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Editorial

“You can hear the dynamics of the glacier unfolding.”

—Matthew Burtner

Greetings on the New Year!!

A series of severe floods ravaged Kerala in July and August 2018. The disaster should be a clarion call that any developmental models the state adopts here on out must to take the vagaries of the climate, which have become the new norm, into account, and strive to make human progress without triggering ecological disaster- a Vision of New Kerala.

The year 2018 marks the 14th year of publication of Scientia (ISSN: 0976-8289), an annual Peer Reviewed National Science Journal from Mercy college, Palakkad. We are happy to bring out this issue of Scientia which features 11 articles from various areas of science.

We are delighted to present this issue with an opening article on *Boerhaavia diffusa* L Nephroprotective Activity in Drug Induced Nephrotoxicity Scenario and interesting contribution on the injudicious use of vehicles on climatic pattern.

With this new issue, we will be contributing on the Studies on the physico-chemical parameters of mangrove vegetations after deluge in Thrissur, Kerala, India. An interesting report on identification of sex-specific markers in *Calamus brandisii* of Western Ghats was described. Study on distribution profile of angiosperms across elevation discontinuity of Palghat Gap in Southern Western Ghats provides an added attraction of this issue. Comb building behaviour of worker bees during colony division reminds the social life of Insects.

A narration of Lip print patterns of human population of Palakkad was attempted. Another study focuses on seed borne fungi associated with some stored seeds and their bio-control by aqueous medicinal plant extract. Group effort on comparative study of proximate composition and nutritional status of major fresh water food fishes during post monsoon season in the culture and capture fisheries of Alappuzha District was an added knowledge in this issue.

We invite your continued support and contribute articles/news items of interest/research papers etc on science.

With warm personal regards

Dr. Jayasree S.

Chief Editor

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Nephroprotective Potential of *Boerhaavia diffusa* L. in Drug Induced Nephrotoxicity Scenario

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Abstract

Kidney is considered as a major target organ for exogenous toxicants. Drug induced nephrotoxicity is more common in patients receiving certain medication and undergoing certain specific clinical treatments resulting in deterioration of kidney functions. Some of the common nephrotoxic drugs include aminoglycosides, sulphonamides, NSAIDs, anti-cancer drugs which further leads to acute kidney injury. *Boerhaavia diffusa* L. is used in herbal medicines as well as functional food in many countries such as Vietnam, Philippines, Brazil and India. This medicinal plant contains phytochemical constituents such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoproteins. The plant has been used in the treatment of nephrotic syndrome and urinary disorders. Ayurvedic literature indicates that *B. diffusa* improves the function of impaired kidneys and also helps the normal kidneys expel the excess fluid out of the body thereby illustrating its diuretic and possible nephroprotective effects against drug induced nephrotoxicity. Administration of compounds with antioxidant activity used for ameliorating drug induced nephrotoxicity have shown positive results in several experimental models but have either failed to show constant amelioration or proved ineffective when used for a long term. Given these limitations of modern medicine, plant based therapeutics can be considered a better alternative.

Keywords: *Boerhaavia diffusa*, *Punarnava*, Nephroprotective, Nephrotoxic drugs, Acute kidney injury.

Introduction

Kidney performs several important functions such as the maintenance of homeostasis, detoxification, and excretion of toxic metabolites and drugs and thus inescapably exposed to high concentrations of both endogenous and exogenous toxins.¹ One of the underlying causes of nephrotoxicity is the usage of nephrotoxic drugs. Gentamicin, cisplatin, non-steroidal anti-inflammatory drugs, ifosfamide-induced acute renal failure resembles the renal failure resulting from administration of drugs in the clinical set-up leading to complications. Some other nephrotoxic drugs include aminoglycosides, sulphonamides, amphotericin-B, neomycin, polymyxin, chloro-tetracyclines, ibuprofen, acetaminophen, heavy metals (lead, mercury, uranium and arsenic), anti-cancer drugs (cyclosporine, cisplatin

and cyclophosphamide), which may lead to acute kidney failure.^{2,3} Intrinsic renal damage due to cytotoxins is a common cause of acute kidney injury (AKI). AKI is characterized by the sudden impairment of kidney function resulting in the retention of nitrogenous and other waste products normally cleared by the kidneys. Nephrotoxicity can be diagnosed through blood test and the levels of blood urea nitrogen (BUN), concentration of serum creatinine, glomerular filtration rate and creatinine clearance are used as parameters for evaluating nephrotoxicity.

Drug induced nephrotoxicity mechanism

Drugs can exert direct toxic effects on renal tubules, inducing cellular injury and death in acute tubular necrosis, or induce inflammation in the renal interstitium

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in acute interstitial nephritis (AIN). General mechanisms leading to nephrotoxicity include changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. Nephrotoxicity is enhanced by the positive charge of polycationic aminoglycosides, which are attracted to the negatively charged proximal tubular membrane phospholipids. This facilitates drug binding to the megalin/cubilin receptor complex. Medications and endogenously produced substances compete with each other for transport proteins and influx/efflux transporters, which may increase intracellular drug concentration and risk for kidney injury.^{4,5,6} These drug-drug interactions increase kidney injury and overall drug toxicity. Accumulation of high concentrations of the polycationic aminoglycosides within intracellular lysosomes causes lysosomal injury, which is associated with phospholipid membrane injury, oxidative stress, and mitochondrial dysfunction. This promotes proximal tubular cell apoptosis and necrosis with clinical manifestations such as an isolated proximal tubulopathy.^{7,8} The lipid/liposomal formulations to a lesser degree, cause kidney injury by disrupting tubular cell membranes and increasing permeability to cations, which result in tubular dysfunction due to cell swelling/dysfunction. The polymixin antimicrobial agent induced nephrotoxicity is related to their D-amino content and fatty acid component, which increases cellular membrane permeability and allows cation influx.⁹ This effect causes tubular cell swelling and lysis followed by AKI development. The acyclic nucleotide phosphonates (adefovir, cidofovir) enter the cell *via* basolateral human organic anion transporter-1 (hOAT1-) and promote cellular injury primarily through disturbing mitochondrial function. Mitochondrial injury is manifested by mitochondrial enlargement, clumped cristae, and convoluted contours that impair cellular energetics. Tenofovir, which is employed widely to treat hepatitis B virus and HIV infection, is associated with proximal tubulopathy and AKI.^{10,11} Reduction in VEGF levels or signaling pathways

by antiangiogenic drugs promotes loss of the healthy fenestrated endothelial phenotype and promotes microvascular injury and thrombotic microangiopathy, causing proteinuria and AKI. By interfering with local alternative complement pathway regulators, these drugs may also activate complement and increase risk for thrombotic microangiopathy.

Researchers have tested different approaches like atrial natriuretic peptide, low dose dopamine, endothelin antagonists, loop diuretics, prostaglandin analogues, sodium bicarbonate, and α -lipoic acid to manage AKI. However, the current treatment of AKI is still pragmatic.^{12,13,14} Though these agents have shown favorable results in several experimental models of ischemic or nephrotoxic AKI, they have either failed to show consistent benefit or proved ineffective when used therapeutically for a long term.^{15,16,17} Owing to the limitations of these agents of modern medicine, researchers are exploring the traditional system of medicine for compounds that are already being used by ayurvedic physicians for treating patients having impaired renal function.¹⁸

B. diffusa in Ayurveda

The science of Ayurveda does not describe the kidney disorders in terms of acute or chronic renal failure. However, it mentions disorders of renal function in terms of various symptoms and signs like dysurea (*Mutrakrucchra*), suppression of urine (*Mutraghata*) and retention of urine (*Mutravrodha*). *B. diffusa* alone or in combination with other herbs has also been used to relieve edema (*shotha*) of several causes. *B. diffusa* is a part of several polyherbal preparations such as *Vidarighrita* and *Bhadravahaghrita* which are used to relieve symptoms presumably occurring due to renal dysfunction.¹⁹ As per the Ayurvedic literature, it is believed to rejuvenate the urinary system. Ayurvedic texts also mention that *B. diffusa* improves the function of impaired kidneys and in edematous conditions, it helps the normal kidneys expel the excess fluid out of the body very effectively.²⁰ Various experimental

studies have also illustrated its diuretic and possible nephroprotective effects against acetaminophen-induced renal damage. However, the exact mechanism of diuresis and nephroprotective potential has not been understood.

B. diffusa : An Overview

Boerhaavia is a highly polymorphic genus of Nyctaginaceae also known as four-o'clock family because most of the species open their flowers four hours after noon i.e. in early evening or morning. It is commonly known as *Punarnava* in Sanskrit, which means 'renews the body'. Nyctaginaceae constitutes 391 species across 32 genera. Most *Boerhaavia* species possess worldwide medicinal uses and hence occupied positions in different systems of medicine including Indian Ayurveda, Siddha and Unani, African medicine, traditional Chinese medicines as well as Brazilian traditional medicine. Six important species i.e; *B. diffusa*, *B. repens*, *B. chinensis*, *B. erecta*, *B. elegans* and *B. reniformis* are found in India. *B. diffusa* shared about 131 compounds out of 180 compounds isolated from this genus and for most of these compounds, it is currently an exclusive source.²¹ The compounds from *Boerhaavia* genus include characteristic chemoalkaloids, quinonolizidine alkaloids, flavonoids, phenolic glycosides, phenolic acids, sterols, organic acids, triterpenoids, lignins and glycoproteins. Besides nephroprotective and diuretic activity, *B.diffusa* also possesses antioxidant, immunomodulatory, anti-cancer, anti-diabetic, hepatoprotective, anti-inflammatory, anti- fibrinolytic, antimicrobial, spasmolytic, anti-asthmatic and anticonvulsant activities.

Taxonomic description

Punarnava is a herbaceous perennial with a large root and highly branched stems that are prostrate or ascending to a height of up to a metre. The leaves are simple, ovate-oblong, acute or obtuse at the tip and rounded or subcordate at the base, glabrous above, white with minute scales below. The small rose or white coloured flowers are borne in small umbels arranged

in corymbose, axillary and terminal panicles, giving way to a detachable indehiscent seed with a thin pericarp.²⁰

Systematic Position

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Caryophyllidae
Order:	Caryophyllales
Family:	Nyctaginaceae
Genus:	<i>Boerhaavia</i>
Species:	<i>diffusa</i>



Fig.1. *Boerhaavia diffusa*

Source: Plant Description bioinfo.bis.res.in

Geographical distribution

Boerhaavia species are widespread and their dispersal is aided by birds and human activity. The distribution of *Boerhaavia* species is in the warmer parts up to an altitude of 2000 m. Besides this, they are found in disturbed areas, waste places, roadsides, dry pinelands, among scrub on tropical reefs. Although native to India and Brazil, *B. diffusa* is found in the tropical, subtropical and temperate regions of the world.²¹ This suggests that the worldwide distribution of *B. diffusa* may have helped in establishment of its broader ethnomedicinal

spectrum. In India, *Rakt punarnava* (*B. diffusa*) is known to possess more medicinal importance than *Shweta punarnava* (*B. erecta*).²²

Nephroprotective Activity

The effect of aqueous ethanolic extract on *E. coli*-induced acute pyelonephritis in rats at a dose 50mg/Kg p.o. administered twice orally showed 42.85% decrease in number of animals showing signs of renal changes. Administration of the extract (50mg/Kg p.o.) twice orally showed 99.09% decrease in bacterial count per mL of urine.²¹ The study on antioxidant potential of BD extract in urinary stones by means of inhibition of oxidative trauma and kidney cell damage and showed decrease in calcium oxalate deposition.¹⁸ Studies approving diuretic and kidney stone dissolving properties of BD extracts along with the isolation of a diuretic alkaloid, punarnavine, describe the use of BD in urinary disorders. The studies in mercury chloride toxicity rats demonstrated that 200 mg/kg b.w. aqueous leaf extract of *B. diffusa* was given orally for 5 days effectively protect kidneys from damage.²³ In eupalitin-3-O- β -D-galactopyranoside treated Koi carp (*Cyprinus carpio*) fish, the levels of urea, creatinine and marker enzymes were normal and no pathological changes in renal tissue were observed.²⁴ Although these studies lack of positive controls, their results suggested the traditional significance of *Boerhavia diffusa* in urinary disorders. A recent study in gentamicin-induced nephrotoxicity rats was carried out in two parts for different parameters. Among five different groups of rats in each part, a positive control group received α -lipoic acid in 0.5% CMC while test groups received 200

mg/kg and 400 mg/kg of aqueous extract of *B. diffusa* orally for 10 days. Assessment of parameters such as blood urea nitrogen (BUN), serum creatinine level, kidney malondialdehyde (MDA), and glutathione (GSH) levels, kidney injury on histopathology. However, their results demonstrated that *B. diffusa* did not show significant improvement in PAH clearance, which was reduced due to gentamicin damage. The results of the study showed that, aqueous extract of *B. diffusa* showed comparable results with positive control. *Boerhavia diffusa*, at a dose of 100 μ g/ml, protected oxidative damage against quinolinic acid (QA), 3-nitropropionic acid (NPA), sodium nitroprusside (SNP), and Fe (II)/EDTA complex induced oxidative stress in rat brain homogenates.²⁵ *B.diffusa* has shown nephroprotective potential with special relevance to its antioxidant mechanism of recovery from renal damage.²⁶

Discussion

Previous experiments have evaluated the nephroprotective effects of *B.diffusa* crude extract against various drug induced nephrotoxicity models and have suggested *B.diffusa* to be an effective plant based therapeutic for amelioration of renal damage. However, the specific mechanisms governing these therapeutic effects and similarities and or differences in their mode of action under both *in vivo* and *in vitro* conditions are yet to be studied in detail. Additionally, isolation and purification of new bioactive compounds with therapeutic properties have to be further explored in order to utilize the medicinal properties of *B.diffusa* to the fullest.

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A review on influence of injudicious use of vehicles on climatic pattern

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Abstract

In spite of the various measures taken by the government at meticulous level, the quality of our atmosphere is nose diving at an alarming pace. Atmospheric temperature is roaring up year by year and the rain is non-seasonal or erratic. These are attributed to huge amount of green house gases released to the atmosphere leading to global warming as well as varying precipitation patterns. Across the globe, the personal automobile is the single greatest polluter of atmosphere as seen from the data obtained from Vyttila. The paradox is that the rich and the educated are causing more harm to the atmosphere. The atmosphere is now a cocktail of chemicals, and not much late, we will have to buy breathing air in bottles and our very existence will become a costly affair. Urgent campaigns must be carried out to sensitize the public about the graveness of the situation

Keywords: automobile, air pollution, greenhouse gas Introduction

Introduction

Under the Paris Agreement, India has committed that the greenhouse gas emission intensity of its GDP will be reduced by 33-35% below 2005 levels by 2030. Also, 40% of India's power capacity would be based on non-fossil fuel sources and that India will create an additional 'carbon sink' of 2.5 to 3 billion tonnes of CO₂ equivalent through creating additional forest and tree cover by 2030.

The report indicates that India is well on way to achieve the target for emission intensity of the economy and share of non-fossil fuel-based power capacity. In fact, at current rates of improvement on both fronts, India could achieve these targets ahead of the 2030 deadline. The emission intensity of India's GDP came down by 21% below 2005 levels by 2014 recording slightly more than 2% annual average improvement in emission intensity. The rate of improvement recorded between 2010-2014 was higher than that recorded between 2005-2010. Even at this rate, India could reach its Paris Agreement commitment ahead of the 2030 deadline.

No doubt, in the past few years India has seen a tremendous spurt in concerns over environmental degradation. Numerous public interest litigations in the courts in support of having cleaner air, especially in heavily polluted cities like Delhi, saw some defining steps taken to curb the issue of pollution.

The government took some key initiatives including promotion of emission-free electric vehicles as an alternative to the traditional fossil fuels. Promotion of public transport, car-free Sunday, network of metro, buses, e-rickshaws and promotion of car pooling, lane discipline, vehicle maintenance, Odd-Even Policy On Vehicles in 2016 and 17 etc. are some other steps taken by the Government.

Following public interest litigation the Supreme Court imposed a ban on sale of all diesel personal vehicles having engines more than 2000cc. Though the ban was imposed in Delhi-NCR, governments of other states explored the idea of imposing a similar ban. The ban was later lifted only after slapping of a 1 percent cess on such vehicles, the amount to be deposited with the Central Pollution Control Board. During the 2016

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budget, Finance Ministry proposed a 'green tax' of 2.5 percent on small cars and 4 percent on bigger cars and SUVs.

In a welcome move, the Union Ministry of Petroleum and Natural Gas has announced a 2-year advancement of the introduction of Bharat Stage (BS)-VI fuel norms from April 1, 2018 in Delhi, currently suffering from its worst smog crisis in years. BS-VI norms are scheduled to be implemented across India in April 2020. BS-VI fuel will bring down sulphur by 5 times from the current BS-IV levels—a whopping 80 per cent reduction and would make fuel extremely clean.

Post the successful implementation of BS-VI norms across the country in 2020, India will remain the only country to have completely skipped an emission norm (BS-V) to jump directly to the next one. While India was originally supposed to adopt BS-V norm in 2019 and BS-VI in 2024 not only did the government decide to skip a norm but it decided to advance the date of BS-VI roll-out, too. Emissions from BS-VI vehicles will be even lesser than the present generation BS-IV vehicles.

Faster Adoption and Manufacturing of Hybrid and Electric vehicles (FAME) formed under the National Electric Mobility Mission Plan was constituted by the Government little less than a year after coming to power. This scheme subsidized purchase of hybrid and electric vehicles by giving direct cash benefits to buyers. Till date, 1.46 lakh vehicles have benefited from the scheme through disbursement of Rs 182 crore worth of incentives resulting in a direct saving of 12 million litres of fuel and a reduction of 31 million kilograms of CO₂, as claimed by the government.

Besides incentivising buyers, the government itself is pulling out all stops for promoting clean technology mobility solutions. Electrification of transport remains one of the main agenda of the government for which it has already started using electric vehicles. While some of its ministries are set to use electric vehicles for transport, it is also asking state governments to replace their ageing diesel/CNG-powered buses

with next generation all-electric buses. An all-electric bus could cost around Rs. 2 crore and the government has promised to pitch in Rs 65 lakh. The Center's blue print states that it wants to be able to allow only electric vehicles in India by 2032.

The government has plans to incentivise and encourage existing owners of old trucks and buses which are more than 11 years old to trade in for new replacements. Plans could involve exemptions on payments of duties or discounts on taxes. Such exemptions would help reduce the acquisition cost of new trucks, boost production and sales of new trucks, reduce emissions, boost fuel-efficiency, minimise fuel consumption and also cut down instances of vehicle breakdowns. The Ministry of Road Transport and Highways had proposed a total monetary benefit of nearly Rs 5 lakh per truck, of which half would come from state and central governments.

Use of liquefied natural gas is a relatively a new concept in India and is aggressively pushed forward by Ministry of Road Transport and Highways. While LNG is already used in the industrial sector, it is not commercially available for the automotive industry. However, the government is setting up LNG storage depots at ports to help make the fuel use as a conventional alternative to CNG. LNG is much cleaner than CNG even as CNG itself is a bigger polluter than petrol.

In spite of all these measures taken by the government, the reality is heart-breaking. One out of eight premature deaths in India is due to breathing polluted air, as reported by The Lancet Planetary Health, the journal by ICMR in its December issue¹. Fifteen of the twenty most polluted cities in the world are in India—as per Global Air Pollution 2018 Report by the environmentalist group, Green Peace in The Hindu 6th March 2019².

Case study conducted at Vyttila

What are the causes of these frightening situations and who are responsible for it. This article presents a review of the case study conducted at Vyttila – the biggest

traffic junction of Kerala. No doubt, vehicles are now a multifaceted sword, which geometrically magnifies the menace of environmental pollution. Increase in population of vehicles, exhaustion of fossil fuels and change in climate are seen to be linked as shown by the cross sectional study at Vyttila. The study could bring to lime light astonishing facts about the extent of greenery sacrificed for setting up fossil fuel outlets and that the sale of fossil fuel is going up steeply as population of vehicles is increasing at an alarming rate. Study confirms that Kochi is contributing a significant share in making the global environment malicious.

