



# INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS | ISSN: 2320 - 2882

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## **IMPACT OF COVID-19 ON FINANCIAL POSITION OF SELF-EMPLOYED WOMEN**

Published In IJCRT ( [www.ijert.org](http://www.ijert.org) ) & 7.97 Impact Factor by Google Scholar

Volume 9 Issue 1, Date of Publication: January 2021 2021-01-27 00:02:46

PAPER ID : IJCRT2101429

Registration ID : 202702



  
EDITOR IN CHIEF

Scholarly open access journals, Peer-reviewed, and Refereed Journals, Impact factor 7.97 (Calculate by google scholar and Semantic Scholar | AI-Powered Research Tool), Multidisciplinary, Monthly Journal

**INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS | IJCRT**  
*An International Scholarly, Open Access, Multi-disciplinary, Indexed Journal*  
Website: [www.ijcrt.org](http://www.ijcrt.org) | Email id: [editor@ijcrt.org](mailto:editor@ijcrt.org) | ESTD: 2013

Publication

IJCRT | ISSN: 2320-2882

13214

SIB

SPECIAL ISSUE

# Shodh Sarita

An International Multidisciplinary Quarterly  
Bilingual Peer Reviewed Refereed Research Journal

• Vol. 8 • Issue 29 • January to March 2021



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# INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

## IMPACT OF COVID-19 ON FINANCIAL POSITION OF SELF-EMPLOYED WOMEN

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### Abstract

The COVID-19 pandemic is harming health, social and economic well-being of women. Women face high risk of job and income loss during times of crisis. The battle of pandemic is against the women. Their jobs, businesses, incomes and standard of living may be affected due to COVID-19. It is our aim to form more equal and balanced economy by doing everything during and after COVID-19 crisis. This includes placing women's economic lives at the heart of the pandemic response and recovery plans. This research study focuses on the financial position of women before pandemic COVID-19, during the quarantine and after the pandemic COVID-19.

**Key words:** COVID-19, Self-employed women

### Introduction:

Self-employed women are most likely to work at risky sectors. However, COVID-19 virus has caused chaos across all facets of life and all sections of society. Everyone has been adversely impacted. Our startup and micro companies across the nation are inherently young, less strong and most vulnerable. Many of them face likely destruction during this extraordinary economic downturn. Everything we do during COVID-19 crisis, must aim to build sustainable economies.

### Objectives

1. To know the financial position of self-employed women before pandemic COVID-19.
2. To understand the financial position of self-employed women during Quarantine of COVID-19.
3. To study the financial position of self-employed women after pandemic COVID-19.

### Financial Impact of COVID-19 on Self-employed women:

COVID-19 is pitching the world economy. The impact of COVID-19 across the global economy will be profound. Women and girls who generally earn and save less and hold insecure job or who live close to poverty feel economic impact of COVID-19. The situation is worse in developing economies where 70% of women's employment is in the informal economy with few protections against dismissal or for paid sick leave. They have limited access to economic and social protection.

### Financial Position of Self-employed Women during COVID-19

Self-employment looks different during COVID-19. Time has changed. COVID-19 is hardest hit to women. In the current situation of COVID-19, there is more risk of loss of income for self-employed women. Since they take the main responsibility of caregiving in their households. Previously poor rural women were engaged in stitching school uniforms but now they are sewing masks. Since past couple of weeks, these women are engaged in sewing cotton masks, helping police personnel and health workers, while earning something for themselves also. With huge numbers of self-employed women, losing their livelihoods during the lockdown and food supply chains are getting disrupted in some areas, SHGs (Self Help Groups) have set up over 10,000 community kitchens across the country to feed stranded workers.

## Synergistic Effect of Plant Extracts against *Malassezia furfur*

Abhijit Sahasrabudhe\*<sup>1</sup>

Received: 23 Nov 2020 | Revised accepted: 19 Jan 2021 | Published online: 05 Feb 2021

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**Key words:** Dandruff, *Malassezia furfur*, Inhibition, Fruit extracts, Synergistic effect

Dandruff (pityriasis, capitis, seborrheic dermatitis confined to scalp) is a disease that has been around for centuries despite of several treatment options. It is a common scalp disorder affecting almost half of the pubertal population of both genders but most prevalent in male population between age group of 20 to 60 years [1]. It is the major cosmetic problem which causes a great public health concern both in the developed as well as developing countries. Dandruff is characterized by slight to moderate scaling of the scalp with varying degrees of sensations of dryness. Characteristics flaking and scaling of the scalp suggest impairment in the desquamation process. In most of the dandruff affected people, hair fall is a very common problem.

Dandruff and dry scalp are mostly used interchangeably by almost everyone because of similar symptoms. Dry scalp lacks moisture which causes dryness and itchy scalp followed by the shedding of small flakes of dead scalp cells due to scratching. The causes of the dry scalp can be dehydration in the body, poor diet or environmental conditions. Dandruff occurs due to the overproduction of the sebum and excessive action of yeast-like fungus known as *Malassezia*. This yeast feeds on the excessive oil sebum and on dead scalp cells resulting in the faster renewal process and further leads to the frequent shedding of scalp cells which fall off in the form of visible flakes.

Dandruff can occur due to any reason such as dirty scalp, over use of hair styling products, product build up (shampoo, wax, gel etc.) on the scalp, excessive oil production etc. However, the most common causes of dandruff are;

- Change in the climatic condition
- Dry skin
- Skin conditions, such as psoriasis, eczema, or seborrheic dermatitis
- Reaction to a hair product or shampoo

*Malassezia* (formerly called as *Pityrosporum*), yeast like lipophilic basidiomycetous fungi is considered to be the chief cause of dandruff problem which is present as scalp commensal [2]. Lipid dependant *Malassezia* yeasts are commonly found on human skin in particular in the upper part of the body, where sebum secretion is highest [3]. Though

dandruff is associated with scalp, flakes may also appear on face, nose and eyebrows as well as on the skin behind the ears and neck. Due to impact of male hormone testosterone, the sebaceous glands are stimulated to secrete more sebum which enhances the microbial growth and also associated formation of dandruff on scalp.

### Action of the fungus

Though there are seven different species of *Malassezia* found, till date the species *M.globosa*, *M.restricta* and *M.furfur* have been mostly related with dandruff in human beings [4]. *M.furfur* is an important causal factor for dandruff. Synthesis of lipase by species *Malassezia* hydrolyzes triglycerides which then release oleic acid that attracts neutrophils towards them. As a result, neutrophils release the reactive oxygen species and cytokines that aggravate scalp by causing the dermal inflammation and tissue damage [5]. *M.furfur* is the most likely initiating organism by virtue of its high lipase activity, and that an *M.furfur* lipase is expressed on human scalp. As a result, the corneocytes present in the epidermis clump together to form large flakes on the skin which causes irritation and uneasiness (Fig 1). Therefore, effective treatment is the need of the hour for people suffering from dandruff formation [6].

