

Science Last Fortnight

Artificial Rain

Varshadhare in Karnataka

The state of Karnataka is divided into two by the Western Ghats. The eastern part receives little rain and there are pockets where the monsoon is often deficient. In 2017, the state government launched a massive cloud seeding programme called Varshadhare. Last fortnight, scientists from the Indian Institute of Tropical Meteorology, Pune reported evaluating the programme.

The seeding plan targeted areas on the basis of soil moisture as derived from satellite data. The lower the soil moisture the greater the need for rain.

Since earlier data indicated rain in the early morning, peaking at about 4 a.m. and higher amounts in the afternoon at about 4 p.m., the operational plans started at 8 a.m.

The weather forecast of the day is taken into account, to plan target areas. The plan is communicated to the Air Traffic Control at the Hubballi airport by 9 a.m. Hubballi has less air traffic than the Bengaluru airport.

By 2 p.m., the pilots are ready on the runway. The presence of clouds and their predicted movements are confirmed by radar data. The pilot and the meteorologist on board get their brief.

Up in the air, the team on board goes under the targeted cloud and circles through the cloud to gather *in situ* data on updrafts and liquid water.



Image: STEAMindia Reports

The scientists used two types of cloud seeding. On top of vertically high clouds that have water below freezing point, silver iodide is useful to provide nuclei to form ice crystals. In clouds with sufficient moisture, calcium chlo-

ride, a hygroscopic substance that attracts moisture to form water, is better. The plane carries 12 flares of each. Depending on the data, the right amount and kind of seeding is deployed. And the plane veers off to the next target site.

The cloud that is seeded is monitored on radar by the ground team. They can 'see' the transformation inside the cloud since the water content reflects the radar waves. Rain may start 15 min after seeding and perhaps last for 4 h. But, since the cloud keeps moving, the rain comes down about 10–20 km on an average, from the point of seeding.

Karnataka has about 6000 rain gauges. So a 5 km resolution of rainfall data can be measured through the rain gauge network, capable of collecting data every 15 min. This, says J. R. Kulkarni, has given data that was not possible to collect earlier.

Seven hundred and eighty-one taluks were thus seeded. Comparing station levels after four hours of cloud seeding with rainfall before cloud seeding, the team found that 618 showed an increase in rainfall – nearly 28% above natural rainfall.

The scientists also analysed natural rainfall that occurred without cloud seeding using floating control-target area rainfall analysis. The mean increase in rainfall is 1.2 mm above natural rainfall, which amounts to something like 28% at regional average rainfall.

The mean increase in the method using calcium chloride was about 12 mm/target-area, whereas, in the method using silver iodide, it was approximately 20 mm/target-area. The scientists say that the higher performance is due to the release of the latent heat of freezing by super cooled water in bigger clouds.

Cloud seeding is useful in drought. The reported efficiency is sufficient to improve agricultural production. The scientists say that Karnataka is planning a two-year programme from 2019.

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Coastal Reservoirs

Storing flood waters

India gets about 4000 billion cubic metres of rain every year. Though the southeast monsoon stretches from June to September, most of the actual rainfall occurs within a span of two weeks. So flooding is quite common. And the floodwaters drain off through the network of both perennial and seasonal rivers. Before India manages to heave a sigh of relief after floods, it's time for the dry season where there is not enough water for irrigation and for drinking.

India has about 4800 dams, mostly built between 1970 and 1989. Environmental concerns have slowed down the construction of storage facilities for water.

Considering that about half the rain water received over land drains into the sea, Sreevalsa Kolathayar from the Vellore Institute of Technology and T. G. Sitharam from the Indian Institute of Science (IISc), Bengaluru have come up with a solution: Harness excess river floodwater by constructing impermeable sea dikes and creating coastal reservoirs. This, they say, is more cost effective and environment friendly than making dams, desalination plants and interlinking rivers to provide water for the needy.

A three-sided impermeable sea wall, constructed in the seabed, creates a reservoir to store flood waters. And one or two barrages with sluice gates at the river mouth to let water in during high tide. If it rains excessively during low tide, the waters can be discharged into the sea.

'India is not running out of water, but water is running out of India', says T. G. Sitharam.

No submergence of precious land, no displacement of people. But enough fresh water for the seventy-odd coastal districts.