Kerala has been bestowed with a pleasant and equable climate throughout the year. The climate of Kochi—the queen of Arabian Sea also has been nothing different and has an invigorating climate. But unfortunately, the climate of Kochi is changing over the past few years along with the global climatic deterioration. During April 2014, Kochi has experienced the highest maximum temperature in the last 10 years^{3,4}. Vyttila, in Kochi is the busiest and the biggest junction of the state and on an average 150000 vehicles ply through the junction everyday, according to the study conducted by the National Transportation Planning and Research Centre (NATPAC). The unprecedented growth of vehicular traffic causes the drastic deterioration of the quality of the environment. The right for pure air to breathe and water to drink is becoming a dream. What is called development is now leading to the tragedy of common man.

In cities across the globe, the personal automobile is the single greatest polluter, as emissions from a billion vehicles on the road add up to a planet-wide problem. Driving a private car is a typical citizen's most air polluting activity. The negative effects of automotive emissions are maximum, when one sit in traffic surrounded by cars, their engines idling. The Green House Gases emitted by the vehicles include carbon dioxide, methane, nitrous oxide and chlorofluorocarbons⁵.

Data from RTOs of Kochi, reveal an astonishing, unprecedented increase in the number of private vehicles –both two and four wheelers –getting registered every year. In order to maintain these lakhs of vehicles plying on the road, numbers of fuel filling stations are also increasing in parallel and new ones emerges at a rate of one per year within a 5 km radius of Vyttila. Inception of one fuel filling station is by sacrificing about 50 cents of greenery and each station is selling on an average 7000 L petrol and 12000 L diesel per day. The newly started filling stations together with the already existing ones, are acting as outlets for crores of litres of petrol and diesel every day. The result is not only fossil fuels will get exhausted in near future but also its combustion is flushing huge amount of toxic gases and particulate matters into the atmosphere. It is idiotic to see that the permissible standards are set according to Rule 115 of CMV Rules 1989 and the permissible standards seems to be very high i.e., 3.5% CO for two wheelers and 0.5% for four wheelers. Moreover, the pollution testing centres are testing only CO₂, CO, Hydrocarbon and O₂. But, in addition to these, vehicles are also emitting SO₂, Nitrous oxide, particulate matter etc. The condition is more aggravated by the fact that most of the vehicles are not even turning up at the pollution testing centres for monitoring their emissions. No doubt “many a mickle makes a muckle”. Even if the emissions of individual vehicles are under permissible limits, lakhs of vehicles plying through a point makes the toxic gases above permissible levels. The present irresponsible situation will definitely make the atmosphere a cocktail of toxic emissions. Fortunately, the levels of sulphur dioxide and oxides of nitrogen are still below the maximum permitted levels, as per the data given by the Kerala State Pollution Control Board⁶. Thanks to the copious rain for the lion share of the year and also the wind pattern. But, the level of suspended particulate matter is increasing year by year and also it has surpassed the maximum permissible limit. In spite of any agricultural activity or sewage outlets, water samples from a domestic well at

Vyttila and another sample from Oil Tanker Jetty, Vembanadu lake, showed increasing levels of nitrate and nitrite for the last five years. Atmospheric nitrogen fixation and consequent leaching out may be contributing to the condition.

The average rain fall during the last years varied considerably. This may be attributed to the change in the amount of the erratic or intermittent rains usually received during the summer season, which in turn may be due to the local influences. The variation from the good old annual average value of 3000 mm may be not due to change in either the south- west or north- east monsoons which are more or less global phenomena. No wonder, the climate of Kochi also is changing over the past few years along with the global climatic deterioration. SkyMet Meteorology Division in India has reported that during April 2014, Kochi has experienced the highest maximum temperature in the last 10 years and the average temperature of every month in 2014 is higher than its preceding year⁷. This may be due to the Green House Gases acting like blankets wherever their concentration increases. Local concentrations increase local heat and increased differences between hotter and colder regions drive weather events into more extreme ranges. The heat retention by the Green House gases is having a serious impact on local climates right now.

It is really pathetic to see that the more educated people are causing more pollution to the air by regular injudicious use of private vehicles. It is ironical that, from among the educated employees of Ernakulam, staff of educational institutions, are far ahead in using private vehicles regularly. Surveys have also revealed a double fold increase in the percentage of private vehicle users in nongovernmental-i.e.,private and management sectors.

According to Ravikant Mahajan (*Your Quest for Being Better*), one day a human being breathes oxygen equivalent to 3 cylinders which on an average costs 3x700=Rs.2100. Yearly cost comes to about Rs. 7,66,500 and for a man of average life span

70 years it becomes Rs.53.66 million. All this oxygen we get free from trees, which we mercilessly cut down and establish fuel filling stations. Since the trees are cut down, the sink of atmospheric carbon dioxide is no more!!!

Until a few years ago, fresh air was the only thing that was available for free to us. But now even that seems to be a luxury.. In 2016, the Gas Authority of India Limited (GAIL) sponsored a campaign which was motivated by a famous YouTube video on a social experiment which introduced the idea of selling freshly packaged air in Delhi. At a time when Delhi was choked and fresh air was a need, this video did wonders. But back then it was only a warning and now it has become a sad reality.

Many companies like Auzair, Vitality Air and Indian brand Pure Himalayan Air have actually come up with bottled fresh air. Pure Himalayan Air is selling a can of 10 Litres fresh air for Rs 550. The idea originally came from Vitality Air, a Canadian company that started selling bottled air. Then came Australia-based brand Auzair who are also selling their products in India. Their bottle of 7.5 litres of fresh air costs Rs 1500⁸.

Breathing fresh, clean air out of a bottle is a rather expensive affair which is impossible to achieve for most of us. We don't understand where our future is headed if this is where we have already reached.

Conclusion

Foregoing discussions reveal how the injudicious use of vehicles makes our breathing air filthy. This, along with other luxuries such as use of air conditioners round the clock, compel us to conclude that the rich and the educated-i.e., the privileged section of the society is responsible for the crime of polluting air, which is a global resource, thus sweeping away the right for pure air for themselves and also for the innocent underprivileged of the society.

Let us join hands to sensitize the residents about the benefits of public transport and to sweep away the misconception that

vehicles are status symbols. Let us form a low carbon foot print society and thus live in harmony with nature. Beware, the present local warming up as well as conversion of water and air to a chemical cocktail can

be the initial step towards the anthropocene defaunation or more technically called a sixth mass extinction. And.... it is not far away.....

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Potency of plant extracts in mosquitocidal activity

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Abstract

Mosquitoes have annoyed man and undermined his health for centuries. The present study tried to assess the mosquitocidal efficacy of leaf extracts of *Ocimum basilicum* and *Simarouba glauca* on the developmental stages of *Culex* species of mosquitoes. The present investigation showed that higher the concentration of the extract, higher was the percentage larval mortality and lower pupal emergence. Maximum larval mortality (93.3%) was observed in 120h (5th day) in 10,000ppm concentration of extracts of *Ocimum basilicum*, while minimum mortality of 3.3% in 2000ppm at 48h. Many abnormal developmental stages were also obtained in *S. glauca* leaf plant extracts.

Key Words: Mosquitocidal, Non-melanized pupae, hyper-melanized and pupal-adult intermediates

Introduction

Mosquitoes are the most important single group of insects in term of public health because of their ability to transmit a number of outrageous diseases like Japanese encephalitis, filariasis, malaria, chickungunya and dengue, causing millions of deaths every year throughout the world^{1,2}. Mosquito species are abundant in the tropics and almost unbelievably large swarms of them occur in the Arctic. In India, these ubiquitous insects occur at elevation of 4,300 meters in Kashmir and 1,160meters below sea level in gold mines of south India³.

Transmission of mosquito-borne diseases is governed by a variety of specific ecological, epidemiological, geographical and social factors. The north-eastern state of Assam is endemic for the mosquito borne diseases with uneven distribution pattern. Malaria is highly endemic in Barak valley and lower Assam areas⁴.

Most of the present day research on mosquitoes is concerned directly with methods of killing them. The control of mosquitoes is complicated by the fact that breeding places of the larvae are often inaccessible or in public water supplies. Burning problem

in mosquito control operation is development of resistance by the mosquito to several chemical insecticides and repellents. Extract of neem seed kernel⁵, leaf extract of *Lucas aspera*, *Ocimum sanctum*, *Azadiracta indica* and *Allium sativum*, Rhizome extract of *Curcuma longa*⁶ and fumigation by peel of different Citrus species⁷ have been tried.

Larvicidal efficacy of ethanolic extracts of *Annona squamosa* (Annonaceae) over the filarial vector, *Culex quinquefasciatus* was tested. The results revealed that ethanolic leaf extracts of *A. squamosa* plant can be used effectively as a potential, eco-friendly, biodegradable and economic larvicide in integrated mosquito control programme⁸. Bioassays against larvae of *Ae. aegypti* with neem seed kernel extracts obtained by extraction with water and organic solvents. Permanent exposure of first instar larvae to treated water resulted in a conspicuous growth disrupting effect, mainly characterized by morphogenetic effects⁹. The effectiveness of the extracts increased with decreasing polarity of the solvents used for extraction. The seed kernel extract caused an extreme prolongation of the larval period when first instar larvae were continuously exposed to treated

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water until adult emergence. As Angamaly is a mosquito prone area, an investigation was undertaken to assess the potency of selected botanicals against *Culex* species of mosquito.

Materials and Methods

Collection of larvae

Hay infusion method was adopted for collecting the mosquito larvae¹⁰. Eggs were laid by mosquitoes after 1-2 days. The rafts were collected and maintained in the laboratory. The 2nd instar larvae emerging from the culture medium were collected and maintained separately. A pinch of dog biscuit was provided as food for these larvae. When the larvae moulted and entered into 3rd instar, they were segregated and used for the study.

Collection of test materials

Leaves of *Ocimum basilicum* (Fig. 1) and *Simarouba glauca* (Fig. 2) were collected from Morning Star Home Science College campus Angamaly, Ernakulam.

a) Preparation of leaf powder

Healthy green leaves were collected,

washed once in water and shade dried. 100g of dried leaves were weighed and ground to powder.

b) Preparation of extracts

10g of leaf was weighed in an electronic balance. Leaf powder was mixed in 200ml of distilled water. The mixture was stirred for 16 h in an electrical shaker at 120rpm and left to stand for 48 h at room temperature. The mixture was centrifuged at 3000rpm for 20 minutes twice. The supernatant was collected and used in this study.

Experimental Setup

The experimental set up consisted of six treatments ranged from 2000ppm to 10,000ppm each with three replications. For bioassay studies, the required quantity of different concentrations of leaf extracts were introduced into 250ml beaker containing 50ml of tap water and thoroughly mixed. 10 newly emerged 3rd instar larvae were introduced into each beaker containing the rearing medium for specific treatments. Dog biscuit was provided *ad libitum* as food. In control only tap water was used. Concentrations of 2000ppm, 4000ppm, 6000ppm, 8000ppm and 10,000ppm were taken as treatments.



Fig1: *Ocimum basilicum*



Fig 2: *Simarouba glauca*

Table 1 Effect of *O. basilicum* leaf extract on the developmental stages of *Culex* species.

Treatments	Larval mortality (%)						Pupal emergence (%)	Pupal mortality	Adult emergence (%)
	24h	48h	72h	96h	120h	144h			
2000ppm	0.00	3.3	6.66	10	33.3	50	50	30.00	20.00
4000ppm	16.6	16.6	16.6	40	63.3	80.0	20	-	20.00
6000ppm	10	10	13.3	33.3	50	60	40	23.4	16.6
8000ppm	13.33	13.3	13.3	50.0	86.6	-	10.4	10.4	-
10,000ppm	36.6	40	43.3	53.3	93.3	-	3.3	-	3.3
Control	0.00	0.00	0.00	0.00	0.00		100.00	-	100.00

Observation was made at every 24h duration on larval and pupal mortality. Dead larvae and pupae were removed at 24h interval after the exposure. Various abnormalities noted during the period and moulting were also recorded. Pupae emerging out were counted, killed and preserved. A record of number of adults emerging also was maintained.

Results

Ocimum basilicum

The effect of *O. basilicum* leaf extracts on larval mortality was found to increase in proportion to the increase in the concentration of the leaf extract (Table 1). Maximum mortality of 93.3% was observed in 120h (5th day) in 10,000ppm concentration, while minimum mortality of 3.3% in 2000ppm at

48h. No mortality was recorded in the control as well as in 2000ppm at 24h. In control 100% pupal emergence was observed while in 10000ppm treatment minimum pupal emergence of 3.3% was observed. Adult emergence could be seen in all treatments except 8000ppm. Minimum adult emergence could be observed in 10000ppm and it was 3.3%.

Simarouba glauca

Effect of *S. glauca* leaf extract on larval mortality indicated 10,000ppm treatment as an effective one producing maximum mortality in 240h (10th day)(86.6.00%) (Table 2)

Larval extension could be observed and it was maximum in 2000 ppm (312h; 13th day). The pupal emergence was 100% in control and 10,000ppm recorded minimum pupal



Fig 3. Non-melanized Pupae



Fig 4. Hyper-melanized pupae



Fig 5. Pupal- Adult intermediates

Table 2 Effect of *S. glauca* leaf extract on the developmental stages of *Culex* species

Treatments	Larval mortality (%)				Pupal Emergence(%)	Pupal Mortality(%)	Adult emergence (%)
	24h	48h	72h	96h			
2000	0.00	0.00	0.00	30.00	20.00	3.3	16.7
4000	3.33	13.3	16.6	16.6	23.00	3.3	20.4
6000	0.00	0.00	0.00	6.6	20.00	3.3	16.7
8000	0.00	0.00	0.00	3.3	16.00	3.3	12.7
10,000	20.00	30.00	30.00	40.00	10.00	3.3	6.7
Control	0.00	0.00	0.00	0.00	100.00	0.00	100.0

emergence (3.3%). Pupal – adult intermediate was observed in 2000ppm (3.3%). Adult emergence was maximum in 4000ppm (20.4%) and minimum in 10000 ppm (6.7%).

Abnormalities observed

Abnormalities were observed in pupal stage. Few pupae were non-melanized and they were white in colour (Fig.3), many were hypermelanized (Fig.4) and pupal adult intermediates (Fig. 5) were also observed.

Discussion

In the present study aqueous leaf extracts of *O. basilicum* and *S. glauca* were found to be potent agents for the control of *Culex* spp. Both these leaf extracts had larvicidal properties and larval extension was observed in *S. glauca* and it was extended up to 13th day.

In the present investigation higher the concentration of the extract, higher was the percentage larval mortality and lower was the percentage of pupal emergence. The optimal dose which brought about maximum mortality and minimal emergence was found to be 10,000ppm in *O. basilicum* leaf extract and in *S. glauca*. In addition to this, no adult emergence was observed in 8000 ppm of *O. basilicum*. In *S. glauca*, the

lower concentration showed larval extension showing its efficacy over other treatments.

Studies using acetone extracts of neem seed coat showed pupal mortality in *Cu. quinquefasciatus* even in lower concentrations, but not in *Ae. aegypti*. In the present work, in comparison with the leaf extract treatment of *O. basilicum* and *S. glauca* pupal emergence was minimum and pupal mortality was significantly high in higher concentrations¹¹.

Various defective stages such as unmelanized pupae, partially melanized pupae and hyper-melanized pupae and pupal-adult intermediate were observed in this bioassay study^{9,12}. The induction of morphogenetic abnormalities is generally attributed to the interference of active ingredients of extracts with the endocrine system. The incidence of endocrine forms in different extracts of *O. basilicum* and *S. glauca* in the present study might be due to the juvenomimetic activity of substance present in the extract.

Conclusion

O. basilicum and *S. glauca* species are available in plenty in our locality and will be a cost-effective mosquitocidal material to be used by all. The present investigation is an attempt to identify some commonly

available effective botanicals that can be used by both rural and urban people in mosquito management. Further studies may be undertaken to study the active ingredients of the extracts and field trials can be undertaken.

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Studies on the Physico-chemical parameters of Mangrove vegetations after deluge in Thrissur District, Kerala, India

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Abstract

Mangroves are the most productive and complex ecosystems on earth, which can fertilise the sea, potentially protect the coastal zone and serve as the breeding, feeding and nesting ground for many animals. The present study was conducted in mangroves of Thrissur District analyze various Physico-chemical characteristics of water and soil after deluge. Rainfall brings vital changes in the hydrological characteristics of estuarine and marine environment. The present study was conducted September 2018 to February 2019.

Key words: Physico-Chemical parameters, Deluge, Mangroves

Introduction

Mangroves are the most productive ecosystems, which can fertilize the sea, potentially protect the coastal zone and serve as breeding grounds for many animals. The mangrove forests are sometimes called as “tidal forests”, “Oceanic rain forests” and “Coastal woodlands”. These plants exist in very inhospitable conditions. They exist in conditions like high salinity, tidal extremes, wind velocities, high temperatures and muddy anaerobic soil. They have “breathing roots” or “prop roots” and also have other adaptations such as support roots, viviparous germination, salt excreting leaves, knee roots etc. Mangroves in Kerala are distributed in eight coastal districts of which Kannur (755 ha) has the largest followed by the districts of Kozhikkode (293 ha), Ernakulam (260 ha), Alappuzha (90 ha), Kottayam (80 ha) and Thrissur (22 ha). The present study deals with the Physico-chemical parameters of Mangrove vegetations after deluge in Thrissur District, Kerala, India

Materials and Methods

The present study conducted from September 2018 to February 2019. The

Experimental sites was visited many times to collect samples. Three index stations were established in the mangrove vegetations in Thrissur. Composite sampling method was adopted, that is collecting the samples at various times from a locality undergoing fluctuations and combining that to analyze seaverage chemical characteristics. Water samples of one litre, each were collected in sterile bottles and he dissolved oxygen content was estimated for. BOD bottles were used for the collection and determination of dissolved oxygen. All bottles were washed thoroughly and rinsed with water samples before collecting it. Care had been taken to avoid contamination during collection and transportation. All the collected samples brought to the laboratory and different Physico-chemical parameters determined by routine laboratory methods.