In the current scenario, many synthetic chemical substances are used for treating dandruff. The main active agents present in it are imidazole derivatives such as ketoconazole and other compounds such as selenium sulphide, zinc pyrithione, piroctone olamine, ciproxirox olamine and many others. They act by removing the scalp thereby reducing *Malassezia* species adherence to corneocytes and inhibit its further growth. Some studies also suggest the involvement of staphylococci bacteria in dandruff disease pathogenesis [7].

Pharmacological properties of medicinal plants may be used as leads in developing novel therapeutic agents. Today herbal products and extracts are widely used to control various human diseases. Medicinal plants are providing an efficient local aid to the health care and disease-free life. They contain physiological active constituents that over the years have been exploited in traditional medicine for the treatment of various ailments [8].

India is rich in biodiversity and has a wide spectrum of habitats from tropical rainforests to alpine vegetation and from temperate forests to coastal wetlands. About one third of the country's recorded flora is endemic and is concentrated mainly in the North-East, Western Ghats, and North-West Himalaya. Western Ghats of India are known for their valuable

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biodiversity and has been considered as one amongst the top most important eight hotspots in the world [9]. This hotspot of

biodiversity is a treasure house of genetic resources of many plant species.

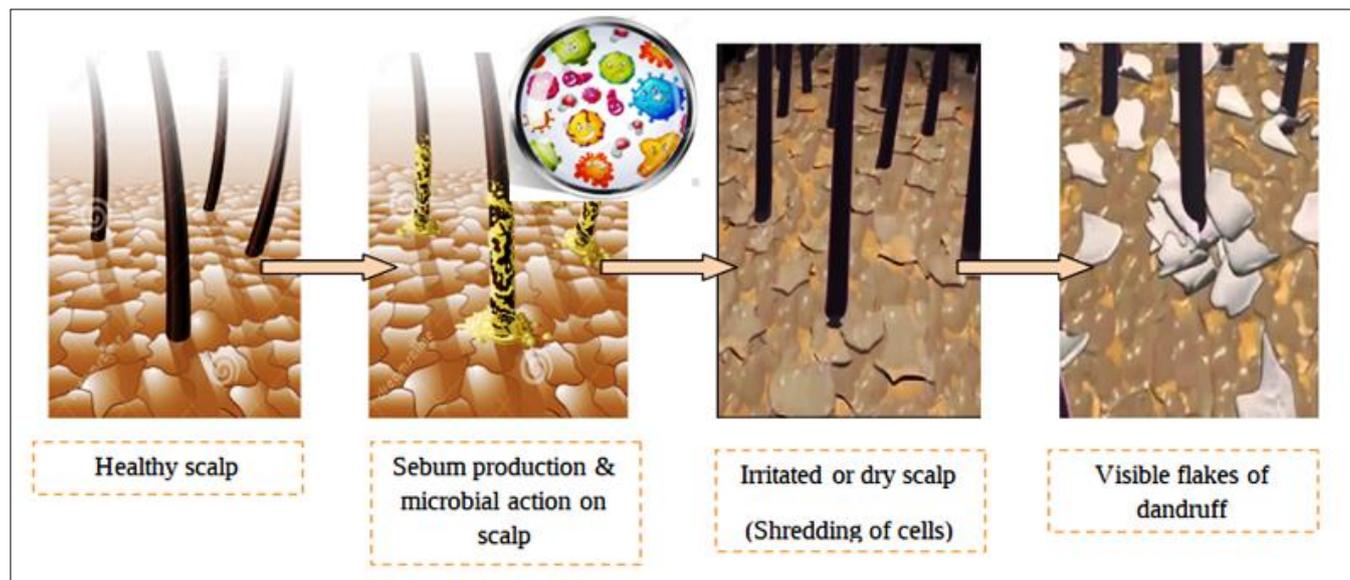


Fig 1 Mechanism of action of fungus on scalp

*Garcinia indica* (family- Clusiaceae) is one such tree species endemic to tropical rain forests of Western Ghats of India. Its fruits are a rich source of Hydroxycitric Acid (HCA), an important biologically active plant metabolite used as anti-obesity and anti-cholesterol drug. The fruits are also used to prepare a pleasant attractive beverage which has bilious action. The fat extracted from the seeds is used in cosmetics as emollient. A lot of work has been carried out on various aspects of extracts separated from fruit rinds of *G. indica*. Fruit rind extracts have shown good anti hyaluronidase and anti-elastase properties [10]. Researchers demonstrated anti-microbial and cytotoxic effects of fruit rinds of *G. indica*. Garcinol and Hydroxycitric Acid (HCA) present in *G. indica* have showed significant anti-oxidant and anti-hyperlipidemic activity [11]. Preliminary work on antidandruff activity of *Garcinia indica* showed promising results [12]. Taking this into consideration it was decided to screen synergistic effect of fruit extracts of various plants against *M. furfur*.

The ripe fruits of *G. indica*, *Terminalia chebula* and *Terminalia bellarica* were collected from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra.

**Microorganism used:** The test organism used in this study was *Malassezia furfur* (MTCC- 1374). The culture was purchased from the Microbial Type Culture Collection, Chandigarh, India.

**Reagents and chemicals:** This work was carried out in Research Laboratory of Department of Botany, K. V. Pendharkar College, Dombivli. Organic solvents used were of analytical grades (Merck and Qualigen). Sabouraud's Dextrose agar (M286) was purchased from Hi Media, Mumbai.

**Preparation of powdered extracts:** Shade dried fruit materials were powdered. 2gms of each powder is dissolved soaked in 100 ml of distilled water with addition of 1 ml of concentrated HCl (v/v). These extracts were air dried in evaporating dish with muslin cloth covering and once dried completely stored in air tight containers for further analysis.

**Separation of ethyl acetate fraction:** One gram of powdered extract was dissolved in 50 ml of D/W. To this 50 ml of ethyl acetate was added and two fractions were allowed to separate in a separating funnel for at least one hour. Ethyl acetate fraction (EAF) and water fraction (WF) were separated. Both the fractions were air dried and used for antidandruff study.

#### Antifungal susceptibility testing by well diffusion method

**Antifungal Susceptibility Testing:** *Malassezia furfur* strain (MTCC-1374) was grown on Sabouraud's dextrose agar supplemented with 2% corn oil for one week at 30°C in an incubator. *M. furfur* culture was then further maintained on the same medium with subcultures being carried out every alternate week. Loopful colonies of the organism were transferred to 100 ml of sabouraud's dextrose broth and maintained for seven days at 30°C on a shaker till the culture became 70% confluent. The broth culture of *M. furfur* was swabbed over the sabouraud's dextrose agar by using sterile cotton buds. Sterile 5 mm diameter Whatman no. 32 filter paper discs were dipped into all three extracts with various concentrations ranging from 25, 50, 75 and 100%. *Embllica officinalis* (Amla) fruit extract was used as the positive control in the same concentrations as other tested extracts. The replicates were maintained. These plates were incubated at 30°C and the zone of inhibition was observed after seven days. Control was maintained with filter paper discs dipped in sterile distilled water.