The concept is not entirely new. Though more primitive in design, the Thanneermukkom Bund constructed in the 1970's that divides the Vembanad Lake into a freshwater lake fed by rivers and a brackish water lake

fed by ocean currents, is proof of concept. Singapore, Hong Kong, China and the UK also have their models to show that it is feasible.



Satellite view Thanneermukkom Bund
Image: via Wikimedia Commons

Irrigation and drinking water in the region for Saurashtra and the Central Gujarat regions of India will soon be provided for by the Kalpasar Project, as per the Gulf of Khambhat development plan. Feasibility studies have been conducted for a coastal reservoir in Mangaluru in Karnataka and in Kollam, Kerala.

Given that India has more than 7000 km of coast, it can be garlanded with such freshwater reservoirs, say the researchers.

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Bacteria from Fish Gut

One fish is probiotic for another

Disease outbreaks often lead to losses for the aquaculture industry. Antibiotics to combat infections lead to the development of drug-resistant pathogens, further aggravating the problem. It is simpler to improve disease resistance in fish. This can be achieved by using probiotics to improve fish health.

Probiotics for humans are now well known. But what microorganisms can serve as efficient probiotic agents in fish? Recently, researchers from the Central University of Kerala, Kasaragod collaborated with researchers from the Mahatma Gandhi University, Kottayam to look for probiotics among fish. And they found a bacterial strain, *Bacillus licheniformis* HGA8B, in the gastrointestinal tract of the climbing perch, *Anabas testudineus*. They decided to test it on Nile tilapia, the world's second largest cultivated fish.

The team formulated a feed supplemented with two different concentrations of the bacterial culture. After feeding the fish with this formulation

for 60 days, they observed better growth and feed utilisation in fishes supplemented with higher concentrations of the probiotic.

When they tested the activities of digestive enzymes, it was found that the diet supplemented with probiotic bacteria did not enhance the activities of digestive enzymes. So how did the fish grow so well?

When they examined antioxidant enzymes and lipid peroxidation in the liver and intestine, they found that the production of these enzymes had increased.

Then, they studied immune and growth-related gene expression. And found that the probiotic supplement enhanced the expression of immune-related and growth-related genes.

Thus the researchers have come up with a solution to improve the immune system and minimise losses due to infections in Tilapia. But how about other aquaculture fishes? Can diet supplemented with this *Bacillus licheniformis* strain improve the health and productivity of other farmed fishes? More research is awaited.

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Diabetes Risk

Vitamin D deficiency?

In 2015, according to a WHO report, about 70 million in India suffered from type II diabetes – a condition where body cells become resistant to insulin. The number is expected to increase. So it is important to figure out how to tackle it.

Recently, Neetu Mishra and team from the Symbiosis School of Biological Sciences, Pune collaborated with the Madhunayani Diabetes and Eye Laser Centre, Pune to report a correlation between type II diabetic patients and their vitamin D levels. Vitamin D has been shown to play a role in biochemical pathways involving insulin receptor genes.

The team collected data for over two years from a group of diabetic patients and a non-diabetic control group. They found that the diabetics had higher body weight, blood pressure, and blood sugar levels – symptoms common to diabetics.

Interestingly, they also found a correlation between vitamin D levels and type II diabetes. Seventy per cent of diabetic patients had vitamin D deficiency, while it was only forty per cent in the control group.

'Our results show that a person with severe vitamin D deficiency has about forty times higher risk of being diabetic', says Neetu Mishra.

Since type II diabetes is becoming very common, people need to be made aware of ways to control it – physical exercise, proper diet and a little bit of sunshine in your life.

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Septic Shock – Child Death

Caused by vitamin D deficiency?

Septic shock or sepsis is a potentially life-threatening condition caused by the body's response to an infection. The body releases many chemicals into the bloodstream to fight infection. But when the balance of these chemicals is changed, it leads to blood poisoning. Many organs are affected and death can occur.

In the new born, septic shock is often fatal. According to the WHO, 80% of deaths in children under four are caused by sepsis-related illness.

Recently there have been reports suggesting that vitamin D plays a major role in maintaining a healthy immune system. Vitamin D is necessary for producing defensins and cathelicidins, proteins which aid the body's innate and adaptive immunity against various infections. However, does vitamin D deficiency contribute to the development of sepsis?

A team from the All India Institute of Medical Sciences, New Delhi, collaborated with researchers at the Ram Manohar Lohia Hospital to assess the relationship between sepsis and vitamin D deficiency in children under 17.