Water Quality Parameters

Different parameter of water was analyzed by standard estimation procedures and calculations.

a) Atmospheric and water temperature

The temperature of atmosphere and aquatic system were recorded using

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thermometer at the time of sample collection. b) Estimation of Dissolved oxygen

Dissolved oxygen content in water was analyzed using Winkler's method. The water was titrated with 0.01N sodium thiosulphate, by the addition of $MnSO_4$, KI, and $Con.H_2SO_4$ with starch as indicator and the values can be calculated using the formula

$$DO = \frac{\text{Vol of } Na_2S_2O_3 \times N \text{ of } Na_2S_2O_3 \times \text{Eq. Wt of } O_2}{\text{Effective N of } H_2O}$$

c) Estimation of Carbon dioxide

CO_2 content in the water can be observed by titrating the water sample against NaOH. Phenolphthalein is used as indicator.

$$DO = \frac{\text{Vol. of } Na_2CO_3}{r^2}$$

d) Estimation of Hardness

Ammonia solution and Eriochrome black 'T' indicator is added to 25ml of water sample and titrated against 0.02N EDTA. Hardness in ppm is calculated with the formula

$$\text{Hardness} = \frac{0.001 \times \text{Vol of EDTA} \times 10^6}{\text{Vol of water sample}}$$

e) Determination of PH

Value of PH can be observed using PH meter. Before determining the PH value, PH meter can be standardized against buffer solution.

f) Estimation of Chlorinity and Salinity

Chlorinity and Salinity was determined by titrating 10ml of water sample against 0.0141N silver nitrate by the addition of 2 drops of potassium chromate.

$$\text{Chlorinity} = \frac{\text{Vol of } 0.0141N \text{ AgNO}_3 \times N \text{ of } AgNO_3 \times \text{Eq. wt of } AgNO_3}{\text{Vol of water sample used}} \times 1000$$

$$\text{Salinity} = \frac{0.3 + 1.805 \times \text{Chlorinity of water in mg/l}}{1000}$$

Soil Quality Parameters

a) Determination of PH

PH can be analyzed using PH meter. Before determining the PH, PH meter standardized with appropriate buffer solutions.

b) Determination of moisture content

Moisture content was determined by drying 1gm of soil in a petridish to about 1 hour in oven. Weigh the petridish before and after drying. Percentage of moisture content was calculated by the formula,

$$\text{Moisture} = \frac{0. \text{Wt of petridish with moist soil} - \text{Wt of petridish with dried soil}}{\text{Wt. of soil sample}} \times 100$$

c) Estimation of Chlorinity

Chlorinity was determined by titrating 0.0141N AgNO₃ against 10ml of water containing soil by the addition of 2 drops of potassium chromate against the prepared blank.

$$\text{Chlorinity} = \frac{V_1 \times N_1 \times \text{Amount of distilled water}}{\text{Vol of water sample}}$$

V₁ = Vol. of AgNO₃ used for (sample-blank)

N₁ = Normality of AgNO₃

d) Estimation of Organic Carbon

To 1gm of dried and sieved soil potassium dichromate, Con.H₂So₄, distilled water, orthophosphoric acid, and diphenyl amine indicator is added and it is titrated against ferrous ammonium sulphate until the colour changes from blue violet to brilliant green. Organic carbon was calculated the formula

$$\text{Organic carbon (\%)} = \frac{10 - (B - S) \times 0.003 \times 100}{B \times \text{Wt. of soil sample}}$$

B = Vol of Fe (NH₄)₂(SO₄)₂ used for Blank

S = Vol of Fe (NH₄)₂(SO₄)₂ used for soil sample

e) Estimation of Calcium

To the weighed soil add 100ml 1N Hcl and covered with cover glass. To 20ml pipetted sample, added bromothymol blue indicator and titrated against 1N NaoH until the colourless solution changed to blue/green. Run a blank at the same time.

$$\% \text{ of CaCo}_3 = (\text{Reading of Blank} - \text{Reading of soil sample}) \times 5$$

Results and Discussion

The observed physico-chemical parameters of water, atmosphere and soil of the mangrove regions were summarized in table 1 and 2.

Table: 1. Various Physico-Chemical Parameters of Water and Atmosphere in Mangrove

Months	Parameters							
	Temperature (°C)		pH	Oxygen (mg/l)	CO ₂ (Mg/l)	Chlorinity (ppt)	Salinity (ppt)	Hardness (ppm)
	Atm.	H ₂ O						
Sep	28	29	8.01	0.956	5.32	2.60	4.99	692
Oct	22	25	7.45	0.973	5.32	0.035	0.363	0.02
Nov	25	27	7.10	0.910	5.95	0.255	0.760	0.08
Dec	23	28	8.10	0.829	8.8	7.48	13.80	1840
Jan	28	29	8.22	0.747	9.21	13.40	26.30	4700
Feb	29	31	8.45	0.747	9.36	18.54	33.77	7004

Table: 2. Physico-Chemical Parameters of Soil Observed During Study Period

Months	PH ^H	Salinity	Organic Carbon	Calcium	Moisture
September	6.57	0.145	1.357%	2.8%	43%
October	6.97	0.039	0.5%	1.5%	64%
November	6.62	0.069	1.690%	3.4%	45%
December	6.41	0.219	1.666%	3.2%	46%
January	5.6	1.479	2.159%	3.6%	36%
February	4.2	1.514	2.307%	4%	55%

Temperature of atmosphere and water was maximum in February and minimum in October. Although there was no marked variations in temperature between atmosphere and water, but the temperature shows fluctuations because of strong land

Remaining two months shows slightly neutral PH, it was the result of rainfall, fresh water inflow and precipitation. It was noted that December onwards the alkaline condition increases and reach the value from 8.10-8.45

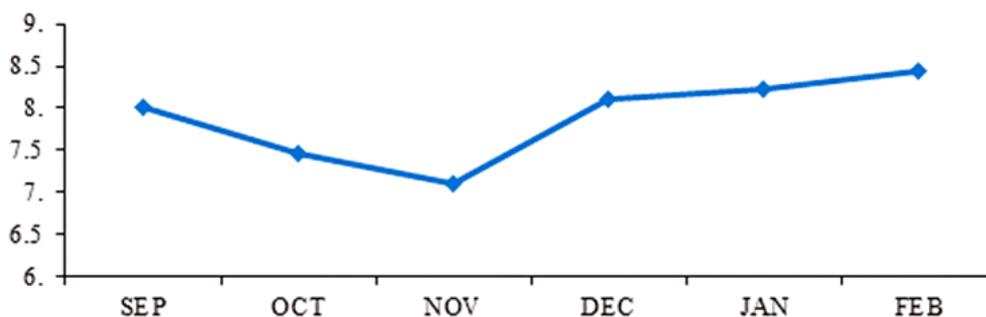


Fig.1.Graph showing PH of water during the study period

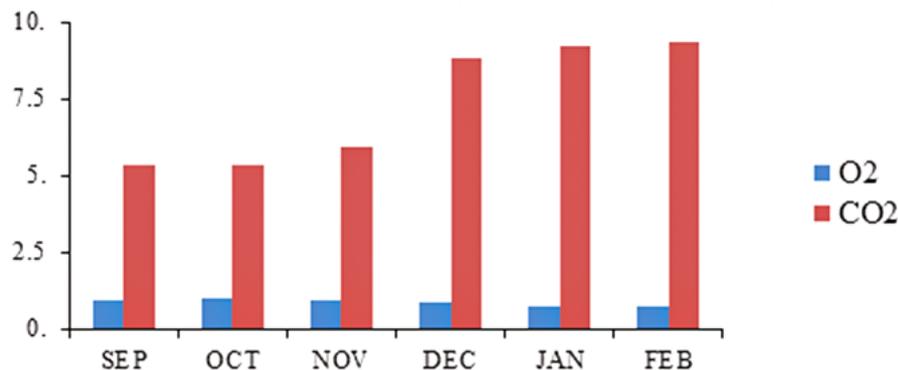


Fig.2.Graph showing Dissolved O2 an Co2 of water during the study period

breeze, monsoonal clouds, humidity and precipitation (Table.1).

During observation the PH shows small variations (Table.2 and Fig.1). In September, December, January and February, the PH value indicates the alkaline condition.

Dissolved oxygen showed higher value in October, which may be due to the cumulative effect of higher wind velocity coupled with heavy rainfall and the resultant mixing of fresh water and its value is minimum in February. The variation of dissolved

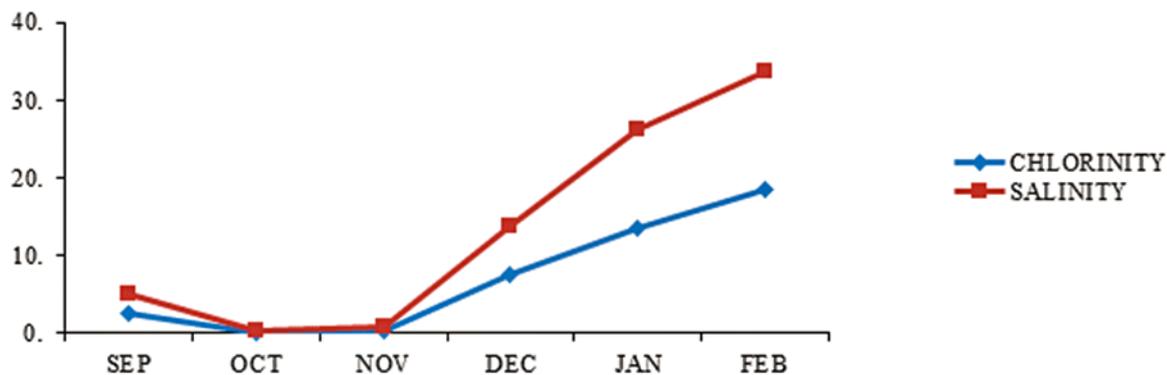


Fig.3. Graph showing Chlorinity and Salinity of water

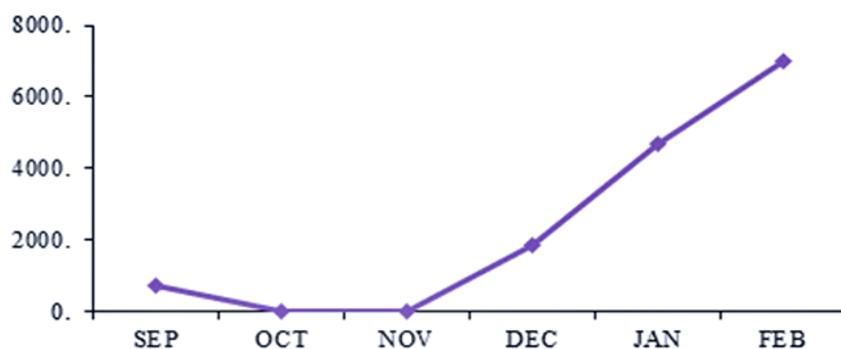


Fig.4. Level of Hardness of Water during the study

oxygen is mainly due to fresh water inflow and outflow and terrigenous impact of sedimentation. Like this CO₂ also shows variations. Maximum value is obtained in February and minimum in October. There is an inverse relationship between CO₂ and DO. Whenever CO₂ level increased, DO level decreased. The discharge of sewage and garbages was leadto O₂ deficiency and thereby increase in CO₂ (Fig.2).

Chlorinity and Salinity of Water

In the present study Chlorinity was maximum in February and minimum in October(Fig.3). This minimum value is due to precipitation and less evaporation. It directly related to salinity. So salinity also showed high value in February and low in October. These two parameters show slight variations. Salinity varies according to topography, low and high tides and fresh water inflow. It was clearly observed that there is a positive correlation between salinity and chlorinity.

The water hardness is very high during September, December, January and February in the ascending order (Fig.4). In the middle two months the water was very soft due to fresh water inflow, rainfall, mixing etc. A high concentration of multivalent cations may be the reason behind increased hardness. List of Physico-chemical parameters of soil studied. Mangroves are salt tolerant plants evolved various adaptations to survive and thrive in harsh conditions. They have pneumatophores to absorb oxygen, and provide shelter, protection and food for numerous organisms.. It acts as barriers to shoreline erosion and it also serve as important nurses, feeding and refuge areas for a wide variety of organisms.

The present study was conducted in mangroves of Thrissur to analyze various physico-chemical characteristics of water and soil. Rainfall brings vital changes in the hydrological characteristics of estuarine and marine environment. In this study temperature of atmosphere and water shows

slight variations due to climatic changes. Atmosphere and water shows maximum temperature in February and minimum in October and may be due to monsoonal clouds. In December atmospheric temperature was low. During October – December water temperature recorded was low because of strong land breeze and precipitation. The high temperature could be attributed to high solar radiation. Throughout the study period PH showed a wide range of values, it varied from neutral to slightly alkaline. This is a typical character of estuarine environment. Alkaline PH recorded in certain months may be due to the removal of carbon dioxide by photosynthetic organisms. So there is a positive correlation between alkaline PH and enhanced rate of photosynthesis and slightly neutral PH could be attributed to the dilution of saline mangrove water while fresh water inflow. Salinity varies according to different factors salt water inflow and out flow, low and high tides, fresh water inflow and mixing. In the present study saline condition of water was high in February due to fresh water evaporation and it is low in October because of rainfall and less evaporation. Chlorinity also showed changes with respect to increased and decreased value of salinity. So, chlorinity is high in February and low in October. Based on hardness, water can be classified into soft, medium and hard. In September, December, January and February the hardness of water was lying in the range of 692-7004. During these months rate of evaporation and salt content was very high. But in October and November water was soft because of dilution of salt water with high rainfall. In the present study dissolved oxygen indicates higher value in October which could be due to the cumulative effect of higher wind velocity coupled with heavy rainfall and the resultant mixing of fresh water. Minimum amount of oxygen was present in February and January. Seasonal variation of dissolved oxygen is mainly due to fresh water flow and terrigenous impact of sedimentation. Carbon dioxide level in water showed fluctuations. It reaches its maximum in February and minimum in October. Mathew (1978) observed an inverse relationship

between carbon dioxide and oxygen. Whenever dissolved oxygen decreased, carbon dioxide increased. The discharge of sewage and waste materials will lead to oxygen deficiency and there by increase the carbon dioxide. There is a positive correlation between temperature and carbon dioxide. PH of soil was slightly acidic. Acidic PH was observed in February and slightly neutral PH observed during other months. That was during summer, soil was more acidic than monsoon this occurs due to decomposition of dead animals and plant parts and less water contents.

High moisture content was observed in this area because soil is partially or completely submerged in water. Slight variations are observed during monsoon and high tide soil is completely submerged in water and during summer and low tide soil is partially submerged in water. So moisture content shows slight fluctuations based on tides and climatic conditions. Maximum salinity was observed in February and minimum salinity in October. This is due to the climatic changes and intrusion of salt water. That is during summer much evaporation and percolation were occurred and during monsoon evaporation and percolation was less. So salinity is high during summer and low during monsoon. Maximum organic carbon was observed in February and minimum organic carbon content was observed in October. The soil collected from study area showed high organic carbon in all months except October. Presence of low organic carbon may be due to heavy rainfall. High organic carbon content may be due to the decomposition of dead animals and plants and climatic changes. Present study reveals that organic carbon was positively correlated with salinity and negatively correlated to PH. When organic carbon increases, salinity also increases which in turn decreases PH.

Calcium present in the soil is absorbed by plants. There is a balance calcium budget existing in ecosystem. Maximum calcium content was recorded in February and it was minimum in October. The calcium content of the acidic soil is generally high,

regardless of texture as a result of low rainfall and little leaching. Calcium is also positively correlated with organic carbon.

The present study showed that the terrible flood in the month of August was not

completely affected the Physico –Chemical parameters of the ecosystem in comparison of previous studies reported in other areas. But long term studies should be needed in this area to identify and mitigate the issues of the precious ecosystem.

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Preparation of TiO₂ film by anodisation of sputtered titanium film

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Abstract

Titanium films were prepared on ITO (Indium Tin Oxide) substrate by RF magnetron sputtering. These films were anodized to obtain titanium dioxide thin films. In-order to obtain an optimum thickness for the titanium layer, sputtering was optimized for different sputtering power (100W-150W) and for various sputtering time. The anodisation of Ti film was carried out at room temperature. In this electrochemical process, titanium film was used as anode and platinum as cathode. The electrolytes used were HF solution in aqueous base and NH₄F solution in Ethylene Glycol base. The formation of TiO₂ was strongly influenced by the concentration of the electrolyte, applied voltage and duration of oxidation. The morphology of the oxidized film was analysed using Scanning Electron Microscope (SEM). Evaluation of band-gap supported the formation of Titanium-di-oxide by the process of anodisation of Titanium. The Rutile phase was confirmed using XRD. Resistivity measurement of the titanium films and anodized films of various thickness was carried out using Keithley Source measuring unit. Attempts were made to use this TiO₂ films in Dye Sensitized Solar Cells (DSSC). Though the output of the cell was not appreciable, parameters like short circuit current, open circuit voltage, fill factor and efficiency confirmed the functioning of solar cell.

Key Words: Sputtering, anodization, titanium dioxide, dye sensitized solar cell

Introduction

Titanium dioxide (TiO₂) is one of the most studied metal oxides, which finds many applications in photo catalysis, dye-sensitized solar cells, self-cleaning, electrochemical devices, batteries, gas sensors display, photochromic devices and so forth. Nanostructured form of TiO₂ is currently under intense investigation, as they provide highly active surfaces with a large surface to volume ratio and unique properties¹. Physical and chemical manipulations in nano dimensions can be applied to enhance/alter the performance of TiO₂ when it is used in the fabrication of micro/nano-size devices for commercial applications². There are many routes for the production of TiO₂ nano-forms, of which, one of the most promising routes, even for commercial processes, is anodization. In this method, Ti in the form of a film or foil is anodized in fluoride-ion-containing electrolytes to form TiO₂. In the case of foils it has been reported that by controlling the anodization voltage and time, the dimensions and aspect ratio of TiO₂ can

be varied. It also requires optimum concentration of NH₄F and H₂O in the electrolyte³.

Initial research demonstrated the possibility of forming self-organized, nano tubular surfaces of titanium oxide by anodic oxidation of a Ti foil. Later many groups have conducted comprehensive studies on Ti foil anodization. However, the use of foil limits their potential applications, particularly in the fabrication of microscale devices, where thin films need to be deposited and patterned. Several research groups have developed alternative methods for the fabrication of nanoporous TiO₂ via the anodization of titanium films. One of the earliest studies was conducted by Moret al. who used electrolyte containing acetic acid and hydro-fluoric acid for the anodization of sputtered Ti films. They achieved 20-30 nm diameter nanotube arrays on RF sputtered films at 500°C using a variety of substrates including glass, silicon and alumina⁵. Anodization of thin films is a much less well-known process than foils due to its specific complications. The type of substrate is an important

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factor in the morphological formation and lattice orientation of Ti films. In addition, the deposition temperature, the stress mismatch of the substrate and Ti films, the thickness of the films, and the type of deposition all affect the films which in return affect the anodization process. Different types of fluoride-ion containing electrolytes such as acidic, neutral, aqueous, or non-aqueous electrolytes can be used in the anodization process. Grimes et al. first showed that well-ordered arrays of nanotubes with high surface to volume ratio can be obtained using fluoride-ion-containing neutral non-aqueous electrolytes such as ethylene glycol. There are many other methods for the manipulation of TiO₂ such as the usage of multistep anodization or altering the water content in the electrolyte.

In this work, we have investigated the formation of nanotubes on RF sputtered Ti films on indium tin oxide (ITO) coated substrates⁵. The conductivity and transparency of ITO are the key properties which are utilized in the fabrication of solar cells, displays, optical based chemical and biosensors, as well as many other devices. Knowing how to control the specifications of nanostructured metal oxide films, such as TiO₂ layers, which are formed onto these transparent and conductive substrates, is the key for the successful fabrication of the electro-optical and chromic devices. The formation of nanotubes in Ti films deposited onto ITO glass substrates is worth investigating⁶.

Anodization Process

Anodization of titanium occurs as a result of a competition between electrochemical oxide formation and chemical dissolution of oxide by fluoride ions. For anodization, the Ti material should be coated in a conductive slide along with a counter electrode. When there is no F⁻ ion in the media, a thin barrier metal oxide is formed on the metal surface as



The reaction can be enhanced by the application of an electric field which aids ion transport (O²⁻ and Ti⁴⁺ ions) through the growing oxide. However, as the oxide layer thickness increases in the anodization process, the electric field across the film is progressively reduced. This limits the oxidation process, and eventually the oxidation

current (transient current) drops. In this process, Ti⁴⁺ ions, arriving at the oxide/electrolyte interface, are not made soluble by complexation, and a hydroxide layer (Ti(OH)_xO_y) precipitates on the surface. This layer is typically loose and porous, and results in further diffusion-retarding effects. In the presence of fluoride ions, the situation is different.

Fluoride ions impose two effects:

- (i) They directly complex with the transported cations at the oxide electrolyte interface (thus preventing Ti(OH)_xO_y precipitation):



- (ii) They also have the ability to react with the oxide to form water-soluble [TiF₆]²⁻ complexes (thus, dissolution and breakdown of the barrier layer occurs along a random path through the barrier layer):



This means that the etching will continue, which causes the current to increase at the initial stage. Eventually, the rate of titanium oxide growth (first equation) assisted by electric field equals the rate of dissolution (second and third equations) by fluoride ions, which results in the constant barrier layer thickness. The current eventually decreases due to various effects such as a decrease in the diffusion of fluoride-containing species in and out of the tubes.

Materials and methods

For this study, titanium films of few μm thickness were first deposited onto ITO glass substrates using RF sputtering technique from a 99.99% pure Ti target⁵. RF power of 100-150 W was applied during the deposition process. The deposition time was varied from 1-15 minutes. Hence the film thickness was optimized. The sputtering chamber was pumped down to the background pressure of 10⁻⁶ torr before introducing the sputtering gas of 100% Ar with a pressure of 10-20 SCCM. The working pressure was about 8*10⁻²Torr.