*Malassezia furfur* is pleomorphic yeast like fungus. It is also referred to as *P. orbicularae* and *P. ovale* depending on the morphology of the cells. When the yeast like cells are rounded and budding form with narrow neck then are called as *P. orbicularae* while on the other hand when yeast cells are oval with broad neck are called as *P. ovale*. However, in recent years the name *Malassezia furfur* is widely accepted for yeast like cells produced by *P. orbicularae*. It is also well known that the optimum requirement of physicochemical parameters varies depending on the species and the habitat in which they grow.

Antifungal activity of certain bioactive compounds extracted from medicinal plants has attracted a lot of attention within the scientific community largely as a result of the growing problem of multidrug resistance among pathogenic fungi [13]. In addition to this medicinal plant extracts are the promising sources of antifungal drugs, even though they have relatively mild effect against human pathogenic fungi when compared with the commercial synthetic drugs [14].

It was reported that *Terminalia chebula* and *Terminalia bellerica* exhibited a significant inhibition activity against *Malassezia furfur*. They also showed that *Lantana camara*

which was less effective against the fungus, but if used in combination with *Terminalia chebula* showed good synergistic effects against the fungus [15].

Anti *Pityrosporum* activity of herbal drug, a combination of *Wrightia tinctoria* and *Hibiscus rosasinensis* was tested *invitro* against the isolates of *Pityrosporum ovale* recovered from dandruff [16]. In another study, screening with four plants (*Aloe vera*, *Eucalyptus globulus*, *Phyllanthus embilca* and *Wrightia tinctoria*), *E. globulus* ( $30 \pm 1.14$ ) and *Aloe vera* ( $29 \pm 0.94$ ) were found to be very much effective against this dandruff causing fungus [17].

Table 1 Antifungal activity of ethyl acetate fraction of various plants

Ethyl acetate fraction	Concentration of fraction (%)			
	Zone of inhibition (mm)			
	25	50	75	100
<i>Garcinia indica</i>	$2 \pm 0.2$	$5.6 \pm 0.7$	$12.2 \pm 0.6$	$26.4 \pm 0.1$
<i>Terminalia chebula</i>	$7 \pm 0.5$	$9.6 \pm 1.2$	$20.4 \pm 0.2$	$24.5 \pm 0.3$
<i>Terminalia bellarica</i>	$5 \pm 0.2$	$10.3 \pm 0.9$	$18.2 \pm 1.2$	$25.5 \pm 0.98$
<i>Emblca officinalis</i>	$6 \pm 0.44$	$11.5 \pm 0.2$	$18.5 \pm 0.4$	$22.5 \pm 0.2$

Table 2 Antifungal activity of combined plant extracts

Combination of crude extracts	Zone of inhibition (mm)
<i>Garcinia indica</i> + <i>Terminalia chebula</i>	$20.6 \pm 0.6$
<i>Terminalia chebula</i> + <i>Emblca officinalis</i>	$22.6 \pm 0.7$
<i>Terminalia bellarica</i> + <i>Garcinia indica</i>	$26.4 \pm 1.4$
<i>Emblca officinalis</i> + <i>Garcinia indica</i>	$28.4 \pm 1.4$

*Garcinia indica* commonly known as Kokam plant has already gained a lot of attention due to its various anti-inflammatory, anti-oxidant, free radical scavenging activities. In earlier studies, ethyl acetate fraction separated from fruit rinds of *G.indica* has shown significant inhibition activity against *M. furfur*. In the present study synergistic effect of ethyl acetate fractions separated from three different fruits were examined in combination against the growth of *Malassezia furfur*. It was observed that *G. indica* at lower concentration is not effective against fungus as compared to other fruit extracts (Table 1). A marked difference in inhibition activity was observed when concentration was in the range of 25 to 75%. At 50%, zone of inhibition for *G. indica* was  $5.6 \pm 0.7$  mm while others it was in the range of 9 to 12 mm. Up to 75% concentration there was an increase in zone of inhibition for others as compared to *G. indica*. *T. chebula* had the maximum zone of inhibition ( $20.4 \pm 0.2$  mm). Surprisingly at 100% concentration of ethyl acetate fraction, *Garcinia indica* showed maximum zone ( $26.4 \pm 0.1$  mm) as compared to others. Four different combinations from these four extracts were made and checked for their synergistic activity against *Malassezia furfur*. *T. chebula* and *G. indica* showed less activity ( $20.6 \pm 0.6$  mm) while combination of *G. indica* with *E. officinalis* showed maximum zone of inhibition ( $28.4 \pm 1.4$  mm) as compared to other combinations.

## SUMMARY

Dandruff is a common disorder affecting the scalp. The genus *Malassezia* is the main causative agent of dandruff. Out of 17 different species, *Malassezia furfur* and *Malassezia globosa* are the main cause of dandruff. In recent years plant-based products are widely used as therapeutic weapon to cure human disorders. Earlier studies showed fruit extracts of *Garcinia indica* (Kokam) have significant effect against fungus growth. In the present study an attempt was made to know the combine effect of different fruit extracts against dandruff causing organism *Malassezia furfur*. It was observed that ethyl acetate fraction of *G. indica* was effective at 100% concentration as compared to other extracts. Combination of *G. indica* and *E. officinalis* showed maximum zone of inhibition ( $28.4 \pm 1.4$  mm). Fruit extracts showed good activity against dandruff causing organism *Malassezia furfur*. From the results, we conclude that plant extracts have antifungal activity and could be safely used for treating dandruff. Further studies can be made on the active molecules of plant extracts responsible for antidandruff activity.

**Acknowledgement:** The author thanks Principal and Head, Department of Botany of K. V. Pendharkar College for providing the laboratory facilities.

## LITERATURE CITED

1. Ravichandran G, Shivaram, Kolhapur SA. 2004. Evaluation of the clinical efficacy and safety of "Antidandruff Shampoo" in treatment of Dandruff. *The Antiseptic* 201(1): 5-8.
2. Mistry Z, More B, Shah G. 2016. Anti-dandruff activity of synthetic and herbal shampoos on dandruff causing isolate: *Malassezia*. *Int. J. Appl. Res.* 2(7): 80-85.
3. Ranjith M, Gokul SS, Ranganathan S, Shivramkrishanan M, Natarajan V, Rasool S. 2002. Role of ABO blood group in the infection rate of dandruff caused by *Pityrosporum ovale*. *I.J.D* 47(1): 21-23.
4. Gupta A, Batra R, Bluhm R. 2004. Skin diseases associated with *Malassezia furfur*. *Journal of American Academic Dermatology* 51(5): 785-798.

