popularity.

Now, henna is a colouring agent that is also reported to provide UV protection. And it is antibacterial and insect repellent.

Used henna dye effluent still retains colour and is not fully exhausted. Large quantities of such effluent cause ecological damage.

Now, Ankit Sharma and team from the IIT, Delhi report reusing unexhausted henna for up to four dyeing cycles in linen fabric.

After each cycle of reuse, the effluent load reduced due to drastic reduction in total dissolved solids, chemical oxygen demand, conductivity and redox potential, says the team.

They also observed an acceptable level of colour and rubbing fastness in the fabric. Though henna dye has less colouring ability after four cycles, it retains functional properties, they say.

The team observed enhanced free radical scavenging properties and UV protection in the dyed fabric. They attribute this to the presence of lawsone and other polyphenolic compounds in henna, which effectively absorb UV-light.

These findings can be economical for dyeing industries and reduce the use of freshwater.

To conserve water resources and keep the environment clean, this effective method suggested by the team can easily be adopted by textile wet processing industries.

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Controlling Air pollution

A comprehensive study

Air pollution is on the rise in recent decades. Pollutants such as O_3 and particulate matter in the atmosphere make Indian cities some of the most polluted in the world. Rapid increases in O_3 and $PM_{2.5}$ levels, as well as prolonged exposure to such compounds, have directly influenced mortality rates in India.

Many studies indicate that inorganic particulate matter accounts for 80% of $PM_{2.5}$ in the atmosphere. However, the previous studies failed to provide comprehensive strategies by examining all possible pollution settings nationwide. So, Indian researchers collaborated with scientists from the USA and China, to investigate 14 possible pollution scenarios and their potential health hazards. To do this, they examined various pollution scenarios including emissions in the energy sector, emission due to residential activities, agricultural activities and industrial emissions.

The team used the Community Multi-scale Air Quality model, an open source suite of programmes for air quality simulation. They also used SAPRC-07, software that analyses how primary pollutants interact to form secondary pollutants such as ozone and other oxidants.

The team find that switching from solid fuels to kerosene in the residential sector significantly reduces particulate matter and ozone. Reducing diesel generating sets decreases NO_x by 50% and ozone by three parts per billion. The results also show that $PM_{2.5}$ levels can be reduced by changing brick kilns to zigzag kilns in the industrial sector.

The control strategies can reduce premature mortality rate by 0.69 million and years of life lost by 44%. However, as this study does not consider the effects of meteorology and socio-economic changes, there is scope for improving the estimated strategies, admit the researchers.

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Efficient Oil Refining

Reducing fuel costs

Shifting to cleaner, renewable sources of energy is a gradual process which will take time. Meanwhile, we need to invest in efficient ways to use available energy sources.

A team led by V. Subramanian, from the National Institute of Technology, Calicut, along with collaborators from institutes in Mysuru and Bengaluru, have now come up with an efficient process for crude oil distillation. Crude oil mixture is extracted from the earth and distilled to produce diesel, LGP, gasoline and other fuels. Distilling crude oil in oil refineries is a major cost and energy consuming step. In traditional distillation, the crude oil mixture is heated to very high temperatures and fed into a single large column.

In this column, different components are separated, based on their volatility. More volatile components boil faster and rise up the column. Less volatile components are collected at lower heights of the column.

In the alternative method proposed by the team, instead of a single large column, distillation is carried out in a chain of columns connected to each other.

The first column is heated to temperatures that separate only highly volatile components, which require less heat. They are separated in the first column. The remaining mixture is fed into the next connected column.

Here, the temperature is higher than in the previous column, separating the next lightest component from the crude oil mixture. In such series of columns, all components are separated out from the crude oil mixture.

The researchers ran simulations using the Aspen HYSYS program, available for optimizing the oil refining process. The alternative method reduces heat required for distillation by almost 10%, and increases the purity of the components separated, thus making the whole process cheaper and more efficient.

With the rising cost of oil-based fuel, this alternative distillation process should help policy makers control fuel prices while making the whole process more efficient.

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