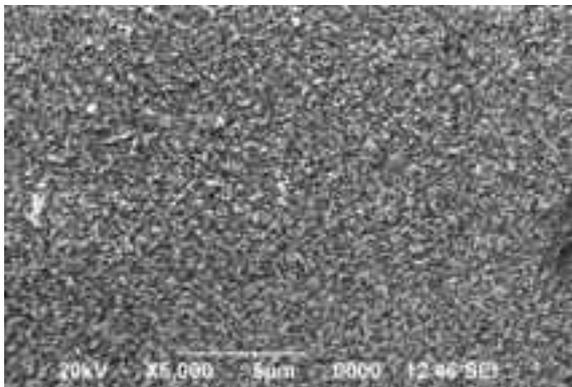


Fig.1 SEM

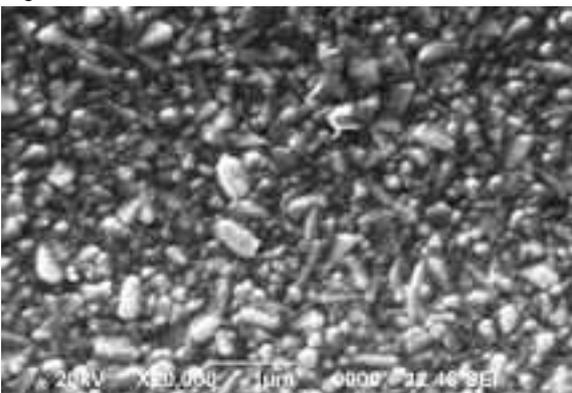


Fig.2 SEM

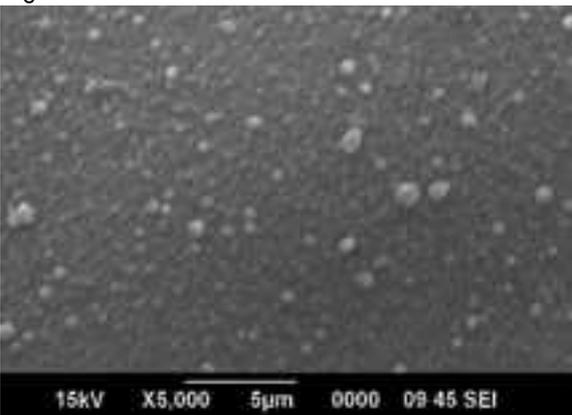


Fig.3 SEM

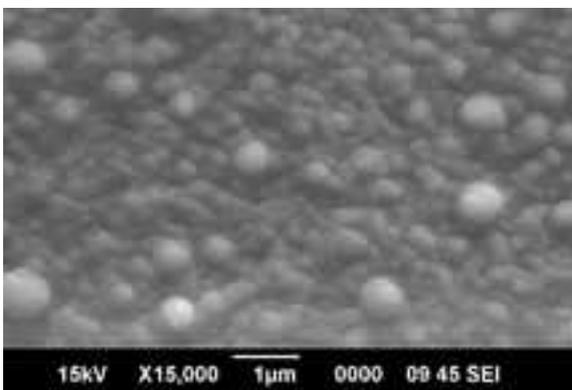


Fig.4 SEM

Anodization was performed in a neutral electrolyte medium of 0.07% (wt) NH₄F/ethylene glycol solution using a platinum wire cathode at room temperature⁶. TiO₂ layers were formed on Ti thin films using anodizing potentials around 1V. The anodizing time was about 30 minutes. Long-term stirring was causing the film to be peeled off during the anodization⁷. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) spectroscopy were employed to determine the surface morphology and the crystalline phase of the TiO₂ surface⁸. Using U-V visible spectrophotometer the absorbance and transmittance spectra of Ti and TiO₂ films were studied⁹. The thickness and resistance measurements were appreciable. Resistance of the film was measured using Keithley source measuring unit. As a device application, these TiO₂ films were tried in dye sensitized solar cell (DSSC)¹⁰.

Results and Discussion

Evaluation of surface morphology:

SEM images of the samples are given in Figures 1 and 2 under different magnifications.

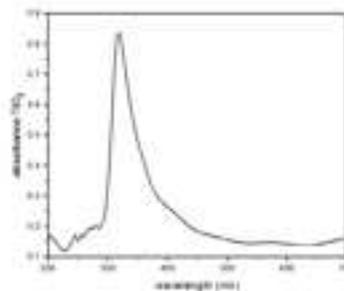


Fig. 5 Absorption spectra

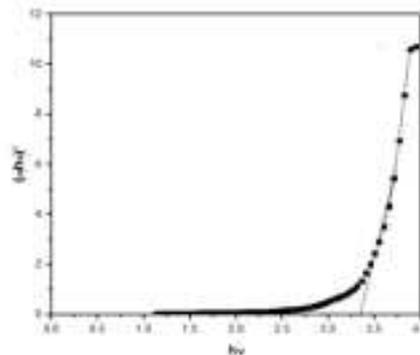


Fig.6 Absorption edge

From these figures, it can be seen that the morphology of the Ti films deposited at room temperature have solid granular deposition.

Figure 3 and 4 shows the morphology of anodized Ti film under different magnification and it can be seen that solid granular nature changes to spherical oxide particles.

Evaluation of optical properties:

From the UV-Vis spectra, figure 5 & 6, the band gap of TiO₂ was evaluated to be 3.34eV.

X-Ray Analysis

From the XRD pattern shown in figure (7) the rutile phase and crystalline nature of TiO₂ structure was confirmed. Also the average grain size was found to be 3.48A⁰.

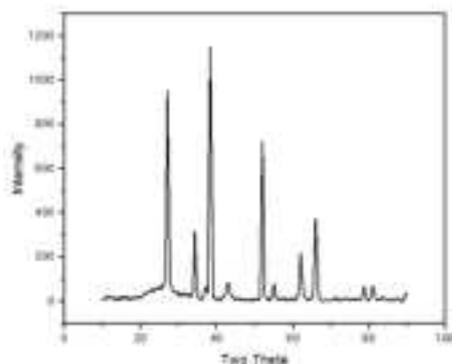


Fig. 7 XRD spectrum of TiO₂

Resistance measurements

Resistance measurements were done using Keithley source measuring unit. In titanium films resistance was found to decrease with increase in thickness, Table 1.

Table .1 Variation of resistance with sputtering time

Sputtered power (Watts)	Sputtered time (minutes)	Measured Resistivity (kilo-ohms per square)
100	1	1.02
100	1.5	0.253
100	2.0	0.118
150	10	0.038

Dye sensitized solar cell (DSSC)

The anodized TiO₂ samples were used for DSSC fabrication. Natural dye from pomegranate was used as sensitizer dye. The V-I characteristics of samples showed output in the power generation quadrant, i.e. the fourth quadrant. This is an appreciable result that hints to the feasibility of a functional solar cell. Here it is to be noted that the order of efficiency of the cell is very poor and it may be attributed to the high series resistance of the TiO₂ layer.

Conclusion

The anodisation parameters play a major role in the formation of well packed uniform layers. Sputtered Ti thin films were anodized by varying the reaction parameters like applied voltage, anodizing time, type of electrolyte and the solution concentration. The surface analysis of the samples was done using SEM and XRD. The best result was obtained for the electrolyte, NH₄F in ethylene glycol base under the optimum solution concentration of 0.07wt%, with an applied voltage of 1 V and the anodizing time of about 20 to 30 minutes. Smooth edged spherical oxide particles were observed in SEM picture.

The band gap of TiO₂ was evaluated to be 3.35 eV. The crystallite size was calculated from the FWHM of the XRD peaks. From the XRD peaks it was concluded that the anodized titanium film resulted in rutile phase. Resistance measurements were done using Keithley source measuring unit. It was found that the resistance of the

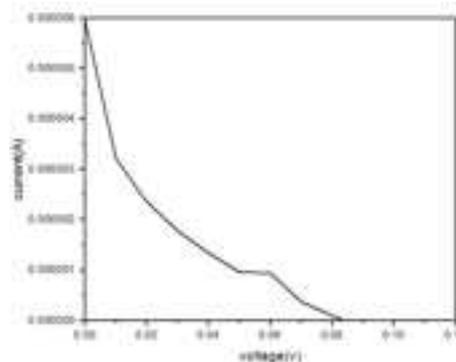


Fig .8 V-I characteristics of DSSC

titanium film decreases with increase in thickness. The resistance of Titanium dioxide film produced by anodisation of sputtered titanium layer was found to be very high. The feasibility of using this TiO₂ film for DSSC fabrication was also tried.

Acknowledgement

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Identification of sex-specific marker in *Calamus brandisii* Becc. (Arecaceae)

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Abstract

Calamus brandisii Becc. (Family Arecaceae) is an evergreen, dioecious threatened rattan widely used for the furniture and handicraft industry. Sexing of rattan seedlings at an early stage before the establishment of the plantations or seed stands can greatly enhance the long term survival and productivity. The present study is to identify molecular markers linked to sex determination using RAPD markers. Of the twenty random primers standardised, the primer OPAW 20 yielded a unique amplicon specific to male plants. It is feasible to identify sex at the early stages of plant life, which is beneficial for improving further breeding programs in *C.brandisii*.

Keywords: Sex determination, RAPD markers, Western Ghats, Dioecious rattan.

Introduction

Dioecy is generally associated with sexual dimorphism, is one of the most striking examples of evolutionary specialization. Although the majority of angiosperm species are hermaphroditic, approximately 6% are dioecious, with sexes segregated in separate individuals¹. Early sex identification in plants is especially those species whose female representatives are more desirable in the production process include *Borassus flabellifer*², *Carica papaya*³, *Hippophae rhamnoides*^{4, 5}, *Myristica fragrans*⁶ etc. In the case of non crop plants especially in rare and threatened Non Timber Forest Products, for planning effective restoration programmes the identification of male and female genotypes are necessary to ensure sufficient large number of productive female plants with only a minimal number of male plants. *Calamus brandisii* Becc. (Family Arecaceae) is a long-living, dioecious evergreen threatened rattan endemic to the Western Ghats of India (Fig.1). This species is distributed in the evergreen forests between 1000-1500 m at Agasthyamala Biosphere Reserve and Shendurney WLS in Kerala and in Kalakkadu and Upper



Fig. 1. *Calamus brandisii* from Shendurney WLS, India.

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Kothayar in Tamil Nadu. This is an excellent small diameter rattan, extensively used in furniture and handicraft industry. Being a dioecious rattan, in which the sex of plants cannot be identified till the commencement of flowering. The existing population has highly fragmented to disjunct patches and natural regeneration is very low and cultivation and breeding programs are urgently required to ensure the survival and sustainable use of this rattan. Maintaining the sex ratio in plantations is crucial for design of seed stands to produce sufficient seeds as planting material for plantations. The number of female plants should be more and early identification of male and female individuals can help to address the limitation of diecocy. In recent years, there have been serious efforts to understand the genetic basis of sex determination in plants and to develop methods to identify sex at an early stage by using molecular markers viz. RAPD, AFLP, ISSR etc. Among these different molecular markers, RAPD markers are widely used for identifying sex linked markers in angiosperms as in *Asparagus officinalis*⁷, *Simarouba glauca*⁸, *Cycas circinalis*⁹, *Myristica fragrans*¹⁰, *Momordica dioica*¹¹ etc. Hence in the present paper, we have attempted to identify sex-specific DNA markers of this rattan using RAPD markers.

Materials and methods

Field surveys were conducted to Bonacaud and Pandimotta forest areas in Kerala part of the Western Ghats and fresh leaf samples and herbarium specimens

Table 1. List of RAPD primers selected for the study

Sl. No	RAPD primers	Sequence (5'-3')
1	OPA-03	AGTCAGCCAC
2	OPA-04	AATCGGGCTG
3	OPA-09	GGGTAACGCC
4	OPA-11	CAATCGCCGT
5	OPA-12	TCGGCGATAG
6	OPA-13	CAGCACCCAC
7	OPA-15	TTCCGAACCC
8	OPA-16	AGCCAGCGAA
9	OPA-17	GACCGCTTGT
10	OPA-18	AGGTGACCGT
11	OPA-20	GTTGCGATCC
12	OPA-10	GTGATCGCAG
13	OPB-15	GGAGGGTGT
14	OPE-02	GGTGCGGGAA
15	OPE-18	GGACTGCAGA
16	OPAU-02	CCAACCCGCA
17	OPAW-07	AGCCCCAAG
18	OPAW-09	ACTGGGTCCG
19	OPAW-10	GGTGTGTTGCC
20	OPAW-20	TGTCCTAGCC

of *Calamus brandisii* were collected from identified male and female plants.

All the herbarium specimens were deposited at Kerala Forest Research Institute (KFRI) Herbarium. Total DNA was extracted from 1g of the leaf tissues using

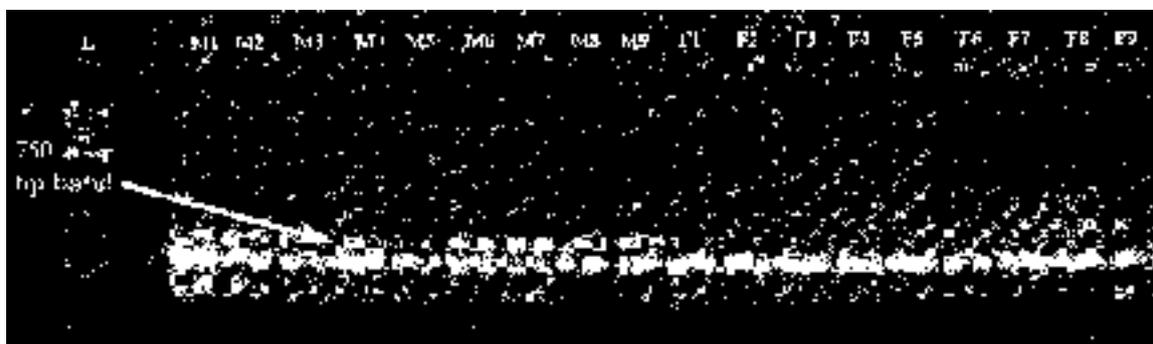


Fig.2. RAPD banding profile of male, female plants obtained by the primer OPAW 20, arrow indicates male specific 750 base pair.

the modified CTAB protocol¹² and the purified DNA was then re-suspended and stored in 100 µl TE buffer. Random primers were selected from the previous screening done on *Calamus* of the Western Ghats¹³. Only those 20 primers (OPA, OPB, OPE, OPAU, OPAW) were selected which have more than 60% polymorphism. These selected 20 polymorphic primers are listed in Table 1. PCR reactions were performed on a PTC-200 Thermocycler (MJ research., USA) in 25µl reaction volumes¹⁴ with 50ng of template DNA, 1mM of dNTP mix, 5 picomoles of primer, 1.5 unit of Taq DNA polymerase, 10mM MgCl₂ and 10X Taq buffer with 15mM MgCl₂. The amplification was performed with an initial denaturation of 95^o C for 4 minutes and 45 cycles of denaturation at 94^o C for 1 minute, 1 minute annealing (36^oC) and extension at 72^oC for 2 minutes. The last cycle was followed by a final extension of 72^oC for 4 minutes.

Results and discussion

Among the 20 random primers used in the present investigation, OPAW 20 yielded a unique amplicon of 750 bp only in male plants (Fig. 2).

This DNA marker is found to be consistent with a potential to develop as a male specific SCAR marker to detect male plants in *Calamus brandisii*. In rattans and other palms, sex-specific molecular markers have been identified in *Calamus simplicifolius*¹⁵, *Borassus flabellifer*¹⁶, *Calamus tenuis*¹⁷ and *Phoenix dactylifera*^{18,19} using RAPD and ISSR markers. Among these¹⁵, standardisation was done in approximate 500-bp male-specific DNA fragment with the S1443 primer using RAPD markers. Molecular studies of dioecious species indicated that most of the sex-determining genes were found in male plants^{20,21} which suggests that males play a vital role in the sex determination of dioecious plants. The characteristics of male plants, including prolonged and earlier flowering cycle²² and more frequent reproduction compared to females, might have suggested that male plants are somehow crucial for sex determination. Generally, in rattans

sex of the plants becomes known only at the time of first flowering, which takes around 4-5 years. Hence, an early identification of sex can help to address the limitation of dioecy and this will be beneficial to improve restoration activities, breeding programmes and developing seed stands. In addition, we have obtained a proper RAPD protocol that is useful for other species of rattan. A RAPD marker OPAW-20₇₅₀ band consistently appeared in male genotypes, suggesting thereby the male associated nature of this DNA marker in *C. brandisii*. OPAW-20₇₅₀ proved to be constant reproducible under a wide variation of amplification conditions and this marker can be used for sex determination of male genotypes which can be used for screening seedlings in forest department nurseries especially for restoration programmes. Even though the reproducibility of RAPD markers are questioned and it is recommended that RAPD markers are converted to specific SCAR markers which possess several advantages over the former one, such as: (1) robust reproducible PCR amplification²³ with minimum effect of reaction conditions and (2) locus specificity amenable to easy detection/scoring on agarose gel. In the case of *C. palustris*, a subtractive library was constructed for male floral tissue to understand the genetic mechanism for gender determination and concluded that the male floral genes may play a vital role in sex determination²⁴. In the present study, the screened RAPD primer (OPAW 20) gave reproducible results for the discrimination of male plants. The generated unique bands from male plants can be sequenced and could be used further for identification of sex at early stage of seedlings. Further, more specific primers can be designed from these generated sequences which could be used for sex identification of *C. brandisii* in a more precise way at seedling stage.

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Comparative study of proximate composition and nutritional status of major fresh water food fishes during post monsoon season in the culture and capture fisheries of Alappuzha District, Kerala, India

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Abstract

The basic biochemical constituents of fish are often referred to as proximate composition. Five species of fish (*Channa striatus*, *Oreochromis mossambicus*, *Anabas testudineus*, *Etrophus suratensis* and *Heteropneustes fossilis*) were collected from a brackish water farm and from a lotic habitat in Alappuzha district for the comparison of proximate composition. The protein content was comparatively less in capture fishes and 16.7% in culture fishes. 17% Protein content average was noticed in capture fishes and 16.7% in culture fishes. And in both capture and culture type, minimum value was seen in *Etrophus suratensis*. In both culture and capture fishes selected, minimal value of carbohydrate content was obtained in *Anabas testudineus* and maximum value is in *C.striatus*. In the present study lipid content in culture fishes have a higher value than that of capture fishes. Average ash content in capture fishes was 7.06% and in culture fishes 6.11 %. Minimum ash content was observed in *O. mossambicus*. Feeding habit were assessed and grouped under various categories. The present study is an attempt to compare the proximate composition of different species of culture and capture fishes during post monsoon season.

Keywords: Protein, lipids, carbohydrates, brackish water, proximate composition

Introduction

Fish can be considered as a natural food resource, not only in terms of bio diversity but also a source of high quality animal protein, therapeutically important polyunsaturated fatty acids, calcium, iodine, vitamins, and several other nutrients¹¹. The major constituents of fish are water, protein, lipid, and ash. Carbohydrate is seen only in negligible amount. Proximate composition of fish generally varies according to season, age, maturity, sex and availability of food⁷. Fish oil may protect the brain from cognitive problems associated with Alzheimer's disease, those with rheumatoid arthritis, psoriasis or other auto-immune disorders and

age related blindness^{4,11} India is presently the world's fourth highest fish producer and the second highest inland fish producer⁴. The south-western state of India - Kerala is gifted with large number of water bodies and different species of fish. Kerala state has a total coastline of 590 Kms. Fishing is a major occupation and a main source of income.

In general, the biochemical composition of fish indicates the quality. So, proximate or biochemical composition of a species showed its nutritional quality and helps to assess its nutritional and edible value⁸. Variation in the composition of fish may occur within same species depending upon

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the fishing ground, age, fishing season, sex of the individual and the reproductive status. From the ancient time onwards, human population include fish as a major part of their daily diet. Due to the presence of high quality proteins, which contain all essential amino acids, they are considered as beneficial nutritional sources¹⁵.