5. DeAngelis Y, Saunders C, Johnstone K, Reeeder N, Coleman C. 2007. Isolation and expression of a *Malassezia globosa* lipase gene, LIPI. *Jr. of Invest. Dermatol.* 127: 2138-2146.
6. Nazzaro- Porro M, Passi S. 1976. Growth requirements and lipid metabolism of *Pityrosporum obiculare*. *Jr. Invest. Dermatol.* 66: 178-182.
7. Leong C, Schmid B, Glatz G, Bosshard P. 2019. In vitro efficacy of antifungal agents alone and in shampoo formulation against dandruff-associated *Malassezia* spp. and *Staphylococcus* spp. *Int. Jr. Cosm. Sci.* 41(3): 221-227.
8. Srinivasan K, Natarajan D, Dheen M. 2006. Anti-bacterial activity of selected medicinal plants, *Ham. Medicine* 2: 5-8.
9. Myers N, Mittermeier R, Mittermeier CG, da Fonseca GAB, Kents J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.
10. Sahasrabudhe A, Deodhar M. 2010. Anti-hyaluronidase, anti-elastase activity of *Garcinia indica*. *Int. Jr. Bot.* 6(3): 299-303.
11. Varalakshmi K, Sangeetha C, Shabeena A, Sunitha S, Vapika J. 2011. Antimicrobial and cytotoxic effects of *Garcinia indica*. *W.J.A.S* 7(2): 193-196.
12. Sahasrabudhe A, Phanse M. 2015. Culturing of *Malassezia furfur* and its growth inhibiting activity of *Garcinia indica*. *I. J. P.* 2(8): 409-413.
13. Tim CT, Lamb A. 2005. Antimicrobial activity of flavonoids. *Int. Jr. Antimicrob. Agents* 26: 343-356.
14. Hammer K, Carson C, Riley T. 1999. Anti-microbial activity of essential oils and other plant extracts. *Jr. Am. Acad. Dermatol.* 51(5): 785-798.
15. Balkrishnan K, Narayanswamy N, Mathews S, Gaurang K. 2011. Evaluation of some medicinal plants for their dandruff control properties. *Int. Jr. Pharma and Bio Sciences* 2(4): 38-45.
16. Krishnamurthy J, Ranganathan S. 2000. Anti *Pityrosporum ovale* activity of herbal drug combination of *Wrightia tinctoria* and *Hibiscus rosa sinensis*. *I.J.D* 45: 125-126.
17. Prabhu M. 2012. Anti-fungal activity of selected plant extracts against *Malassezia globose*. *Int. Jr. Adv. Sci. Res.* 2(5): 162-168.



# **Analysis of Genetically Modified Seeds (GM) with Reference to Nutritional Value Content**

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## **ABSTRACT**

The development of new technologies and inventions in genetic engineering has given rise to genetically modified organisms (GMOs). These crops are the fastest adopted commodities in the agribiotech industry. This market penetration should provide a sustainable basis for ensuring food supply for growing global populations. The successful completion of two decades of commercial GM crop production (1996–2019) is underscored by the increasing rate of adoption of genetic engineering technology by farmers worldwide. Due to this rapid increase of GM crops in the market and great public concern about the safety of seeds and food products derived from these GM crops. Present study shows a preliminary work on proximate content of GM seeds with reference to nutritional values. GM maize showed variation in the nutritional content when compared with conventional maize while with soya results were promising.

**Keywords:** Genetically modified seeds, Health safety, Nutritional value content, proximate analysis

## **INTRODUCTION**

During a conference held at Asilomar in 1975, the concept of risk assessment of genetically modified organisms (GMOs) was first discussed. This concept that allows for the comparison of a final product to one having an acceptable standard of safety is an important element of a GM food safety assessment. This principle was elaborated by the Food and Agriculture Organization (FAO), World Health Organization (WHO), and Organization for Economic Cooperation and Development (OECD) in the early 1990s and referred to as “substantial equivalence” means GM foods can be considered as safe as conventional foods when key toxicological and nutritional components of the GM food are comparable, “substantially equivalent”, to the conventional food (within naturally occurring variability) and when the genetic modification itself is considered safe.

Transgenic technologies lead to genetically modified (GM) crops and promote food quantity and quality [1,2]. GM crops with pest and disease resistance, stress tolerance, edible vaccines, and added vitamins and nutrients are beneficial for humans [3,4]. For example, GM corn containing a gene that encodes for *Bacillus thuringiensis* (Bt) toxin acquired resistance to certain insect herbivores and produced a lower level of mycotoxin [5], which in turn reduced the use of pesticides and improved human and animal health. The global area of production of GM crops has reached 160 million hectares in the last 15 years [6]. Despite the many advantages of GM crops, their acceptance in many countries is controversial, especially in Europe [7]. Besides environmental and ecological issues [8], whether GM crops are safe for human health is a major concern [7].

These organisms carry genetic material that has been altered by insertion or deletion of genes in order to confer pest resistance, herbicide tolerance or to improve the quality of their produce. Number of such products or seeds is available in the markets today. Due to this rapid increase of GM crops in the market and great public concern about the safety of seeds and food products derived from these GM crops, the government of some countries has introduced mandatory labeling legislations of GM food and their derivatives. The labeling threshold levels were established in different countries. The main benefits of labeling system are to inform consumers of the presence of GM content in crops and related products. With these modifications, there is serious concern emerging about health safety and socioeconomic issues related to these GM crops. In addition, there is a lack of evidence regarding the substantial equivalence of antioxidant bioactive compounds, such as carotenoids, polyphenols, and total antioxidant activity. In relation to the hypothesis that genetic modification in seeds and other crops could lead to changes in metabolic pathways. India is the leading exporter of cotton, soybean and



maize and according to International Service for Acquisition of Agri-Biotech Applications (ISAAA) total of 11.4 million ha area is under genetically modified crops. Though BT cotton is primarily used in the textile industry but soybean, maize and other crops are edible.

Soybean seeds (*Glycine max*) belong to family Fabaceae has about 60% protein contents along with sucrose as the primary carbohydrate. Around 6 to 7% of saponins are present in soya seeds. On the other hand maize (*Zea mays*) belongs to Poaceae is rich in carbohydrate content and vitamin B. With the modifications at genetic level, the nutritional composition of these plants also changes which could lead to hazardous effects on mankind. Due to this rapid increase of GM crops in the market and great public concern about the safety of seeds and food products derived from these GM crops, the government of some countries has introduced mandatory labeling legislations of GM food and their derivatives. The labeling threshold levels were established in different countries. The main benefits of labeling system are to inform consumers of the presence of GM content in crops and related products. With these modifications, there is serious concern emerging about health safety and socioeconomic issues related to these GM crops.

The top biotech regulator in India is Genetic Engineering Appraisal Committee (GEAC). The committee functions as a statutory body under the Environment Protection Act 1986 of the Ministry of Environment & Forests (MoEF). It was earlier known as Genetic Engineering Approval Committee. Under the EPA 1986 "Rules for Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells 1989", GEAC is responsible for granting permits to conduct experimental and large-scale open field trials and also grant approval for commercial release of biotech crops. Only those fulfill genetic compliance or norms are exported while others are rejected.

In this study conventional and genetically modified seeds were analyzed for mineral, total ash, fat, crude protein and total carbohydrate content to check if there was any variation in their nutritional value.