They found that 72% of children admitted to hospital for septic shock were severely deficient in vitamin D. And children with severe vitamin D deficiency were unable to recover from septic shock. Planning interventional studies in children with septic shock and severe vitamin D deficiency may reduce child mortality, say the researchers.

These results support previous studies which demonstrated that vitamin D deficiency significantly increases infection rate and mortality risk among critically ill adults with sepsis. Recent studies, including this study, demonstrate that vitamin D deficiency is associated with the development of cardiovascular diseases, autoimmune disorders, and certain types of cancers. Now, in a country like India with adequate sunshine, vitamin D deficiency should be largely preventable.

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Molecular Basis of Pain Relief *Testing an ayurvedic formulation*

Peedantak Vati is a pain relieving ayurvedic formulation containing a total of 23 herbal ingredients! However, can the formulation stand up to the molecular mechanistic yardstick of modern science? Recently, Ravikant Ranjan and team from the Patanjali Research Institute, Haridwar looked into the formulation's molecular mechanism of action using animal cell culture and rat models.

Using animal cell lines Ravikant and team found that the formulation reduced the release of nitric oxide – an inflammatory mediator. It also significantly reduced the release of pro-inflammatory cytokines.

In a carrageenan-induced paw oedema rat model, the *vati* significantly reduced paw inflammation after 3 h of treatment at an oral dose of 200 mg/Kg. With an oral dose of 400 mg/Kg, oedema reduction was significant after 2 h.

The team assessed the pain relieving activity of the polyherbal formulation using four animal models. The results of hot plate and tail flick tests show that the formulation exerts a pain relieving effect via a supraspinal and spinal mechanism.

The researchers used a writhing test model and observed significant peripheral pain relieving activity by the *vati*, which could be due to its action on cyclooxygenase enzymes.

They also chose a formalin test in an animal model due to its biphasic activity. The early phase involves neuropathic pain while the late phase involves pain due to inflammation.

The researchers observed that the *vati* displayed a marginal effect on the first phase and a prominent effect on the second phase of pain.

They also developed high performance liquid chromatography, high performance thin layer chromatography, and liquid chromatography mass spectrometry methods to analyse quality consistency in the production of the formulation.

The team has provided clues about how the formulation may work. But given the relationship between the research team and the producers of *Peedantak Vati*, and the consequent questions related to conflicts of interest, the experiments may need repetition in other labs.

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Digital Differential Count *Hope for haematology*

The differential blood count – the number of red blood cells, leukocytes and platelets – reveals an individual's overall health status. A small sample of blood, from a prick on your finger, is smeared on a microscope slide. Then the pathologist crouches over a microscope, counting the number of each type of blood cell. After counting the cells in a few grids, the pathologist calculates the numbers by extrapolation. After all the time and effort taken, the subjective evaluation and the extrapolation are prone to errors. Is there a way to reduce the time and effort spent to get more accurate results?

A team from the Manipal Academy of Higher Education collaborated with researchers from the Nitte Mahalinga Adyanthaya Memorial Institute of Technology, Manipal to report an efficient automated method for leukocyte detection which works under different colour and illumination variations.

Leukocytes are particularly difficult for automatic detection since they come in various shapes. So the researchers started from the nuclei. Nuclei are easier to detect, being darker. The researchers made it a tad easier by mapping the red colour to green.

'We used G'G'B representation of original RGB images to highlight nuclei regions', says Roopa B. Hegde, who has used the trick in earlier research.

This shift highlighted all the nuclei. Using a combination of arithmetic operations and Zack's thresholding, nuclei could be detected and therefore counted.

Now, leukocytes differ in shape and size. Hence, the team used an active contours method to classify them: Starting from the nuclei, mask the area with increasing radii. The active contours can easily be identified, again using the Zack's threshold method. Though the cell outlines are not that distinct, the method works.

'We obtained an average Dice similarity coefficient of 0.97. The coefficient compares algorithm output against reference masks. And the sensitivity is also high – nearly 0.96', says Keerthana Prasad, MAHE, Manipal.

Brij Mohan Kumar Singh, Kasturba Medical College, Manipal is happy. An automated method to locate cells correctly irrespective of colour, illumination, brightness and staining variations, makes pathology lab work easier.

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Plant DNases *For snake bite necrosis*

The saw-scaled viper is a venomous snake. Even if anti-venom is given, its venom induces local tissue necrosis.