Proper knowledge on the biochemical composition of fish finds application in several areas. Different food products are made from fishes which are quite nutritious for health and are beneficial. For formulating such products, proper data on the biochemical composition is very essential. Another vital area where accurate information on biochemical composition is processing and preservation of fish and fishery products. Fish is an easily perishable commodity and deterioration in quality is due to the changes taking place to the various constituents like proteins, lipids etc. Generally changes in chemical composition of body reflect storage or depletion of energy reserves. Feeding habit, environment and genetic trait are also known to influence chemical composition of fish¹³.

The significance of the investigation relies on many relevant reasons. Different species of fishes are available in our market and they are being cultured in many farms around us. But fishes available in our markets are not of first quality. They are available only after several days of preservation and lose its natural quality. It is being preserved in less amount of Formalin, Ammonia and many other preservatives. Such preservatives are harmful for human health as well as affect the quality of fishes. So culture fishes are more preferable for edible purposes. Culture fishes are devoid of such preservatives and chemicals. They can

be fed with fish feeds and domestic wastes and it is economically more feasible as they attain large size within a short span. Culture fishes grow faster than capture fishes.

Materials and Methods

The major food fishes such as *Channa striatus*, *Oreochromis mossambicus*, *Anabas testudineus*, *Etroplus suratensis* and *Heteropneustes fossilis* were selected with almost equal size and weight (Table 1). The weight of selected capture and culture fishes varied from 15-18 cm in capture and 9-17cm in culture. They were procured from different places and farms in Alappuzha District, Kerala. Muscle between the gills and the dorsal fins were used for analyses in triplicates. In the present investigation, both culture and capture fishes of same species were taken and proximate composition was estimated in which moisture content, ash content, protein, carbohydrate, lipid were estimated and tabulated with graphical representation.

Moisture content was estimated by the method in FAO (1989)⁴. Total lipid was estimated by the method of Folch *et.al*, (1957)⁶. Protein estimation was done by the method of Lowry *et.al*.¹⁰. Dubois *et.al*³. (1956) method was used for the estimation of carbohydrate (Phenol-Sulphuric method).

Estimation of nutritional value

The importance of a species depends on its nutritional value. The fishes were classified into five types based on the fat and protein contents in the muscle¹⁴.

Note :Microsoft Excel 2007 was used for statistical analysis.

Results and Discussion

Species wise variation of the proximate

Category	Type	Oil content (%)	Protein content (%)
A	Low oil- high protein	Less than 5	15-20
B	Medium oil- high protein	5 -15	15-20
C	High oil – low protein	More than 15	Less than 15
D	Low oil- very high protein	Less than 5	More than 20
E	Low oil- low protein	Less than 5	Less than 5

Table 1. Length and weight of selected food fishes under study

Fishes	Channa		Etroplus		Anabas		Oreo-chromis		Heteropneustes	
	Capture	Culture	Capture	Culture	Capture	Culture	Capture	Culture	Capture	Culture
Length (cm)	18	18.5	15.7	16.5	18.2	17.2	16.7	16.4	24.1	26.2
Breadth (cm)	4	3.5	7.2	5.2	4.6	3.3	5.7	4.5	5.7	3.8
Weight (gm)	55.50	38.40	65.24	34.55	70.45	19.68	70.15	33.3	56.10	40.62

Table 2. Proximate composition of selected capture and culture fishes during post monsoon season

FISH SPECIES	Carbohydrate (%)		Moisture content(%)		Lipid(%)		Protein(%)		Ash(%)	
	Capture	Culture	Capture	Culture	Capture	Culture	Capture	Culture	Capture	Culture
<i>Channa striatus</i>	0.22± 0.01	0.26± 0.05	66.34± 0.02	63.77± 0.01	12.9± 0.01	13.6± 0.01	15± 0.01	19.5± 0.02	7.63± 0.02	6.30± 0.01
<i>Etroplus suratensis</i>	0.18± 0.02	0.20± 0.03	71.31± 0.07	64.36± 0.02	10.8± 0.01	12.5± 0.01	12.5± 0.02	17± 0.02	6.52± 0.01	7.32± 0.01
<i>Anabas testudineus</i>	0.18± 0.02	0.17± 0.03	62.5± 0.03	64.85± 0.02	11.4± 0.01	12.1± 0.02	18± 0.02	20± 0.03	8.30± 0.02	6.25± 0.04
<i>Oreochromis mossambicus</i>	0.21± 0.04	0.24± 0.02	64.50± 0.01	57.28± 0.03	11.1± 0.02	12.9± 0.02	21.5± 0.01	24.5± 0.02	5.38± 0.01	5.19± 0.02
<i>Heteropneustes fossilis</i>	0.16± 0.01	0.19± 0.03	59.22± 0.02	54.28± 0.02	18.9± 0.04	19.4± 0.02	18± 0.01	22.5± 0.01	7.48± 0.01	5.51± 0.01

composition of selected five fish species were reported and presented in Table 2 and Fig.1a-e. It has been observed that the species are protein rich during post monsoon season.

The principle constituents are water (66 – 84%), protein (15 – 24%), lipids (0.1 – 22%), minerals (0.8 – 2%) and sugar in very minute quantity (0.3%) at maximum value in fishes⁶. The proximate composition of Indian fishes ranged between 65 – 90% water; 10 – 22% protein, 01 – 20% lipid and 0.5–05% minerals¹³. However main changes in body occur in moisture

and lipid content.

In the present study, moisture content was high in capture fishes. Maximum value was found in *E.suratensis* (71.31%) and minimum value in *H.fossilis* (59.22%), (Table 2). While in culture fishes, it was 64.85% in *A.testudineus* and minimum value 54.28% in *H.fossilis*. Estimations indicate that composition was not similar in selected fishes.

Maximum value was 21.5 % in *O.mos-sambicus* and minimum value was 12.5% in *E.suratensis*. But in culture type,

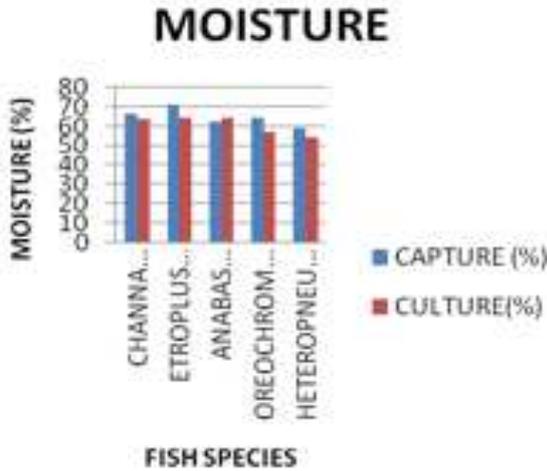


Fig. 1a. Moisture content of selected fishes from culture and capture fisheries during postmonsoon season

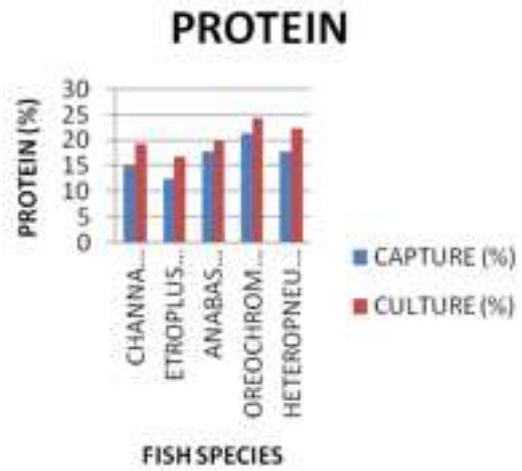


Fig. 1b. Protein content of selected fishes from culture and capture fisheries during postmonsoon season

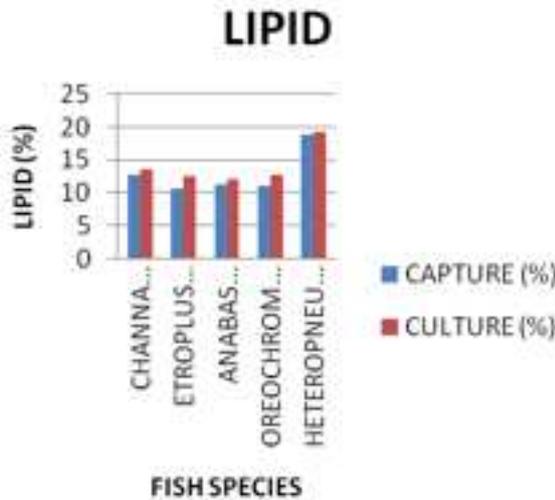


Fig. 1c. Lipid content of selected fishes from culture and capture fisheries during postmonsoon season

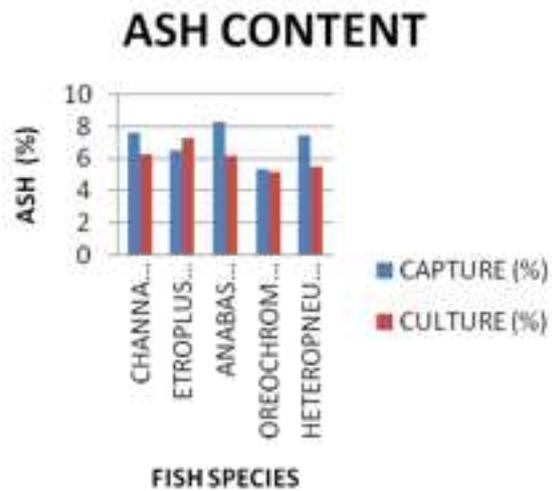


Fig. 1d. Ash content of selected fishes from culture and capture fisheries during postmonsoon season

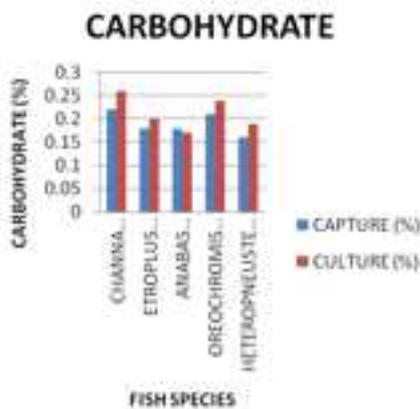


Fig. 1e. Carbohydrate content of selected fishes from culture and capture fisheries during postmonsoon season

maximum protein content is 24.5% and was found in *H. fossilis* and minimum is 17% and was found in *E.suratensis* respectively. Average of protein content is 17% in capture fishes and 16.7% in culture fishes. And it was found that in both capture and culture type, minimum value is seen in *E.suratensis*. According to the studies done before, it is found that, carbohydrates is absent or if present only in very minor quantities in fishes and hence plays a major role in activities⁹. The low values of carbohydrates recorded in present study supports the view that carbohydrate plays an insignificant role as energy

Table 3. Gut contents in the selected fish species studied

Contents	CHANNA		ETROPLUS		ANABAS		OREO-CHROMIS		HETERO-PNEUSTES	
	Capture	Culture	Capture	Culture	Capture	Culture	Capture	Culture	Capture	Culture
Crustaceans	P	A	A	A	A	A	A	A	P	A
Insect Remains	P	P	A	A	P	A	A	P	A	P
Fish Remains	A	A	A	P	A	P	P	A	P	A
Mud & Sand	A	A	P	A	A	A	A	A	P	A
Detritus	A	A	P	A	P	A	P	A	P	A
Algal Remains	A	A	P	P	A	A	P	P	A	P
Shell Remains	P	A	A	A	A	A	A	A	P	A
Spines & fins	A	A	A	P	P	P	A	A	P	P
Exoskeletons of Arthropods	P	A	A	A	P	A	A	A	P	A
Farm food	A	P	A	P	A	P	A	P	A	P
Plant materials	P	A	A	A	P	A	A	A	A	A
Worms & micro organisms	P	P	A	P	A	A	A	A	P	A
Others	P	P	P	P	P	P	P	P	P	P

reserve in aquatic animals⁹. In the present study, carbohydrate content ranged from 0.10-0.30 % and values were very low compared to other fishes. Minimum carbohydrate content was observed in *A.testudineus* (0.13 %) and maximum in *C.striatus* (0.22%) among capture fishes and *A.testudineus* has a low content (0.17%) and highest in *C.striatus* (0.26%) among culture fishes respectively. Average carbohydrate in capture fishes is assessed as 0.216% and 0.212 % in culture fishes. Hence it is found that, both in culture and capture fishes selected, minimal value of carbohydrate content obtained is in *A.testudineus* and maximum value is in *C.striatus*.

Lipids are the primary energy storage material in fish⁹. Lipid content is a good index of future survival in some species. Depending on the level of fat contents, fish can be grouped into four categories: lean fish (<2%), low fat (2-4%), medium fat (4-8%) and high fat (>8%) fish (Ackman,

1989). After estimation of lipid, maximum content is found in *H. fossilis* in capture (18.9%) and in culture also, it has the highest value (19.4%)(Figure 1). At the same time, minimum content is observed in *E.suratensis* in capture fishes and in *Anabas*, it is 12.1% among culture fishes. Lipid values ranges from 10%- 20% and average is 13.02% in capture and 14.1% in culture fishes (Figure 1) In fact, total lipid and its composition in fish vary more than any other nutrient component. Average value of result obtained is 13.02% in capture fishes and in culture fishes it is 14.1%. From the above result, the culture fishes have a higher value than that of capture fishes. The concentration of lipid varies considerably in different parts of the body of the fish⁹.

Ash content of selected fishes were assessed and tabulated. Highest content was observed in *A.testudineus* as (8.30%) and lesser content in *O.mossambicus* (5.38%) among capture fishes while

E. suratensis (7.32%) has higher content and *O. mossambicus* (5.19%) has lesser content among culture fishes. It is found that, both in culture and capture fishes, minimum content was observed in *O. mossambicus*. Average ash content in capture fishes is 7.06% and in culture fishes it is 6.11 %.

Feeding habit was identified using gut content analysis (Table 3). Presence and absence of different types of food materials like crustaceans, shell and insect remains, detritus, fins and fish remains, plant materials, mud and algal remains etc. were observed during the analysis and hence the feeding habit of fishes were identified. *C. striatus* and *A. testudineus* are included under carnivorous type whereas *E. suratensis*, *O. mossambicus* and *H. fossilis* are omnivorous type.

The importance of a species depends on its nutritional value. Fishes are classified the fishes into five types based on the fat and protein contents in the muscle¹⁴. According to the present results, *C. striatus* can be included under the category B (Medium oil-high protein), the oil content recorded was in between 5-15 % and protein content recorded was between 15-20%. In this study *E. suratensis* can be included under the category B with respect to oil and protein content. In *A. testudineus*, it can be included under the category B in which protein is high and less oil content based on the feeding habit. *Oreochromis mossambicus* is included under the category B in which oil content is medium and in category D where protein is very high. *H. fossilis* was also examined and is included under the category C, where oil content as well as protein content is very high. Carbohydrate content ranged from 0.10-0.30 % and values were very low compared to others. The low values of carbohydrates recorded in the present study supports the view that carbohydrate plays an insignificant role as energy reserve in aquatic animals (Love, 1980). Lipid content is a good index of future survival in

some species. The percentage ash content in the fishes analysed is an indication of mineral content in fish. The proximate composition of a species helps to assess its nutritional and edible value compared to other species. The principal constituents are water (66 to 84 %), protein (15 to 24 %), lipids (0.1 to 22 %), minerals (0.8 to 2 %) and carbohydrate in very low quantity (0.3%) at maximum value in fishes⁴. The biochemical constituents are influenced by metabolism, mobility of the fish and geographical area¹⁴. In the present investigation variations were obtained in the biochemical composition of the fish muscles of different fishes under study, which may be the result of the above processes. The main constituent in edible parts is moisture content & protein content is comparatively less in *H. fossilis* from capture and culture. Minimal value of carbohydrate content obtained is in *A. testudineus* and maximum value is in *C. striatus* and culture fishes have higher lipid content than that of capture fishes. Both in culture and capture fishes, minimum ash content was observed in *O. mossambicus*. Present study reveals that culture fishes have comparatively higher nutritional value and quality than capture fishes. Lowest protein content was recorded in *Etroplus suratensis* (12.5±0.02) and highest value (24.5±0.02) was observed in *Oreochromis mossambicus* (both in culture and capture fisheries) which couples with high feeding intensity observed by the gorged conditions of stomach. In the present study, all fishes were fat fishes with moderate fat content.

Conclusion

The knowledge of chemical composition of any edible organism is extremely important as the nutritive value is reflected in its biochemical composition. The principal constituents of fish and mammals are the same. During long starvation period it may utilize protein in its body to survive. In general, the biochemical composition of the whole body indicates the fish quality.

The present study revealed the changes in the body composition of culture and capture fishes. In the present study *Oreochromis* is protein rich so it is an important source of animal protein for the local people. Since the feeding habit showed this species is an omnivorous feeder and can be easily cultivable.

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Distribution profile of angiosperms across elevation discontinuity of Palghat Gap in Southern Western Ghats

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Abstract

Floristic documentation in two forest areas on the north and south of the 32km wide elevation discontinuity of the Southern western Ghats, the Palghat Gap has been undertaken to assess the distribution continuity across the topographic discontinuity. The Kollengode forest range is situated on the South of Palghat Gap and is home to 955 species of angiosperms belonging to 135 families and the Olavakkode range has 655 species of angiosperms coming under 105 families. Of the taxa analysed, 103 families and 302 species are common in both the ranges and the average taxonomic singularity is 0.821 in Kollengode forests while it is 0.845 in Olavakkode range. The endemic taxa recorded in Kollengode forests are 216, while it is 93 in Olavakkode range. It is observed that 8 taxa of Olavakkode range has an exclusive Northward distribution and in contrast, 18 species found in Kollengode range has an exclusive southward distribution. *Strobilanthes lanatus* Nees, *Meteoromyrtus wynaadensis* (Bedd.) Gamble and *Justicia glauca* Rottler from north of Palghat Gap and *Ceropegia thwaitesii* Hook., *Givotia moluccana* (L.) Sreem. and *Diospyros ebenum* J. Koenig from South of the Gap have successfully crossed the dispersal barrier of the mountain pass. The instances of taxa showing habitat sharing on either sides are very less and hence point to the Palghat Gap acting as a barrier in the continuous distribution of taxa.

Key words: Palghat Gap, distribution, dispersal barrier

Introduction

Distribution of vegetation patterns are limited by contrasting climatic regimes. Oceanic foothills in the north and strongly continental inter mountain basins in the south of Siberian mountains revealed that winter and summer temperatures and precipitation exerted a dominant influence on species composition¹. The study also revealed that on a more local scale, the main source of variation in species composition was topography, producing landscape patterns of contrasting plant communities on slopes of different aspects and valley bottoms. More over the inquiry on the abundance of alien species was found to be declining with the altitude and in contrast, species richness

among comparable native taxa appeared to be nearly independent of altitude over the same range².

Palghat Gap in Southern Western Ghats is a natural discontinuity which forms a 32km wide corridor between the political boundary between Kerala and Tamilnadu. The Palghat Gap forms an elevation and topographic discontinuity and whether the distribution of plants across the Gap points to the Gap acting as a barrier for dispersal of plants is debatable. In this backdrop, the floristic analysis of two forest ranges, viz. Olavakkode range in north of Palghat Gap and Kollengode range in south of Palghat Gap have been carried out to understand the distributional continuity of angiosperms

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across the Gap. Special focus has been given to the distribution of threatened and endemic taxa.

Materials and methods

Explorations and collection of angiosperm taxa in the forests of Olavakkode range lying between 10° 45' and 10° 55' North latitude and 76° 50' and 76° 10' East longitude and Kollengode range lying between 10° 25' and 10° 55' North latitude and 76° 35' and 76° 55' East longitude have been done between 2015 and 2018, along with collection of information on distribution of taxa in southern Western Ghats emphasizing Palghat gap. Identification was ascertained with the help of Flora^{3,4,5,6}. The updation of nomenclature was done with and IPNI⁷, Plant list⁸, Tropicos⁹. Threat status was resolved as per IUCN version 13¹⁰ and alien invasive plants were identified from the latest literature¹¹.