#### MATERIAL AND METHODS

1. **Sample Collection:** Conventional soya and maize seeds were purchased from local markets while GM seeds were procured from a known source.
2. **Sample preparation:** Seeds of soya and maize were grounded into fine powder, oven dried and stored in air tight container for analysis.
3. **Chemicals and reagents:** chemicals and other reagents required for analysis were purchased from Merck, India.
4. **Determination of proximate compositions of maize and soya seeds:**

##### a. Moisture content

The moisture content was estimated by drying triplicates 10g weight of the sample at 105°C for 24 hrs and then reweighing after cooling in a desiccator. The moisture content is expressed as percentage of the dry weight.

$$\text{Moisture content} = \frac{\text{Weight loss of sample}}{\text{Original weight of the sample}} \times 100$$

##### b. Ash Content/Mineral content

Two grams of the dried sample was weighed in to a dry porcelain dish and then heated in a muffle furnace at 600°C for 6 hours. It was cooled in desiccators and weighed. The percentage ash content was calculated as

$$\% \text{ ash content} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

##### c. Fat content

The fat content was determined using Soxhlet extraction method (AOAC, 1984). Two gms of the sample was weighed into the Soxhlet extraction thimble. Cotton wool was used as plug to avoid loss of sample. The thimble was transferred into the Soxhlet extractor and sufficient petroleum ether was added until the latter is siphoned into the receiving flask which has been weighed. More ether was poured to cover the thimble completely and flask placed with the extractor on the electric



heating mantle. The reflux condenser was heated gently for 3 hours, switched off and allowed to cool for 10 minutes. Recovered solvent was transferred into an air oven (100°C) for 1 hour and then cooled in desiccators and weighed. The amount of oil produced was calculated and expressed as percentage of original sample.

$$\% \text{ Fat content} = \frac{\text{Weight loss of sample (extracted fats)}}{\text{Original weight of the sample}} \times 100$$

#### **d. Crude Protein**

One gram of each sample was weighed into a digestion flask. Ten gms of potassium sulphate, 0.7g mercuric oxide and concentrated sulphuric acid were added to the sample in the digestion flask. The flask was heated gently at an inclined angle until frothing subsides and boiled until the solution becomes clear. This was continued for half an hour. When the frothing is in excess, a small amount of paraffin wax was added. On cooling, 90 ml of distilled water was added and mixed. A small piece of pumice was added to prevent bumping. 80ml of 2M sodium hydroxide solution was added while tilting the flask so that two layers are formed. The condenser unit was rapidly connected, heated and the distilled ammonia collected in 50ml boric acid / methyl red indicator. Fifty milliliters of the distillate was collected and titrated against 0.1M hydrochloric acid solution.

$$\% \text{ N} = \frac{\text{Volume of acid} \times \text{Molarity of std. acid} \times 0.014}{\text{Weight of the sample}} \times 100$$

$$\% \text{ Crude protein content} = \% \text{ N} \times 6.25$$

#### **e. Total Carbohydrate**

Total carbohydrate can be calculated as,

$$\% \text{ carbohydrate} = (100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ fat} + \% \text{ protein} + \% \text{ fibre}))$$

## **RESULTS AND DISCUSSION**

### **Proximate content analysis:**

In the year 2017, the estimated global area planted with genetically modified (GM) crops reached 1500 million hectares and the main GM crops like cotton, soybean and maize have covered around 250 million hectares all around the world. Apart from these crops there are other GM crops like alfalfa, tomato, potato, rice, papaya, sugar beet, sugarcane etc which are widely produced all over the world. The purpose of production of GM crops varies depending upon their application such as herbicide or insect resistance, pesticide tolerance, antibiotic resistance, to increase the amount of protein or sugar level etc. As mentioned earlier threshold levels are different in different countries. 0.9% in EU, 1% in Australia and New Zealand, 3% in Korea, and 5% in Japan and Indonesia [11]. Protein energy malnutrition is the most lethal form (Food and Agriculture Organization, 2006) of malnutrition and affects every fourth child worldwide, according to the World Health Organization (2006). The Food and Agriculture Organization estimates that 850 million people worldwide suffer from under nutrition, to which insufficient protein in the diet is a significant contributing factor. Most plants have a poor balance of essential amino acids relative to the needs of animals and humans. The cereals (wheat, rice, etc.) tend to be low in Lysine, whereas legumes (soybean, pea are often low in the sulfur-rich amino acids Methionine and Cysteine. Poultry, swine, and other nonruminant animals have specific requirements for each of the essential amino acids while carbohydrates in the diet, it has become clear to the public that not all carbohydrates are created equal. While it is still something of a value judgment to describe “good” versus “bad” carbohydrates, there are clear clinical indications of the value of polymeric versus simple sugars. Plants are effective at making both polymeric carbohydrates (e.g. starches and fructans) and individual sugars (e.g. Sucrose and Fructose).

The proximate composition of maize and soya obtained from the seeds is as shown in the **Table 1**. It was observed that moisture content in GM maize (49.6%) was found to be more as compared to conventional seeds (45%) while that there was not much of difference seen with conventional and GM soya. % of ash content was more with GM soya than conventional seeds but for conventional and GM soya it was more or less same. Fat and fibre content were found to be more in GM maize while for both types of soya seeds it was nearly similar.



With reference to soya seeds, protein content was more in GM seeds (58.9%) than that of conventional seeds (55.6%) (**Figure 1**). But for carbohydrate content, conventional maize (40.2%) showed more value as compared to GM maize (30.4%). For soya, values are encouraging while with maize there was a decrease in the nutritional value.

### CONCLUSION

This was a preliminary work on proximate content of GM seeds showing not many changes in nutritional values of essential components. In further studies we can explore variation in amino acids and sugar contents with reference to proteins and carbohydrates respectively.

### ACKNOWLEDGEMENT

I thank University of Mumbai for sanctioning this Minor Research Project. I also thank Dr. (Mrs.) A.K. Ranade, Principal, K.V.Pendharkar College and entire botany department for their constant encouragement and support throughout the work.

### REFERENCES

- [1]. Bouis, H.E. The potential of genetically modified food crops to improve human nutrition in developing countries. *J. Dev. Stud.* 2007, 43, 79–96.
- [2]. Avni, A.; Blazquez, M.A. Can plant biotechnology help in solving our food and energy shortage in the future? *Curr. Opin. Biotechnol.* 2011, 22, 220–223.
- [3]. Kok, E.J.; Kuiper, H.A. Comparative safety assessment for biotech crops. *Trends Biotechnol.* 2003, 21, 439–444.
- [4]. Farre, G.; Ramessar, K.; Twyman, R.M.; Capell, T.; Christou, P. The humanitarian impact of plant biotechnology: Recent breakthroughs vs bottlenecks for adoption. *Curr. Opin. Plant Biol.* 2010, 13, 219–225.
- [5]. Wu, F. Mycotoxin reduction in Bt corn: Potential economic, health, and regulatory impacts. *Transgenic Res.* 2006, 15, 277–289.
- [6]. James, C. *Global Status of Commercialized Biotech/GM Crops: 2011*; ISAAA Briefs No. 43; ISAAA: Ithaca, NY, USA, 2011.
- [7]. Levidow, L.; Boschert, K. Segregating GM crops: Why a contentious “risk” issue in Europe? *Sci. Cult.* 2011, 20, 255–279.
- [8]. Hsieh, Y.T.; Pan, T.M. Influence of planting papaya ringspot virus resistant transgenic papaya on soil microbial biodiversity. *J. Agric. Food Chem.* 2006, 54, 130–137.
- [9]. AOAC. *Official Methods of Analysis of AOAC (1984)*. International. 18<sup>th</sup> ed. Gaithersburg (MD) A.O.A.C. International
- [10]. World Health Organization (WHO). Report of a WHO Workshop WHO/FNU/FOS/95. 1. WHO, Geneva, Switzerland, 1995
- [11]. Berdal, K.G; Boydler, C., Tengs, T. and Holst-Jensen, A. 2008. A statistical approach for evaluation of PCR results to improve the practical limit of quantification (LOQ) of GMO analyzes (SIMQUANT). *European Food Research and Technology* 227: 1149-1157