Image: via Wikimedia Commons

Since the anti-venom fails to treat this problem, traditional healers use medicinal extracts to try to treat it. One such herbal medicine is red ball snake gourd, *Trichosanthes tricuspidata*. Its anti-inflammatory properties are well known. But is there scientific evidence that it can act against snake bite?

Last fortnight, researchers from the University of Mysore tested whether plant extracts had properties that

could neutralize *Echis carinatus* venom¹. Though the venom itself is a mixture of proteins, the victim's immune system releases DNA and that is what leads to the cascading pathology of tissue necrosis. So the team screened aqueous leaf extracts of well-known medicinal plants from the Western Ghats. They fractionated and purified the proteins in the extracts. Out of all the medicinal plants, they found that a protein from *T. tricuspidata* showed potent DNase activity.



Image: Lalithamba via Wikimedia Commons

The researchers subcutaneously injected lyophilized powder of saw-scaled viper venom into the tails of mice. They recorded the level of injury every day for ten days.

'To evaluate the effects of the plant DNase at the region of necrosis, we injected different fractions of *T. tricuspidata* DNase after 30 minutes of venom injection' says G. V. Rudresha, University of Mysore.

After ten days, they stained the excised mice tail tissue and found that the DNase enzyme reversed venom-induced tissue necrosis and retained normal tail tissue morphology in mice. When they examined collagen fibres in the tissue, they found that the *T. tricuspidata* DNase prevents venom-induced collagen depletion in connective tissues.

'We compared the pharmacological actions of the plant DNase and the commercial enzyme – DNase1. And we found that red ball snake gourd could indeed complement anti-venom therapy in treating rats with snake bite', says B. S. Vishwanath, the leader of the team.

Considering increasing reports of death and permanent disability from snake bite in India², plant DNases and their protective role against venom-induced tissue necrosis makes sense. However, clinical trials are required before doctors can prescribe the therapy.

¹DOI: 10.1002/jcb.28115

²DOI: 10.1371/journal.pntd.0001018

Hiking Entry Fee Saving indigenous forests

Kodagu or Coorg in Karnataka, a hill station on the eastern slopes of the Western Ghats, is a biodiversity hotspot. The tropical forests of Kodagu house around 280 species of indigenous trees and animals – elephants, spotted deer, macaque.

Coffee, the major cash crop of this region, is traditionally grown in the shade of indigenous trees. The coffee plantations with forest-like dense native tree cover are corridors for wild animals between adjoining protected forests or national parks. The plantations could play a major role in conserving plant and animal species native to this region.

But developmental projects, expansion of plantations and excessive tourism have led to an alarming decline in forest cover and subsequent loss of biodiversity in Kodagu over the past decades. To provide shade in the plantations, many coffee growers now grow an exotic tree species, the silver oak, in place of native trees, leading to a loss of native tree diversity. Silver oak is commercially more viable for farmers as it is easier to grow and yields timber. However, these plantations now have less tree cover and fewer wild animals pass through.

Recently, Kavita Sardana, a professor at the TERI School of Advanced Studies, New Delhi decided to find ways to overcome the problems. After discussions with local coffee growers, her team realized that, when farmers plant native trees instead of silver oak, they have to give up income from selling silver oak timber. Compensating for this loss of income may

encourage farmers to grow native trees in their plantations again.

She wondered whether the tourists would pay an increased entry fee to national parks. The national parks and wildlife sanctuaries of Kodagu attract many tourists. The hike, she reasoned, could be used to restore native trees in the agro-forestry of coffee plantations outside protected areas.

So she conducted a survey among tourists who visited the three national parks in Kodagu between January and June 2016. Sardana's team told them about the benefits of native tree cover in coffee plantations surrounding national parks and why plantations were resorting to planting exotic trees.

Participants were not just asked whether they were interested in paying a higher entry fee to compensate local coffee farmers. They were also told how this system would work. The forest department would introduce a surcharge on the entry fee to Kodagu's national parks and sanctuaries. This money would be transferred to coffee farmers through local authorities. The participants felt that their contribution could be significant in conserving the biodiversity of this region. On an average, they were ready to contribute around 200 rupees as surcharge for conserving native trees in plantations.

According to Sardana, this model of using tourism revenue from protected forest areas to restore native trees could be replicated in other coffee growing regions in southern India or tea plantations in the north east. She hopes such case studies could, one day, inform policy decisions regarding forest conservation.

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