Results

Species diversity

The explorations revealed the presence of 1610 species of angiosperms in the forests of the two ranges. The forests in Kollengode range has 955 species belonging to 135 families and the forests in Olavakkode range has 655 species belonging to 105 families.

Distributional discontinuities across the gap

In Kollengode forest range, out of the 955 species, 77 are new distributional record to the district of Palakkad district (Table 1). Of these, 31 species are reported to be present in regions south and north of Palghat Gap, while 23 have been reported only in regions south of Palghat Gap. Three species that were earlier reported only in regions north of Palghat Gap are *Strobilanthes lanatus* Nees, *Meteoromyrtus wynaadensis* (Bedd.) Gamble and *Justicia glauca* Rottler. In Olavakkode forest range out of the 655 species 18 are new distributional record for the district of Palakkad. In the Olavakkode range 16 species are seen that are having exclusively southward distribution and are

examples of range expansion from south of the Palghat Gap to the North. There are 6 species present in Olavakkode range that have a continuous distribution in the northward regions of Palghat Gap and hence have been unable to expand across the discontinuity. The species known to be growing in both these ranges number 302 and are examples of continuous distribution of taxa across the Palghat Gap.

New discoveries

Floristic exploration during this study in Kollengode range has brought to light the presence of taxa hitherto not known to science. *Sonerila nairii* Soumya & Maya¹², *Sonerila victoriae* Soumya & Maya¹³, *Oldenlandia vasudevanii* Soumya & Maya¹⁴, *Impatiens sasidharanii* var. *sasidharanii*^{15a}), *Impatiens sasidharanii* var. *hirsuta*^{15b} are new discoveries from Kollengode range. Olavakkode forest range is the type location for three new species, *Chlorophytum palghatense*¹⁶, *Zingiber sabuanum*¹⁷ and *Justicia gambleana*¹⁸ and an earlier exploration has helped to rediscover *Impatiens concinna*¹⁹ from forests of Olavakkode range

Distribution of taxa with adaptive traits

The insectivorous genera *Drosera* is represented by three species namely, *D. indica* L., *D. burmanii* Vahl. and *D. peltata* Thunb. of which only the former is showing continuous distribution across the Gap. Another insectivorous genera *Utricularia* is represented in the forests of both ranges, with two species, *U. lazulina* P.Taylor and *U. graminifolia* Vahl. showing continuous distribution across the Gap. *U. aurea* Lour. and *U. praeterita* P.Taylor are present in Kollengode range, but not yet recorded in Olavakkode range, while *U. albocaerulea* Dalzell is present in the Olavakkode range, but not in Kollengode range. The Orchid flora of Kollengode range is very diverse consisting of 27 species under 16 genera. Olavakkode range harbors much less species of Orchids with 8 species under 8 genera and the two ranges have very unique orchid flora since none of the species is common to both the ranges.

Table 1. Taxa new distributional record to Palakkad from Kollengode range

Sl. No	Binomial	Family
1.	<i>Alseodaphne semecarpifolia</i> Nees var. <i>malabarica</i> Robi & Udayan	Lauraceae
2.	<i>Andrographis atropurpurea</i> (Dennst.) Alston	Acanthaceae
3.	<i>Andrographis elongata</i> (Vahl) T.Anderson	Acanthaceae
4.	<i>Asystasia crispata</i> Benth.	Acanthaceae
5.	<i>Biophytum intermedium</i> Wight	Oxalidaceae
6.	<i>Blumea laevis</i> (Lour.) Merr.	Asteraceae
7.	<i>Blumea oxyodonta</i> DC.	Asteraceae
8.	<i>Cajanus rugosus</i> (Wight & Arn.) Maesen	Fabaceae
9.	<i>Cardamine africana</i> L.	Brassicaceae
10.	<i>Cassytha filiformis</i> L	Lauraceae
11.	<i>Centranthera tranquebarica</i> (Spreng.) Merr.	Orobanchiaceae
12.	<i>Ceropegia maculata</i> Bedd.	Apocynaceae
13.	<i>Cestrum aurantiacum</i> Lindl.	Solanaceae
14.	<i>Cestrum nocturnum</i> L.	Solanaceae
15.	<i>Cheirostylis parvifolia</i> Lindl	Orchidaceae
16.	<i>Cladopus hookeriana</i> (Tul.) C.Cusset	Podostemaceae
17.	<i>Commelina clavata</i> C.B. Clarke	Commelinaceae
18.	<i>Crinum asiaticum</i> L.	Amaryllidaceae
19.	<i>Crotalaria grahamiana</i> Wight & Arn.	Fabaceae
20.	<i>Cryptocarya stocksii</i> Meisn.	Lauraceae
21.	<i>Cyanotis burmanniana</i> Wight	Commelinaceae
22.	<i>Cyathula tomentosa</i> (Roth) Moq.	Amaranthaceae
23.	<i>Cyperus digitatus</i> Roxb.	Cyperaceae
24.	<i>Diodella teres</i> (Walter) Small	Rubiaceae
25.	<i>Diospyros ebenum</i> J.Koenig ex Retz.	Ebenaceae
26.	<i>Dodonaea viscosa</i> subsp. <i>angustifolia</i> (L.f.) J.G.West	Sapindaceae
27.	<i>Drimia indica</i> (Roxb.) Jessop	Asparagaceae
28.	<i>Echinochloa frumentacea</i> Link	Poaceae
29.	<i>Eriocaulon nepalense</i> Bong. var. <i>luzulifolium</i> (Mart.) Praj. & J.Parn	Eriocaulaceae
30.	<i>Gymnema decaisneanum</i> Wight	Apocynaceae
31.	<i>Hardwickia binata</i> Roxb.	Fabaceae
32.	<i>Impatiens sasiharanii</i> var. <i>hirsute</i> Prabhukumaret al.	Balsaminaceae
33.	<i>Impatiens sasiharanii</i> var. <i>sasiharanii</i> Prabhukumaret al.	Balsaminaceae
34.	<i>Ipomoea indica</i> (Burm.) Merr.	Convolvulaceae
35.	<i>Isodon lophanthoides</i> (Buch.-Ham. ex D.Don) H.Hara	Lamiaceae
36.	<i>Justicia glauca</i> Rottler	Acanthaceae
37.	<i>Lagerstroemia parviflora</i> Roxb.	Lythraceae

38.	<i>Limnophila aromatica</i> (Lam.) Merr.	Plantaginaceae
39.	<i>Linum mysurense</i> B.Heyne ex Benth.	Linaceae
40.	<i>Luisia tenuifolia</i> Hook.f	Orchidaceae
41.	<i>Memecylon bremeri</i> M.B.Viswan.	Melastomataceae
42.	<i>Meteoromyrtus wynaadensis</i> (Bedd.) Gamble	Myrtaceae
43.	<i>Mollugo nudicaulis</i> Lam.	Molluginaceae
44.	<i>Murdannia gigantea</i> (Vahl) G. Brückn.	Commelinaceae
45.	<i>Murdannia crocea</i> subsp. <i>ochracea</i> (Dalzell) Faden	Commelinaceae
46.	<i>Neanotis indica</i> (DC.) W.H.Lewis	Rubiaceae
47.	<i>Oldenlandia vasudevani</i> Soumya & Maya	Rubiaceae
48.	<i>Osbeckia gracilis</i> Bedd.	Melastomataceae
49.	<i>Pandanus kaida</i> L.	Pandanaceae
50.	<i>Phyllocephalum courtallense</i> (Wight) Narayana	Asteraceae
51.	<i>Pinalia mysorensis</i> (Lindl.) Kuntze	Orchidaceae
52.	<i>Piper hapnium</i> Buch.-Ham.	Piperaceae
53.	<i>Premna paucinervis</i> (C.B.Clarke) Gamble	Lamiaceae
54.	<i>Pupalia lappacea</i> var. <i>orbiculata</i> (Heyne ex Wall.) Juss.	Amaranthaceae
55.	<i>Scutellaria wightiana</i> Benth.	Lamiaceae
56.	<i>Sesamum indicum</i> L.	Pedaliaceae
57.	<i>Smithia gracilis</i> Benth.	Fabaceae
58.	<i>Solanum viarum</i> Dunal	Solanaceae
59.	<i>Solanum aculeatissimum</i> Jacq.	Solanaceae
60.	<i>Sonerila nairii</i> Soumya&Maya	Melastomataceae
61.	<i>Sonerila veldkampiana</i> Ratheesh, Mini & Sivadasan	Melastomataceae
62.	<i>Sonerila victoriae</i> Soumya & Maya	Melastomataceae
63.	<i>Spermacoce hispida</i> L.	Rubiaceae
64.	<i>Sporobolus tenuissimus</i> (Schrank.) Kuntze	Poaceae
65.	<i>Sterculia balanghas</i> L.	Malvaceae
66.	<i>Sterculia foetida</i> L.	Malvaceae
67.	<i>Strobilanthes consanguineus</i> Clarke	Acanthaceae
68.	<i>Strobilanthes lanatus</i> Nees	Acanthaceae
69.	<i>Stylosanthes hamata</i> (L.) Taub.	Fabaceae
70.	<i>Taxillus recurvus</i> Tiegh.	Loranthaceae
71.	<i>Tephrosia maxima</i> (L.) Pers	Fabaceae
72.	<i>Tephrosia pumila</i> (Lam.) Pers.	Fabaceae
73.	<i>Thunbergia tomentosa</i> Wall. ex Nees	Acanthaceae
74.	<i>Tiliacora racemosa</i> Colebr.	Menispermaceae
75.	<i>Trichosanthes tricuspидata</i> Lour var. <i>tomentosa</i>	Cucurbitaceae
76.	<i>Tylophora ovata</i> (Lindl.) Hook. ex Steud.	Apocynaceae
77.	<i>Zingiber wightianum</i> Thwaites	Zingiberaceae

Table 2. New distributional record to Palakkad from Olavakkode range

Sl. No.	Binomial	Family
1	<i>Thottea sivarajanii</i> E.S.S.Kumar, A.E.S.Khan&Binu	Aristolochiaceae
2	<i>Miliusa gokhalaei</i>	Annonaceae
3	<i>Dioscorea belophylla</i> (Prain) Voigt ex Haines	Dioscoreaceae
4	<i>Dioscorea hamiltonii</i> Hook.f.	Dioscoreaceae
5	<i>Dioscorea wightii</i> Hook.f.	Dioscoreaceae
6	<i>Phrynium pubinerve</i> Blume	Marantaceae
7	<i>Amomum ghaticum</i> K.G.Bhat	Zingiberaceae
8	<i>Eriocaulon conicum</i> (Fyson) C.E.C.Fisch.	Eriocaulaceae
9	<i>Cymbopogon citratus</i> (DC.)Stapf	Poaceae
10	<i>Ischaemum barbatum</i> Retz.	Poaceae
11	<i>Elatostema cuneatum</i> Wight	Urticaceae
12	<i>Memecylon randerianum</i> S.M.Almeida&M.R.Almeida	Melastomataceae
13	<i>Allophyllus serratus</i>	Sapindaceae
14	<i>Cyathula tomentosa</i>	Amaranthaceae
15	<i>Diospyros ebenum</i> J.Koenig ex Retz.	Ebenaceae
16	<i>Justicia japonica</i> Thunb.	Acanthaceae
17	<i>Andrographis elongata</i> (Vahl) T.Anderson	Acanthaceae
18	<i>Utricularia albocaerulea</i> Dalzell	Lentibulariaceae

Discussion and conclusion

The Kollengode and Olavakkode forest ranges are located respectively at the southern and northern boundaries of the Palghat Gap, a 32km wide natural discontinuity of the Southern Western Ghats in the district of Palakkad of Kerala state. The elevation of Palghat Gap is 210m.a.s.l., while the Western Ghats continue to a height of 2637m.a.s.l.at Doddapeta in the Nilgiris in the Northern side of Palghat Gap and to the height of 2533m.a.s.l. in the Palani hills lying South of the Palghat Gap. The discontinuity of Palghat Gap therefore could be a dispersal barrier effecting the continuous distribution of plants along the stretch of the Western Ghats.

The exploration of forests of Kollengode forest range has revealed presence of 77 taxa so far not been recorded in the district of Palakkad. Three of these species are so far reported exclusively from the northern part of the Gap. *Strobilanthes lanatus* Nees, *Meteoromyrtus wynaadensis* (Bedd.) Gamble, and *Justicia glauca* Rottler and their presence in the Kollengode range across the

Gap cannot be attributed to any common adaptive feature.

Of the 77 new distributional reports from Kollengode, 23 are known to exist only in regions south of Palghat Gap, but the presence of these taxa in the locality can easily be understood as the range expansion of these species along the continuous elevated topography of the Western Ghats. Out of the 77 new distributional reports 34 taxa are reported from both the north and the south of Palghat Gap and can be considered as successful examples of range expansion.

The exploration of forests of Olavakkode forest range has revealed 18 species to be new distributional reports to the district of Palakkad out of which 5 are previously reported only from regions south of Palghat Gap. Clearly the taxa have successfully dispersed themselves across the barrier of the Gap. Three species among the 18 are previously reported only from regions north of gap, while one taxon is reported only from the district of Palakkad, while the rest of the species are seen both to the north and south of the Gap.

Table 3. Taxa showing range expansion across Palghat Gap

Sl. No.	Distribution pattern	Taxa
1	Plants located North of Palghat Gap and in Kollengode & Olavakkode range of Palakkad district	1. <i>Utricularia lazulina</i> P. Taylor 2. <i>Colebrookea oppositifolia</i> Smith 3. <i>Bauhinia racemosa</i> Lam.
2	Plants located North of Palghat Gap and new distribution report from Kollengode	1. <i>Strobilanthes lanatus</i> Nees 2. <i>Meteoromyrtus wynaadensis</i> (Bedd.) Gamble 1. <i>Justicia glauca</i> Rottler
3	Plants of Olavakkode range with only Northward distribution	1. <i>Thottea sivarajanii</i> E.S.S.Kumar, A.E.S.Khan&Binu 2. <i>Miliusa gokhalaiei</i> Narayanan <i>Actinodaphne lawsonii</i> Gamble 3. <i>Cinnamomum palghatensis</i> M.Gangop. 4. <i>Dioscorea belophylla</i> (Prain) Voigt ex Haines <i>Utricularia albocerulea</i> Dalzell
4	Plants located South of Palghat Gap and in Kollengode & Olavakkode range of Palakkad district	1. <i>Calamus rotang</i> L. 2. <i>Cyanotis papilionacea</i> (Burm.f.) Schult. &Schult.f. 3. <i>Chrysopogon nodulibarbis</i> (Hochst. ex Steud.) Henrard 4. <i>Givotia moluccana</i> (L.) Sreem.
5	Plants of Olavakkode range with only Southward distribution	1. <i>Amomum ghaticum</i> K.G.Bhat 2. <i>Crotalaria mysorensis</i> Roth 3. <i>Dalbergia sissoo</i> DC. 4. <i>Trifolium repens</i> L. 5. <i>Givotia moluccana</i> (L.)Sreem. 6. <i>Hopea utilis</i> (Bedd.) Bole 7. <i>Cyathula tomentosa</i> (Roth) Moq. 8. <i>Diospyros ebenum</i> J.Koenig ex Retz. 9. <i>Ceropegia thwaitesii</i> Hook. 10. <i>Toxocarpus kleinii</i> Wight &Arn. 11. <i>Jasminum flexile</i> var. <i>ovatum</i> Wall.ex C.B.Clarke 12. <i>Andrographis elongata</i> (Vahl) T.Anderson 13. <i>Calamus rotang</i> L. 14. <i>Cyanotis papilionacea</i> (Burm.f.)Schult.&Schult.f.) 15. <i>Chrysopogon nodulibarbis</i> (Hochst. ex Steud.)Henrard 16. <i>Bauhinia racemosa</i> Lam.

The range expansion that has been possible for the 95 species shows that 78% of the range expansion has been along the elevation continuity of the Western Ghats while only 23% of the species has been able to expand across the Gap.

Discussing the relationship between tropical climatic uniformity at a given site and the effectiveness of topographical

barriers adjacent to the site in preventing movements of plants, Janzen says that 'topographic barriers may be more effective in tropics'. It is the temperature gradient across a mountain range, which determines its effectiveness as a barrier, rather than the absolute height²⁰. The district of Palakkad which lies in the Gap region records the highest average temperature in the state,

and could be a factor that influences the range expansion of taxa across the Palghat gap of the Western Ghats.

Examination of the disjunct species distribution patterns in the northern Peruvian seasonally dry tropical forests (SDTFs) suggest that either species migration between the Marañón drainage and the Pacific region over the Andes has recently occurred via the Porculla Gap, or these areas were once continuous before the uplift of the Andes. The Huancabamba Depression in which the Abra de Porculla pass is located separates the northern and southern Andes and serves as a biogeographic barrier to species movement. The RioMarañón valley, is located east of the northwestern Peruvian coastal SDF and connected to them by the lowest mountain pass of the whole Andean chain, the Porculla Pass(2,165 m.a.s.l.). It has been suggested, that this pass has favoured the immigration and exchange of SDF biota, which evolved either in the Marañón valley or the coastal SDF²¹.Phytogeographical conclusions are often derived from the data obtained on the distribution of plant groups in topographically isolated sites. The plant groups typical of the AmotapeHuancabamba Zone in Ecuador – Peru region (*Nasa triphylla* group, *N. ser. Alatae*, *Ribes andicola* group, *Nasa picta* subsp. *picta*) find their southeastern distribution limit in the northern part of the province Pataz, and are

replaced by their southern counterparts (*N. poissoniana* group, *Ribes viscosum*) immediately south of this pass height of Abra de Porculla²². Elevation and geographic location were the dominant environmental gradients underlying the variations in species composition in Alpine ecosystems of the Hengduan Mountains, northwest Yunnan, China²³.

Evidently elevation continuity is indeed a favourable element in the successful range expansion of species. The declining abundance of alien species with altitude certainly promotes migration and range expansion of native species. The mountain pass therefore is a barrier to the successful range expansion of taxa as is evident from the fact that 77% of the plants that has succeeded in expanding their habitat range has been unable to do it across the Palghat Gap region.

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Seed borne fungi associated with some stored seeds and their bio-control by aqueous medicinal plant extract

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Abstract

To determine the mycoflora associated with some stored seeds, and their bio-control by aqueous medicinal plant extract. This study aimed to isolate and identify seed-borne fungi associated with seeds in both agar plate method and moist blotter method. The studied seeds were *Oryza sativa* L and *Cajanus cajan* (L.) Millsp. Seven dominative fungal species were isolated from these seeds such as *Aspergillus niger* Van Tieghem, *A. fumigatus* Fresenius, *A. terreus* Thom, *A. Flavus* Link, *Rhizopus nigricans* Ehrenberg, *R. Stolonifer* (Ehrenb.: Fr.)Vuill, *R. oryzae* Went & Prins.Geerl. Percentage of incidents of mycoflora in both agar plate and moist blotter method were studied. Spore suspension of two dominant fungi *A.terreus* and *A.niger* were prepared and frequency of fungal infected seeds was observed to identify the affect of particular fungus on seeds. Four medicinal plants named *Allium cepa* L, *Allium sativum* L, *Zingiber officinale* Roscoe and *Ocimum tenuiflorum* L were screened for their anti-fungal activities. All seeds were treated with aqueous extracts of fresh samples. *Allium sativum* and *Zingiber officinale* exhibited the highest antifungal activity among all plants tested.

Key words: *Aspergillus*, *Rhizopus*, Spore suspension, medicinal plants, antifungal activity.