**Table 1: Proximate content in soya and maize seeds (in %)**

Sample	Moisture %	Ash %	Protein %	Fat %	Fibre %	Carbohydrate %
Conventional maize	45	1.34	4.6	3.4	5.7	40.2
GM maize	49.6	2.9	4.9	4.6	7.8	30.4
Conventional soya	22.4	3.4	55.6	5.6	10.3	4
GM soya	22.9	3.3	58.9	5.8	11	5.4

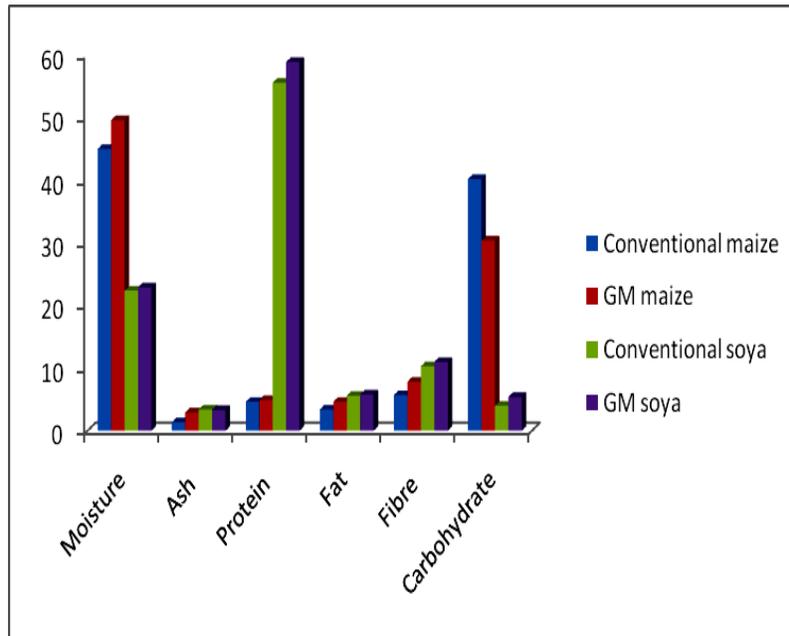


Fig 1: Proximate content in soya and maize seeds (in %)



# Standardization of Rapid Blood Isolation Protocol for PCR Analysis

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## ABSTRACT

Pure DNA isolation is the first and crucial step for many of the available downstream applications used in the field of molecular biology. Whole blood samples are one of the main sources used to obtain DNA, and there are many different protocols available to perform nucleic acid extraction on such samples. These methods vary from very basic manual protocols to more sophisticated methods included in automated DNA extraction protocols. Based on the wide range of available options, it would be ideal to determine the ones that perform best in terms of cost-effectiveness and time efficiency. We have standardized blood DNA isolation with few modifications in SDS based method. The key features are during isolation we have avoided the use of hazardous chemicals such as phenol, chloroform: isoamylalcohol etc. Without the use of RNase we were able to isolate pure and sufficient amount of DNA which proved to be ideal for PCR analysis.

**Keywords:** Whole blood sample, genomic DNA isolation, without RNase, conventional PCR

## INTRODUCTION

Human health studies in the field of molecular biology require the use of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein samples. Successful use of available downstream applications will benefit from the use of high-quantity and high-quality DNA. Therefore, nucleic acid extraction is a key step in laboratory procedures required to perform further molecular research applications. It is essential to choose a suitable extraction method, and there are a few considerations to be made when evaluating the available options [1], [2]. These may include technical requirements, time efficiency, cost-effectiveness, as well as biological specimens to be used and their collection and storage requirements. DNA extraction methods follow some common procedures aimed to achieve effective disruption of cells, denaturation of nucleoprotein complexes, inactivation of nucleases and other enzymes, removal of biological and chemical contaminants, and finally DNA precipitation.

Whole blood is one of many different available sources to obtain genomic DNA (gDNA), and it has been widely used in facilities around the world. Therefore, we will focus on DNA extraction protocols using whole blood samples. Issues regarding collection, storage, and manual handling of human whole blood specimens escape the scope of this publication and will not be covered [3]. However, they are important and they should be considered, as they could potentially impact on the performance and success of any DNA extraction technique chosen. As previously mentioned, lysis of cells is a common step in most DNA extraction protocols, and it is commonly achieved through the use of detergents and enzymes. Sodium dodecyl sulfate (SDS) and Triton™ X-100 (Sigma-Aldrich, St Louis, MO, USA) are examples of popular detergents used to solubilize cell membranes. Enzymes are also combined with detergents to target cell surface or cytosolic components [4]. Proteinase K is a commonly used enzyme used in various protocols to cleave glycoproteins and inactivate RNase and DNases. Other denaturants such as urea, guanidinium salts, and chemical chaotropes have also been used to disrupt cells and inactivate cellular enzymes, but these can impact on quality and nucleic acid yield.

Therefore, in order to fulfill the demand of a rapid and cost effective procedure for obtaining high quality genomic DNA, hereby we have aimed to develop a protocol free from costly enzymes and toxic organic solvents for extracting pure DNA from human blood samples.



## MATERIAL AND METHODS

### Primers used for the study:

Both forward as well as reverse primers were purchased from **Sigma Aldrich, USA**. Both forward and reverse primers were reconstituted in Milli- Q water as major stock of 100 $\mu$ M each. Details of primers and their sequences are given in the

**Table 1: Primers with their sequences used for blood DNA amplification**

Oligo Name	Sequence	Length	GC%
Forward primer	GACCATACCAGTGACG	16	50
Reverse primer	TGTTATCACTGGTGCT	16	44

### Sample Collection:

Blood sample was collected in 20 ml EDTA tubes from pathology laboratories with proper consent.

### Chemicals and other reagents:

Extraction buffers:

Buffer A contains 0.32 M Sucrose, 10 mM Tris HCl, 5 mM MgCl<sub>2</sub>, 0.75% Triton-X-100 and pH was adjusted to 7.6 and Buffer B contains 20 mM Tris HCl, 4 mM EDTA, 100 mM NaCl and pH was adjusted to 7.4

Other reagents: 30% SDS, Proteinase K, 5.3M NaCl, Double distilled water, chilled ethanol and 0.1X TE buffer.

### Blood DNA isolation:

To carry out Blood DNA isolation we tried three different protocols.

#### 1. SSC buffer:

1. Take 1 ml of blood to that add 1 ml of 1 $\times$  SSC buffer. Centrifuge for 12000 rpm for 2 mins at 4 $^{\circ}$ C. 2. Discard supernatant and add 1 ml of 1 $\times$  SSC buffer to the pellet and Mix by inversion. Centrifuge for 12000 rpm for 2 min. at 4 $^{\circ}$ C. 3. Repeat the above step. 4. Remove the supernatant and to the pellet add 500 $\mu$ l of 0.2 M Sodium acetate, 25  $\mu$ l of 10% SDS and 5  $\mu$ l of Proteinase k (10 mg/ml). Incubate 1 hour at 55 $^{\circ}$ C. 5. Transfer the supernatant into a new tube and add 500 $\mu$ l of Phenol/Chloroform/Isoamylalcohol (25:24:1) and mix by inversions. Centrifuge at 4000 rpm for 5 min at 10 $^{\circ}$ C. 6. Transfer the supernatant into a new tube and add 500 $\mu$ l of Chloroform: isoamyl alcohol (24:1) and mix by inversions. Centrifuge at 4000 rpm for 5 min at 10 $^{\circ}$ C. 7. Repeat the above step 2-3 times to remove the Phenol traces. 8. Transfer the supernatant into a new tube and add 50 $\mu$ l of 2 M sodium acetate and 500 $\mu$ l Distilled Ethanol. Invert slowly. DNA will appear in the form of threads. Spin at 5000 rpm for 10 min at 5 $^{\circ}$ C. 9. Allow the pellet to dry completely, dissolve it into 100  $\mu$ l of TE buffer till use.

#### 2. SE buffer:

1. Take 1 ml of blood to that add 3 ml of lysis buffer. Vortex gently and incubate for 30 mins in ice. 2. After incubation centrifuge the sample for 12000 rpm for 10 min. at 4 $^{\circ}$ C. 3. Discard supernatant and add 1 ml lysis buffer to the pellet. Mix it by inversion. Centrifuge for 12000 rpm for 10 min. at 4 $^{\circ}$ C. 4. Remove the supernatant and add 0.5 ml of S.E buffer to the pellet. Vortex gently and centrifuge at 12000 rpm for 10 min at 4 $^{\circ}$ C. 5. Through the supernatant, add 0.5 ml of SE buffer, 4  $\mu$ l Proteinase k (10 mg/ml) and 20% SDS. Incubate the sample for 2 hrs at 55 $^{\circ}$ C. Vortex gently at regular intervals of 20 min. 6. After incubation add 0.5ml SE buffer and 1ml phenol. Vortex gently. Centrifuge at 4000 rpm 5 min at 10 $^{\circ}$ C. 7. Transfer the supernatant into a new tube and add 1 ml of Phenol/Chloroform/Isoamyl alcohol (25:24:1) and mix by inversions. Centrifuge at 4000 rpm for 5 min at 10 $^{\circ}$ C. 8. Transfer the supernatant into a new tube and add 1 ml of Chloroform/Isoamyl alcohol (24:1) and mix by inversions. Centrifuge at 4000 rpm for 5 min at 10 $^{\circ}$ C. 9. Repeat the above step 2-3 times to remove the Phenol traces. 10. Transfer the supernatant into a new tube and add 30 $\mu$ l of 3 M sodium acetate and 1 ml isopropanol. Invert slowly. DNA will appear in the form of threads. Spin at 5000 rpm for 10 min at 5 $^{\circ}$ C. 11. Allow the pellet to dry completely, dissolve it into 100  $\mu$ l of TE buffer till use.

### 3. Standardized protocol:

1. Take 1 ml of blood to that add 1 ml of buffer A and 1ml of cold, sterile double distilled water, vortex gently. Incubate on ice for 2-3 mins.
2. Centrifuge at 4000 rpm for 15 mins at 4°C.
3. Discard the supernatant and to the pellet add 1 ml of buffer A and 3 ml of cold, sterile double distilled water and vortex gently.
4. Centrifuge at 4000 rpm for 10 mins at 4°C.
5. Repeat the above step till the pellet becomes creamish white in color.
6. Remove the supernatant and to the pellet add 2.5 ml of buffer B and 250µl of 30% SDS. Vortex vigorously for 30 sec and add 25µl of Proteinase K solution and incubate at 55°C for 2 hours.
7. Mix it well by inversion with an interval of 20 mins.
8. After incubation add 2 ml of 5.3M NaCl solution. Vortex gently for 15 sec.
9. Centrifuge at 5000 rpm for 15 mins at 4°C.
10. Collect the aqueous layer and add equal volume of ice cold isopropanol. Invert slowly, DNA will appear in the form of threads.
11. Spin the DNA at 5000 rpm for 10 mins at 4°C.
12. Throw the supernatant and allow the pellet to dry overnight. Next day dissolve the pellet in 100µl of 0.1X TE buffer.

### Agarose gel electrophoresis:

Isolated DNA was visualized on 0.8% agarose gel containing ethidium bromide (10mg/ml) in 1x TAE buffer at 60 volts for one and half hours.

### Conventional PCR analysis

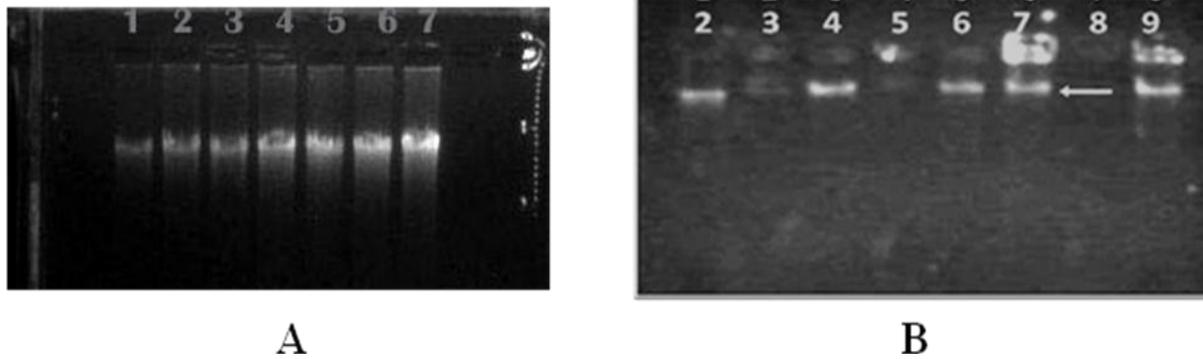
PCR was carried out in thermocycler, Applied Biosystems, USA. All reactions were carried out in 0.2 ml PCR eppendorf tubes. Individual reactions contains 12.5µl of Master mix, 2.5µl of template DNA, 2.5µl of each forward and reverse primer and finally volumised to 25µl using sterile Mili-Q water. Negative template control (NTC) was also run along with the DNA samples to check false amplification. Conventional PCR was carried out with initial denaturation for 10 mins at 94°C followed by 35 cycles of denaturation for 40 sec at 94°C, annealing for 1 min at 55°C, extension for 1 min at 72°C. Finally PCR products were hold for 7 mins at 72°C. PCR products were visualized on 2% agarose gel containing ethidium bromide (10mg/ml) in 1X TAE buffer at 100 volts for half an hour.