Introduction

Cereals and legumes rank among the most important groups of crop plant production. They are an important source of high protein, carbohydrates, minerals, vitamins and fiber and also one of the cheapest food components. Seed-borne fungi have been found to affect the growth and productivity of crop plants including legume and cereal seeds.^{1&2} Presence or absence of seed-borne fungi on seed surface is one of the important aspects that determines the quality of seeds. Seed-borne pathogens causes seed rot, germination failure and seedling mortality are the causes for the reduction in the crop production and results enormous losses to our crop. The infected seeds may fail to germinate and they may, transmit disease from seed to seedlings and from seedling to growing plants.³ Some important seed-borne fungi of pulses which caused reduction in seed germination and they germinate poorly and could be a major source of inoculums for new crops raised from them.⁴

Aflatoxins are poisonous carcinogens that are

produced by certain molds. When contaminated food is processed; aflatoxins enter the general food supply where they have been found in both pet and human foods, as well as in feed stocks for agricultural animals. Children are particularly affected by aflatoxin exposure, which is associated with stunted growth,^[3] delayed development,^[4] liver damage, and liver cancer. This work was designed to identify the seed-borne fungi and their bio-control by medicinal plant extracts.

Materials and methods

Collection of seed samples

Stored seeds of Rice (*Oryza sativa* L. Poaceae) and Pigeonpea (*Cajanus cajan* (L.) Millsp. Fabaceae) were collected from Triprayar market, Thrissur, Kerala.

Detection and identification of seed-borne fungi of stored seeds

The samples of Rice and Pigeon pea were assayed for the presence of seed borne

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fungi by using (A) Agar plate method (B) Blotter method as recommended by ISTA (1966)^{5,6,7&8}

Surface sterilization of seeds

Stored seeds were surface sterilized using 0.1% HgCl₂ (Mercury chloride) for four minutes, rinsed with distilled water for 3-4 times and dried between sterilized filter papers.

Agar plate method (PDA method)

25 ml of sterilized PDA medium was poured in a pre-sterilized borosil glass Petri-plates. ⁹ The Petri-plates were allowed to cool at room temperature 33±0C; then 1 gm of sterilized seeds were plated at equal distances in triplicates and unsterilized seeds were kept as control under aseptic condition. Plates were incubated at room temperature for seven days. On eighth day the seeds were examined under stereoscopic binocular.

Moist blotter plate method (BPT)

In moist blotter plate method; the sterilized seeds were placed on water soaked three layered white blotter papers of 8.0 cm diameter and placed in pre sterilized borosil glass petri-dishes. 1 gm sterilized seeds were placed at equal distance in triplicates and unsterilized seeds keep as control in moist blotter paper under aseptic condition. The triplicates were incubated at room temperature 33±0C for seven days. On seventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought in to pure culture and maintained on PDA plates.

In both Agar plate method and Moist blotter method examine the frequency of seed-born fungi during incubation. The result was expressed in percentage.

Frequency of occurrence % =

$$\frac{\text{No. of seed on which fungal species occurs}}{\text{Total no. of seeds}} \times 100$$

Preparation of spore suspension and suspension culture:

Spore suspension of dominant seed-borne fungi, *A. terreus* and *A. niger* were prepared separately by adding 10 ml of distilled water in to the sporulating pure culture of seed-borne fungi, maintained on PDA slants for seven days at room temperature 33±0C. The slants were shaken and content filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension. 1 gm of test samples were dipped in the spore suspension prepared from the dominant seed-born fungi for few minutes and placed on the water soaked three layered blotter paper in a pre-sterilized Petri-dishes. The plates were incubated at room temperature 33±0C for seven days. Observed the growth of fungus on test samples.

Preparation of Medicinal plant extracts and Effect of medicinal plant extracts on incidence of fungi:

Four fresh samples were used in this study. Samples were Onion bulb, Garlic Bulb, Ginger rhizome and Thulasi Leaves. Water extract of fresh samples were prepared as follow: 10 gm of each plant material were cut in to small pieces using a sharp knife. The cutting plant material was mixed with 25 ml of distilled water by using pestle and mortar. The homogenate was placed in a closed container and was left for 48 hours. ¹⁰After soaking of seeds in medicinal plant extracts for half an hour, they were placed in sterilized Petri-plates on three layer of blotting paper water soaked in distilled water. Each dish contains 1 gm of seeds. Then the Petri-dishes will be kept at 33±0c and data will be recorded seven days after sowing. The percentage of fungal infection and their effects on growth were observed.

Result and Discussion

Seven fungal species were identified on the basis of colony colour and morphological features from rice and pigeon pea seeds through Agar plate method (PDA) and Blotting paper method (BPT). Dominant fungal species are *Aspergillus niger*, *A. fumigates*,

A. terreus , *A. Flavus* , *Rhizopus nigrican*,
R. Stolonifer , *R. oryzae* . The present work reveals the dominance of Aspergillus and Rhizopus sps. (Table .1)

Table 1. Fungal isolates from stored seeds

Sl. No.	Seed sample	Isolated seed mycoflora						
		<i>A.niger</i>	<i>A.fumigatus</i>	<i>A.terreus</i>	<i>A.flavus</i>	<i>R.nigricans</i>	<i>R.stolonifer</i>	<i>R.oryzae</i>
1	Rice	✓	✓	✓	x	✓	✓	✓
2	Pigeon pea	✓	x	✓	✓	✓	✓	x

Note: ✓ Pesent, x Absent

In the present study, the incidents of seed mycoflora were more in controlled seeds than the sterilized seeds. pigeon pea 100 % of incidents of fungi were observed in both types of seeds kept as control and that were subjected to the above mentioned methods . Where as in rice seeds, the percentage of fungal infection were 71.6 % in sterilized seeds of agar plate method and 100 % in control respectively. But in blotter plate method, the infection rate was 15.56 % and 16.7 % observed in sterilized as well as control seed respectively. (Table 2 & Fig 1)

Table.2: Comparative incident of seed borne fungi on different seed in PDA and BPT culture method in percentage

Sl. No.	Seed sample	Incidents of seed borne fungi (%)			
		Agar plate method		Blotter plate method	
		Sterilized seed %	Control seed%	Sterilized seed%	Control seed%
1	Rice	71.6	100	15.56	16.7
2	Pigeon pea	90	100	90	100

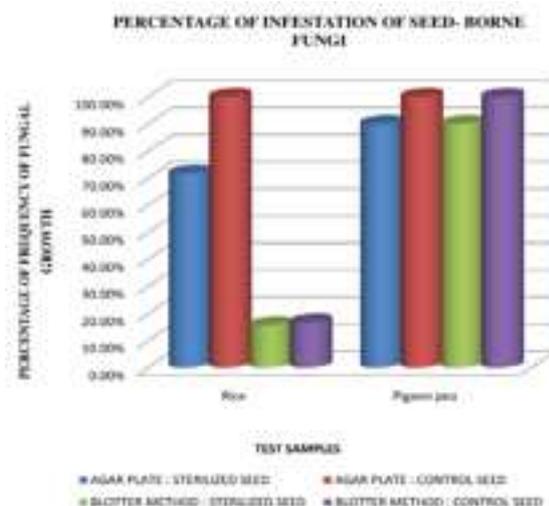


Fig. 1. Comparative incident of seed borne fungi on different seed in PDA and BPT culture method in percentage

Table. 3: Frequency of fungal infection in spore suspension method in percentage

Sl. No.	Seed sample	A. terreus	A. niger
		Fungal incidents	Fungal incidents
1	Rice	100%	100%
2	Pigeon pea	100%	80%

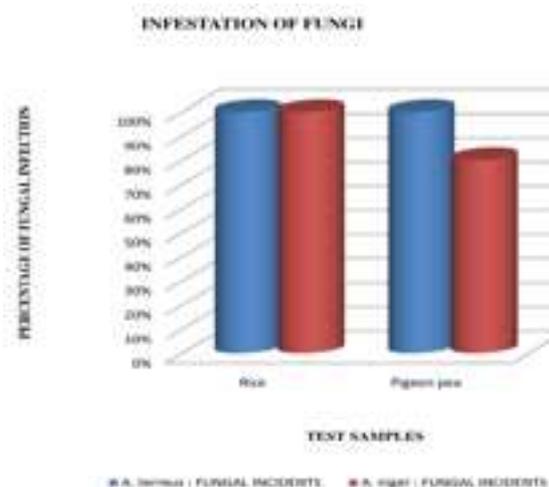


Fig 2. Frequency of fungal infection in spore suspension method in percentage
A. terreus and *A. niger* were commonly found on both seeds. They were isolated and their spore suspensions were prepared. Test

seeds were soaked in the above two suspensions for few minutes and cultured on blotting method. After the treatment of seeds in spore suspension of *A. terreus*, 100% fungal infection is observed in Rice and pigeon pea. Seeds after the treatment of *A. niger* suspension, exhibited 100% infection in both test samples. (Table 3 and Fig 2)

Water extract from four fresh samples onion bulb (*Allium cepa* L. - Amaryllidaceae), garlic bulb (*Allium sativum* L. - Amaryllidaceae), ginger rhizome (*Zingiber officinale* Roscoe.- Zingiberaceae), ocimum leaves (*Ocimum tenuiflorum* L. - Lamiaceae) were prepared and seeds were treated in it as thereafter the frequency of fungal infection was observed after medicinal plant extract treatment. The anti fungal activity of each extract on different samples was tabulated (Table 4 and fig 3).

Table.4. Frequency of fungal incidents after medicinal plant extract treatment

Sl. No.	Test sample	Onion	Garlic	Ginger	Thulasi
		Frequency of fungal incidents			
1	Rice	40%	-	-	30%
2	Pigeon pea	50%	-	5%	60%

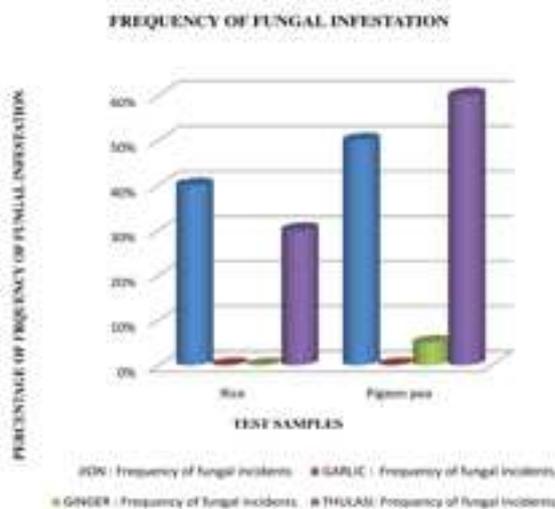


Fig. 3. Frequency of fungal incidents after medicinal plant extract treatment

No fungal infection was observed in seeds, soaked in Garlic extract unlike other three. Seeds treated with Onion extract showed maximum rate of fungal invasion.

Healthy seeds play a vital role in the production of healthy crops. Healthy seed is the foundation of healthy plant; necessary conditions for good yields.¹¹ Many pathogenic fungi are seed transmitted, often reduce the germination ability or killed the infected plants or substantially reduce the productivity. Therefore, control of seed-borne fungi is extremely important and the damaging effects can be revealed through integrated approaches.¹² This study was conducted to determine the prevalence of seed borne fungi of stored seeds were collected from the Tripprayar market for the experiment, different methods were carried out simultaneously employing BPT and PDA method.

Seed health test in two methods yielded seven different fungi *Aspergillus niger*, *A. fumigatus*, *A. terreus*, *A. flavus*, *Rhizopus nigricans*, *R. stolonifer*, *R. oryzae*. A considerable number of seed borne fungi belonging to the two genera *Aspergillus* and *Rhizopus*.¹³

All the seven fungal species were observed in both methods. In PDA culture availability of nutrients continuously along with moisture may be the reason for increased fungal growth.

The present work reveals that after treatment in the both seeds, spore suspension of *A. terreus* and *A. niger* possess 100% infection. Deteriorations of seeds by fungi is due to unhygienic condition of storage and this in turn is associated with initial high moisture content of stored products or absorption of moisture during storage due to defects in the storage system.¹⁵ In present study, the predominant fungi can cause deterioration in stored seeds and reduce the seed quality.

After medicinal plant extracts treatment, showed reduction in seed borne fungus. Similar studies were conducted in

various ways to control seed borne fungi in biological method.¹⁶ intensive.

This study revealed that Ocimum and Garlic are most suitable plants to make extract to control fungi pathogen. Because it had high antifungal activity and make the seed fungal free after treatment. According to Zakaria., 2014.^{17 & 18} all samples extracted from fresh samples, only Garlic bulb exhibited the highest anti-fungal activity against isolated fungi. The most abundant sulfur compound in Garlic is allicin (S-allylcysteine sulfoxide), which is present at 10 mg/g fresh Garlic or 30 mg/g dry weight, water extract of Ginger also suitable one and exhibited anti-fungal activity against all tested fungi.¹⁹ Reported that the gingerols and gingerdiol are the main anti-fungal activity against all tested fungi.²⁰

Onion extract treated seeds were contaminated by fungus when compared to other plant extract . . Medicinal plant extract preparation is very simple and economically and environment friendly approach. Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides

Thus, there is a need to search for alternative approaches to store seeds, cereals for human consumption without toxicity problems that are eco friendly and not capital

Conclusion

Seed-borne fungi appear in sterilized and unsterilized condition. They cause toxicity in human consumption. Moisture rich condition is favorable for their growth and their occurrence on stored seed adversely affect the germination rate and also inhibit the plant growth and also adversely affect the productivity of crop. High seed quality is essential in any crop production venture to attain higher yield and good quality products. Present work reveals that aqueous medicinal plant extracts have antifungal property. The extract obtained from Ocimum, Garlic and Ginger plants. They are more suitable alternatives to minimize the seed-borne fungus on the stored seeds. It is a less expensive and an eco-friendly approach to reduce the severity of seed-borne fungus in agriculture and human consumption. This study recommends the use of natural plant extract because they are safer than chemical fungicides. The use of these extracts cans also increase seed germination during agriculture. Ocimum, Garlic and Ginger extract are recommended as anti-fungal agent for preserving seeds.

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Effect of supplementation of Ascorbic acid on the comb building behaviour of worker bees (*Apis cerana*) during colony division

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Abstract

Honey bees are social insects which lead a fascinating colonial life. *Apis cerana* is an important bee in apiculture. They play an important role in maintaining food security and biodiversity through their pollination services. There is a steady decline in the population of *Apis cerana* due to many reasons. Climate change, use of pesticides, deforestation, pathogenic infections are the major threats for bee population. Their colony consists of a queen, thousands of workers and a few male drones. Worker honey bees, the slave females in the bee colony are always engaged in comb building, hive cleaning, pollen collection, nectar collection, honey making, nursing queen bee and larvae. In the present study we have studied the effect of supplementation of Ascorbic acid on the comb building behavior of *Apis cerana* during colony division. Honey bee colonies were constructed in suitable places and standard procedures were maintained for all colonies. Bee colony with full strength of adult bees was divided in to two colonies, one having Queen and one without queen. Queen right colonies after colony division were selected for further studies. Control colonies were provided with sugar syrup and experimental colonies were supplemented with Ascorbic acid sugar syrup. We observed that in the experimental colonies comb building was increased when compared to that of control group when supplemented with ascorbic acid. This behavior of worker bees can be utilized for increasing the brood area after colony division for the overall growth of the colony.

Keywords: Apiculture, comb building behaviour, ascorbic acid, *Apis cerana*.

Introduction

Honey combs are mass of wax cells built by worker honey bees to care their larvae and to store honey and pollen. The wax is produced by eight wax-producing glands in the abdominal segments of worker bees. The wax is released from the abdomen as wax scales, which are used for comb construction. Honey comb in the lower chamber forms the brood which contains egg, larvae, pupae. Queen, the only reproductive female in the colony lays egg in each cell. Fertile queen lays thousands of eggs per day. Egg hatches to larvae, which is fed by the worker bees. Larva then enters in to pupal stage and then come out of the cell as adult. The brood is cared and monitored by nursing worker bees. Honey comb in the upper chamber of bee box forms the super, in which workers will store honey. Comb

building is an important job of worker bees because they need comb to maintain their colony.

Honey bees require proteins, carbohydrates, lipids, vitamins and water. Bees collect nectar as their carbohydrate source and pollen as their protein source from flowers. During rainy season, beekeepers supplement sugar syrup for the bee colonies to compensate the inadequate supply of nectar from flowers. There is a need for supplemental food as the modern land use practices reduce dependable nectar and pollen supply ¹.

Materials and methods

Apiary construction and management

Artificial bee hives were constructed in suitable places. Standard procedures were

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adopted for all colonies, which is periodically checked to exclude the presence of honey bee diseases. Study was conducted during the period of colony division, from October to December.

Colony selection

Bee colony with full strength of adult bees was selected for the study. Before selecting bee colonies, we have ensured the presence of sufficient number of adult workers, sealed worker cells, adult drones, sealed drone cells and eggs in the comb cells.

Colony division

Selected colonies were subjected to division. All colonies have six frames of comb in their brood chamber. During division of the selected colonies, queen bee along with three frames of comb is placed in another bee box. Now one colony is a queen right colony and one colony is a queen less colony. Then both the colonies are provided with three free frames. Queen right colonies after division are placed in a different location. Control and experimental queen right colonies were set in the same location. Control and experimental queen less colonies were set in another location.

Feeding

Control colonies are fed with 50% sugar syrup and experimental colonies are provided with 0.2% ascorbic acid supplemented sugar syrup. The sugar syrup was provided *ad libitum* to all the colonies every alternate day.

Results

We observed a busy schedule for workers for the construction of new comb in the free frames in all the colonies. We had observed that in the experimental group, workers were very active in

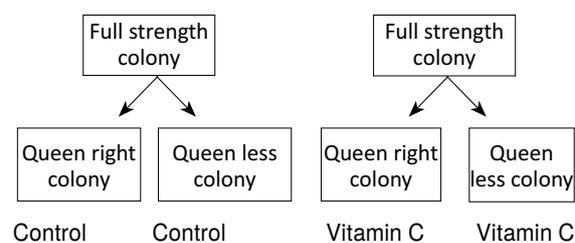


Fig.1 Experimental design

building new comb as compared to that of control group. Table 1 shows the comb building behavior shown by different groups during feeding period. Experimental groups started comb building in the first week after division. But control groups started only in the second week. Experimental groups completed comb building in all the three frames by the fourth week. Control groups took five weeks to complete all the three frames. Table 2 shows the total brood area of control and experimental groups during the test period. Total comb area is found to be highest in the test colonies which are supplemented with Vitamin C(Figure 1). The total comb area of Queen Right test colonies is significantly high when compared to that of all other groups. Figure 2 shows the extra combs constructed by the worker bees in the Queen Right colony when supplemented with Vitamin C. These extra combs are found to be attached to the top of the bee box.



Fig. 2. Additional comb constructed by worker bees in Queen Right colony supplemented with Vitamin C

Discussion

In the present study we have studied the effect of supplementation of Ascorbic acid on the comb building behavior of *Apis cerana* during colony division. Table 1 shows the behavior of worker bees in control and experimental groups during the feeding period. During the first week after division we observed that in control groups, worker bees are focused on building platform in the first frame. But in experimental colonies, worker bees already constructed platform and started to extend new comb. In the Queen less colonies workers constructed queen cells to raise a new queen. Table 1

Table 1. Comb building behavior of control group and experimental group during feeding period

	Control (Queen right)	Control (Queen less)	Test colony (Queen right)	Test colony (Queen less)
Week 1	Started comb building in frame 1	Started comb building in frame 1. Three queen cells present.	Started comb building in frame 1	Started comb building in frame 1. Presence of more than three queen cells.
Week 2	Building comb in frame 1	Building comb in frame 1. presence of new queen.	Completed comb building in frame 1. started comb building in frame 2	Building comb in frame 1 and started comb building in frame 2. Presence of new queen.
Week 3	Completed frame 1 and Started comb building in frame 2.	Completed frame 1 and started in frame 2.	Completed comb building in frame 2 and started comb building in frame 3.	Completed frame 1 and frame 2 .started comb building in frame 3.
Week 4	Completed frame 2 and Started comb building in frame 3.	Completed frame 2 and started frame 3.	Started building combs towards the top of the box.	Completed frame 3.
Week 5	Completed comb building in frame 3.	Completed frame 3.	Attached extra combs to the top of the bee box.	

Table 2. The total brood area of control and experimental groups during test period.

	Control (Queen right)	Control (Queen less)	Test colony (Queen right)	Test colony (Queen less)
Week 1	Frame 1-0 Frame 2-0 Frame 3- 0	Frame 1-0 Frame 2-0 Frame 3-0	Frame 1- 20cm ² Frame 2- 0 Frame 3- 0	Frame 1- 12cm ² Frame 2- Frame 3-
Week 2	Frame 1- 40cm ² Frame 2-0 Frame 3-0	Frame 1- 24cm ² Frame 2-0 Frame 3-0	Frame 1- 91cm ² Frame 2- 60cm ² Frame 3- 0	Frame 1- 40cm ² Frame 2- 12cm ² Frame 3- 0
Week 3	Frame 1- 187cm ² Frame 2- 21cm ² Frame 3-0	Frame 1- 150cm ² Frame 2- 32cm ² Frame 3-0	Frame 1-150cm ² Frame 2- 126cm ² Frame 3- 105cm ²	Frame 1- 132cm ² Frame 2- 130cm ² Frame 3- 36cm ²
Week 4	Frame 1- 187cm ² Frame 2- 160cm ² Frame 3- 44cm ²	Frame 1- 192cm ² Frame 2- 165cm ² Frame 3- 18cm ²	Frame 1- 204cm ² Frame 2- 176cm ² Frame 3- 150cm ²	Frame 1- 165cm ² Frame 2- 160cm ² Frame 3- 140cm ²
Week 5	Frame 1- 187cm ² Frame 2- 176cm ² Frame 3- 180cm ²	Frame 1- 192cm ² Frame 2- 187cm ² Frame 3- 160cm ²	Frame 1- 204cm ² Frame 2- 192cm ² Frame 3- 150cm ²	Frame 1- 180cm ² Frame 2- 187cm ² Frame 3- 176cm ²

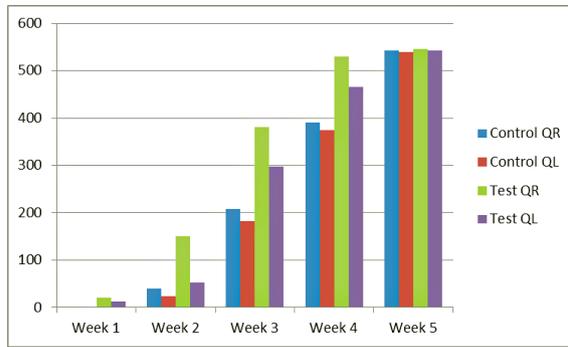


fig. 3. The total brood area of control and experimental groups during test period.

shows that when Queen less colonies are provided with Vitamin C, they constructed more number of queen cells when compared to that of control Queen less colonies.

Table 2 shows the total brood area of control and test groups during the feeding period. Significant increase in the total brood area was observed in test groups by week 4, which was supplemented with Ascorbic acid. Ahmadi Andi *et al*² studied the influence of vitamin C in sugar syrup on brood area, colony population, body weight, and protein in bees. They fed the control group with sugar syrup while experimental groups with different concentrations of soluble Vitamin C. They found that the highest

average brood area was in the experimental group provided with 2000 mg/L Vitamin C. Herbert J³ studied the effect of dietary vitamin C on brood rearing of honey bees using both free-flying and confined colonies. In this study they reported that colonies showed significantly more brood when the diet was supplemented with 2000 mg/L ascorbic acid. Marek Farjan *et al*⁴ reported the diet supplementation with vitamin C positively influenced some of the physiological and biochemical indicators in emerging worker bees. These results suggest that vitamin C can be recommended as a natural, safe, and relatively cheap diet supplement, elevating resistance to stress factors of wintering bees and spring generation of worker bees.

Conclusion

The present study indicates that supplementation of Vitamin C to the colonies after colony division shows a significant increase in the total brood area when compared to that of control colonies which are provided with sugar syrup. Total brood area increase in the bee colonies can be considered as an indicator of colony growth. So the supplementation of Ascorbic acid containing sugar syrup can be used to enhance the colony growth after colony division.

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Lip Print Patterns of Palakkad, Kerala

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Abstract

Personal identification is important not only in legal medicine but also in criminal investigation, identification and genetic research. Several methods are available for personal identification out of which, the best and commonly used one is fingerprints. Another emerging identification tool is Cheiloscopy or Lip Pattern study. Cheiloscopy involves the study of the grooves and furrows present on the red part of the human lips. These grooves occur as distinct patterns or types and are unique to each individual and thus can be used to fix the identity of a person. The grooves present on human lips are unique to each person and can be used to determine identity. A study carried out to study the lip print patterns in Palakkad, Kerala revealed that Type -I pattern was the most prominent pattern among the females and Type-II and Type-III pattern was more common among the males. The least encountered lip print pattern was Type-V. Detailed study in the four lip quadrants revealed the potential of using Cheiloscopy as an identification tool. The presence of just 1.08 % Type V pattern in upper left quadrant and its absence in males is worth further study. The absence of Type-V pattern in the left lower quadrants of both sexes also needs to be further investigated. The various patterns and distribution is discussed. A detailed study with more samples and inclusion of transgender may bring out a clearer picture. This may soon find a place as an economical identification tool or biomarker.

Key Words: Cheiloscopy, Lip print patterns, Personal Identification tools, Lip quadrants

Introduction

Personal identification is important not only in legal medicine but also in criminal investigation, identification and genetic research¹. Several methods are available for personal identification out of which, the best and commonly used one is fingerprints. Another emerging identification tool is Cheiloscopy or Lip Pattern study. Any tool useful for biometric study is always appreciable and should be tested. "Cheiloscopy" derived from Greek words *chelos*-lips and *skopein*-see is the name given to the study of lip prints. Cheiloscopy involves the study of the grooves and furrows present on the red part of the human lips. The grooves present on human lips (*Sulci labiorum*) are unique to each person and can be used to determine identity².

Lip prints are as unique as fingerprints and do not change during the life of a person³. Cheiloscopy is a potentially useful tool

for personal identification. The lip prints of parents and children and those of siblings have been reported to show some similarities⁴. Research studies and information regarding the use of lip prints as evidence in personal identification and criminal investigation in dentistry, although age old, are scanty⁵. Variations in patterns among males and females could help in sex determination. The arrangement of lines on the red part of the human lip is individual and unique for each human being⁶.

The legal system typically requires two different types of corroborative evidence in order to confirm placement of a suspect at a crime scene. Collection of lip prints along with other crime scene evidence is always useful and may help to confirm matters. In a crime scene investigation, lip prints can link a subject to a specific location if found on clothes or other subjects, such as glasses, cups or even cigarette butts¹⁰. Traces of lipstick smears found on these objects or parts

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of body may all be significant forensic evidence⁸. Lip prints may be found in cases of sexual assault or even homicides⁹. The lipstick itself can be analysed for various oils and waxes and an identification of the lipstick type can also be obtained¹⁰.

The use of lip prints in forensic identification is preferred because of their low utilization cost, simplicity, reliability and because it is not time consuming. It could be easily employed by a general dentist, as it does not require expertise.

Martin Santos was the first person to classify lip grooves. He divided them into four types namely: Straight line, Curved line, Angled line and Sine-shaped curve.

Another accepted classification method of lip prints is as follows⁶

Type I - A clear-cut groove running vertically across the lip

Type I - Partial-length groove of Type I

Type II - A branched groove,

Type III - An intersected groove

Type IV - A reticular pattern

Type V - Other patterns

Cheiloscopy hasn't been attempted among individuals of Palakkad, Kerala. Hence this study was planned to document common lip patterns among individuals of this region of Kerala.

Materials and Methods

Subjects and Study Area

A cross sectional survey was conducted among college students from different parts of Palakkad to identify the target population. This study was conducted among 132 subjects, which comprised of 92 females and 40 males aged between 18 and 24. They were briefed about the objectives of the study and written consent was taken from them before undertaking the study. Subjects undergoing orthodontic treatment, having congenital abnormalities, inflammation or trauma and known hypersensitivity to lipstick and students who were not from Palakkad were

excluded from the study.

Students (92 females and 40 males) coming from different taluks of Palakkad district were randomly selected for the study. A majority of students from our college who were from different panchayaths of Alathur gave written consent for this study. Male students were not willing to apply lipstick; hence we had to be satisfied with just 40 of them who obliged to be a part of this study. Alathur is a village and grama panchayat in the Palakkad District, state of Kerala, India. It is the headquarters of the Alathur Taluk and is situated at the south west end of the district 24 km from the district headquarters at Palakkad. Alathur Taluk is predominantly rural and has an agricultural economy.

Alathur is located along the Coimbatore-Cochin national highway NH 47. It consists of 111 Villages and 9 Panchayats. It is in the 65 m elevation (altitude). This Place is in the border of the Palakkad District and Thrissur District. Thrissur District Pazhayannur is west towards this place. Total population of Alathur Taluk is 422,229 living in 88,397 houses, spread across total 111 villages and 9 panchayats. males are 204,994 and females are 217,235.

Methodology

The subjects were asked to wash their mouths thoroughly and wipe it dry with neat dry towel. The lipstick was then applied with a single stroke, evenly on the vermilion border. The subjects were asked to rub both the lips to spread the applied lipstick. After two minutes, a strip of cellophane tape was cut out with scissors. Over the lip stick, the glued portion of cellophane tape strip was placed and the subject was asked to make a lip impression gently in the normal rest position of the lips.

Then the tape was carefully lifted from the lip from one end of the strip to the other, and then the strip of cellophane tape cellophane strip was stuck to the white bond paper for permanent record purpose (If the print was not satisfactory, the above steps were repeated). Lip Impression was taken

on bond paper too for safety sake. The subject's name age, sex and serial number were written on the bond paper beside the stuck strip of cello tape comprising the lip impression. The subject was provided with tissue paper to clean the lips. The lip prints were then examined with the help of 10X magnifying lens and the analysis carried out.

The grooves of lip prints were classified according to Suzuki and Tsuchihashi classification (Table 1 and Fig 1). This classification is the one most commonly used for recording lip patterns.

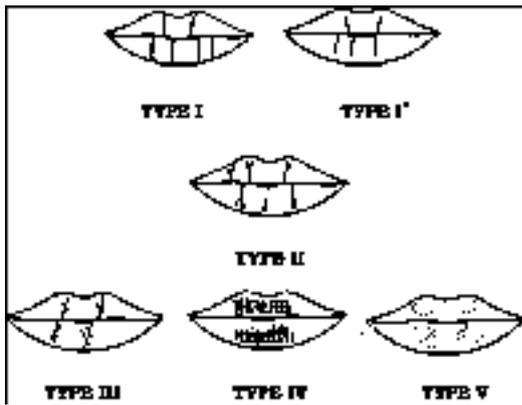


Figure 1. Suzuki and Tsuchihashi (1970) Classification Of Lip Prints

Table 1. Suzuki And Tsuchihashi (1970) Classification Of Lip Prints

Types Of Lip Print	Patterns Of Lip Prints
Type I	Vertical grooves
Type I'	Partial length across the lip grooves of type I
Type II	Branched grooves
Type III	Intersecting grooves
Type IV	Reticular grooves
Type V	Other patterns

For clear categorization of the lip pattern, each lip print was measured for its length and divided four quadrants in a lip print: Upper lip right, upper lip left, lower lip right, lower lip left.

Total numbers of horizontal lines, vertical lines, partial vertical lines, Y shaped

Table 2. Percentage Of Lip Print Patterns In Palakkad

Lip patterns	Female		Male		Total	
	No.	%	No.	%	No.	%
Type I	56	60.87	7	17.5	63	47.73
Type I'	9	9.78	4	10	13	9.85
Type II	1	13.04	12	30	24	18.18
Type III	5	5.44	13	32.5	18	13.64
Type IV	7	7.61	2	5	9	6.81
Type V	3	3.26	2	5	5	3.79

Table 3. Lip Patterns of Males And Females of Palakkad

Pattern	Males (40)		Females (92)	
	No.	%	No.	%
Type I	46	28.75	233	63.31
Type I'	15	9.37	21	5.7
Type II	44	27.5	69	18.8
Type III	45	28.12	28	7.6
Type IV	8	5	13	3.52
Type V	2	1.26	4	1.07

pattern, intersected pattern, number of boxes and undetermined pattern were counted by using 10X magnifying glass in each part of every lip print of all the subjects and data tabulated. To avoid bias, all the lip prints were compiled, analyzed and interpreted.

ULRQ – Upper Lip Right Quadrant

ULLQ - Upper Lip Left Quadrant

LLRQ– Lower Lip Right Quadrant

LLLQ– Lower Lip Left Quadrant

Results

Lip Patterns in Palakkad

All five types of lip patterns were observed in Palakkad. The most encountered lip pattern was Type I (47.73%). It was followed by Type II (18.8%), Type III

Table 4. Percentage Of Lip Print Patterns In Palakkad In Different Lip Quadrants

Lip Pattern	Sex	ULRQ	ULLQ	LLRQ	LLLQ
Type I	Males	35	30	30	20
	Females	73.9	71.74	57.61	50
Type I'	Males	7.5	10	7.5	12.5
	Females	3.26	6.52	4.34	8.69
Type II	Males	30	22.5	30	27.5
	Females	13.04	11.95	25	23.91
Type III	Males	20	30	27.5	35
	Females	3.26	3.26	10.87	13.04
Type IV	Males	5	10	5	5
	Females	4.34	3.26	2.17	4.34
Type V	Males	2.5	0	0	0
	Females	2.17	1.08	1.08	0

(13.64%), Type-1'(9.85%), Type IV (6.81 %) and just (3.79%) of Type V (Table.2).

Comparison of Lip Patterns of Males and Females in Palakkad

Among 92 females, 60.87% had Type I lip pattern. The pattern least encountered was Type V i.e. 3.26%. Among the males only 17.5% showed Type I pattern. The most prominent pattern among the males was Type III (32.5%). While 30% of males had Type II pattern, 10% showed Type I',

5% showed Type IV and 5% showed Type V pattern too. This clearly brings out a picture of variation in lip print pattern among males and females (Table-2).

Most Prominent Lip Pattern in Palakkad

The most prominent lip pattern among individuals in Palakkad was Type I (47.73%). Among males, Type II and Type III patterns were observed in almost equal numbers. The least encountered pattern was Type V (5%). Among females Type I dominated. The least encountered pattern

was Type V (3.26%) (Table-2).

Variation of lip patterns in the four lip quadrants among individuals of Palakkad

Upper Lip Right Quadrant

In the upper lip, among females 73.9% had Type I pattern in the right quadrant. In the upper lip, among males only 35 % had Type I pattern in the right quadrant. In the upper lip, among females 3.26 % had Type I' pattern in the right quadrant. In the upper lip, among males only 35 % had Type I' pattern in the right quadrant. Type II pattern was observed in the upper lip right quadrant of 13.04 % cases of females and 30% of males. In the right upper quadrant, Type III was seen in 3.26 % females and 20% of males. Type IV and Type V patterns were observed in 4.34% and 2.17% of female lips respectively. Type II pattern was seen 30% of male lips in this quadrant. Type III was observed in 20% cases. Type IV and Type V patterns were observed in 5 and 2.5% of male lips respectively (Table-4).

Upper Lip Left Quadrant

In the upper lip, among females 71.74 % had Type I pattern in the left quadrant. In the upper lip, among males only 30 % had Type I pattern in the left quadrant. In the upper lip, among females 6.52 % had Type I' pattern in the left quadrant. In the upper lip, among males only 10 % had Type I' pattern in the left quadrant. Type- II pattern was observed in the upper lip right quadrant of 11.95 % cases of females and 22.5 % of males. In the left upper quadrant, Type III was seen in 3.26 % females and 30% of males. Type IV and Type V patterns were observed in 3.26 % and 1.08 % of female lips respectively. Type IV pattern was seen 10% of male lips in this quadrant. Type V pattern was not observed in this quadrant in males (Table-4).

Lower Lip Right Quadrant

In the lower lip, among females 57.61% had Type-I pattern in their right quadrant. In the lower lip, among males only 30 % had Type-I pattern in the right quadrant. Type I' pattern was observed in only 4.34% cases of females in this quadrant. 7.5 % cases of males had Type I' pattern in the right quadrant. 30% of male lips had Type II pattern in this quadrant compared to 25% of female lips. Type III was observed in 30% cases of male lips but just 3.26% of female lips. Type V pattern was observed in 1.08 cases of female lips and completely absent in male lips (Table-4).

Lower Lip Left Quadrant

In the lower lip, among females 50% had Type I pattern in the left quadrant. In the lower lip, among males only 20 % had Type I pattern in the left quadrant. Type I' pattern was observed in only 8.69 % cases of females in this quadrant while 12.5 % cases of males had Type I' pattern in the left quadrant. 27.5 % of male lips had Type II pattern in this quadrant compared 23.91% of female lips. Type III was observed in 35% cases of male lips but just 13.04% of female lips. Type V pattern was not observed in this quadrant in both female and male lips (Table-4).

Discussion

Personal identification tools are very essential in these days of increasing crime, thefts, frauds and natural calamities. It is necessary for detecting unknown dead people in homicide, suicide, accident or a mass disaster. It is also very useful to trace down missing individuals and also to identify culprits or fraudsters hiding their identity. If a definite description of the different parts of the upper lip and the lower lip are established for an individual through a detailed study, this ante-mortem record can be used for matching the details of lip

prints in postmortem records for personal identification¹¹.

Type I pattern was observed more in all quadrants especially in the case of females. Our results agree with the work of authors¹². Type I was the most frequent pattern encountered in Mumbai. Our results agree with the results among Nepalese population of other researchers¹³. Surprisingly Type IV pattern was found to be prominent among Keralites in their work carried out at Bangalore³. But they have specified that the reticular pattern prominent in Type IV was observed in the middle red portion of the lip. The quadrants have not been specified. It is likely that a clearer picture will emerge if more number of individuals are screened. In all likelihood a new method of cheiloscropy should be designed in which, instead of the four quadrant format, each lip can be marked out into three regions – right, middle and left regions. Thereby six regions can be screened and a clear pattern may emerge.

This study was carried out on 132 individuals only. In the present work, Type IV pattern was observed in just 5% of males and 4.34% females. Other works on Indian subjects have yielded varying results. Type III was predominant in a study of lip prints of Indo-Dravidian population¹³. Type I' is the most predominant pattern in first and second quadrant, while Type II is predominant in third and fourth quadrant among males and females, Type I pattern was predominant in all the quadrants¹⁴. Type III pattern is the most predominant pattern in the study population (31.3% males and 33.3% females) followed by Type I, Type II, Type IV then Type V pattern¹⁵.

The most encountered lip pattern was Type-I (47.73%). It was followed by 18.8% Type II, 13.64% Type III, 9.85% Type I', 6.81 % Type IV and just 3.79% of Type V.

Among 92 females, 60.87% had Type-I lip pattern. The pattern least encountered was Type V i.e. 3.26%. Among the males only 17.5% showed Type I pattern. The most prominent pattern among the males was Type-III (32.5%). While 30% of males had

Type II pattern, 10% showed Type-I, 5 % showed Type IV and 5% showed Type V pattern too. The rarity of Type V observed in Palakkad has also been reported by another researcher⁵. The most common lip print pattern among males and females both was the intersecting type while the least common was the reticular groove pattern V. This was in contrast to study done among Goan population, where Type V pattern was most predominant pattern by researcher¹⁶. Type III pattern was predominant in a study of lip prints of Indo-Dravidian population¹³.

Various studies have shown that the lip print patterns formed revealed a population wise dominance. In other words, particular populations show predominance of a particular lip print type. This makes it

a potentially useful tool for identification. This may soon find a place in Aadhar cards, which is now used as an authentic identity card in India.

Cheiloscopy is a relatively new field among the large number of identification tools available to the forensic expert. Work on this subject has already elicited useful information such as that lip prints are unique to an individual and can be used to fix the identity of a person; that they remain stable over time and that lip prints show gender difference^{7, 12, 17}. Further work on the subject can help us understand why cheiloscopy can become a practical reality at the ground level of the forensic identification process.

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