## RESULTS

The isolation of genomic DNA from blood typically involves digestion of nuclei with a combination of Proteinase K and SDS followed by deproteinization with organic reagents such as Phenol and Chloroform [6]. To isolate pure DNA from Blood samples we tried three different protocols. During extraction, we encountered many hurdles such as interference of hemoglobin, proteins and other components of blood. Inconsistent results were observed when we used SSC buffer for DNA isolation. Either very less DNA was isolated or there was no DNA yield. In case of SE buffer, though DNA was isolated but the pellet was reddish in color and gelatinous which was very difficult to get dissolve in TE buffer. Electrophoresis pattern showed presence of RNA and proteins along with DNA (**Figure 1A**) (**Table 2**). On the contrary, our standardized protocol yielded pure and good amount of DNA (**Figure 1B, depicted with arrow**). There was no protein or RNA contamination associated with the isolated DNA.

**Table 2: Different protocols used for blood DNA isolation**

Protocol	Result	Remarks
1. SSC buffer	No DNA isolated	No band was observed
2. SE buffer	Very less DNA was isolated	A feeble band with lot of RNA and protein contamination
3. Standardized protocol	DNA was isolated consistently	A sharp band was observed. DNA was suitable for Real Time PCR analysis.



**Figure 1: A: DNA isolated using SE buffer B: DNA isolated using standardized protocol**

To isolate pure blood DNA we made few modifications in the protocol,

1. During first step we found use of Milli-Q water instead of distilled water which removed hemoglobin more efficiently. Hence subsequent washing were reduced.
2. To remove proteins, we used 15, 25, 50 $\mu$ l of Proteinase K for 1 and 2 hours incubation and observed that incubation with 25 $\mu$ l for two hours removed proteins completely.
3. We found chilled isopropanol precipitated more and pure DNA than that of chilled ethanol.

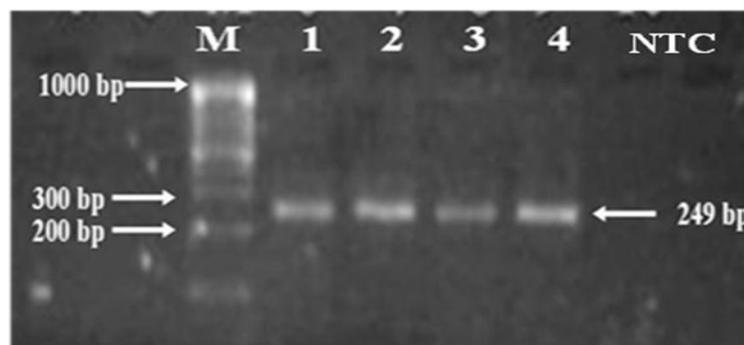
The key feature of the protocol was we neither used phenol nor chloroform: isoamylalcohol (24:1) for subsequent washings which avoided the traces of phenol throughout the procedure. During initial step we used sucrose which created osmotic pressure in the red blood cells causing rapid removal of hemoglobin and decolorization of the pellet. It also helped to lyse erythrocytes [5] [6]. With these modifications we obtained pure and large amount of blood DNA (**Figure 1, lane 4, and 7**).

#### Conventional PCR analysis:

Using conventional PCR the desire segment of DNA was amplified. No amplification was observed in NTC confirming that there was no false amplification.

### DISCUSSION

Earlier methods were laborious, technically demanding and expensive. To overcome these difficulties it was decided to standardize a rapid, reliable cost effective method for PCR analysis. To begin with, first we have standardized rapid blood DNA isolation without the use of hazardous chemicals such as Phenol and Chloroform: isoamylalcohol etc. Even no RNase was required as isolated DNA was very much purified (**Fig 1, lane 4 and 6**). Bands were sharp and consistent. For conventional analysis we have initially followed the method described [6]. But we couldn't get satisfied amplification product. Hence we modified the PCR cycle as initial denaturation was carried out for 10 mins instead of 5 mins at 95 $^{\circ}$ C. At the same time we carried out 40 cycles instead of 30 and final extension for 7 mins at 72 $^{\circ}$ C. Proper amplification was observed with above conditions (**Figure 2 Lane 1 to 4**).



**Figure 2: PCR product of 249 bp resolved on 2% agarose gel**



## CONCLUSION

The protocol mentioned in this study may prove to be efficient in yielding considerable amount of genomic DNA from human blood samples. Furthermore, the elimination of time consuming steps such as enzymatic incubation (RNase) and avoiding the use of toxic organic solvents made the protocol time- saving and economical without affecting the quality of the DNA samples which could be reliable enough for applications in advanced molecular biological techniques. Lastly it is a cost effective method

## REFERENCES

- [1]. Albarino CG, Romanowski V. Phenol extraction revisited: a rapid method for the isolation and preservation of human genomic DNA from whole blood. *Mol Cell Probes*. 1994;8:423–427
- [2]. Carpi FM, Di Pietro F, Vincenzetti S, Mignini F, Napolioni V. Human DNA extraction methods: patents and applications. *Recent Pat DNA Gene Seq*. 2011;5(1):1–7..
- [3]. Holmes FL. *Meselson, Stahl, and the Replication of DNA: A History of the Most Beautiful Experiment in Biology*. New Haven, CT: Yale University Press; 2001.
- [4]. Meselson M, Stahl FW. The replication of DNA in Escherichia coli. *Proc Natl Acad Sci U S A*. 1958;44(7):671–682.
- [5]. Duarte GR, Price CW, Littlewood JL, et al. Characterization of dynamic solid phase DNA extraction from blood with magnetically controlled silica beads. *Analyst*. 2010;135(3):531–537.
- [6]. Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2001.
- [7]. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res*. 1991;19(19):5444.



## ANTIMICROBIAL ACTIVITY OF *GARCINIA INDICA* AGAINST FOOD SPOILING BACTERIA

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Article Received on  
20 Dec. 2020,

Revised on 10 January 2021,  
Accepted on 01 Feb. 2021

DOI: <https://doi.org/10.17605/OSF.IO/ZWSSJ>

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### **ABSTRACT**

The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem. *Garcinia indica* (Kokam) native to India is one of such plants which has shown many therapeutic uses. The present study shows the antimicrobial activity of fruit extracts of *G.indica* against *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli* (gram negative bacteria). Out of three screened fractions (Chloroform, ethyl acetate and water fraction), ethyl acetate fraction was found to be more effective against *S.aureus* and *E.coli* as compared to other fractions.

**KEYWORDS:** Food spoilage, Antibacterial activity Natural preservatives, Fruit extracts, *Garcinia indica*.

### **INTRODUCTION**

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption. Food borne sickness is any illness resulting from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that infect food & we consume that spoiled food consciously or unconsciously.<sup>[1]</sup> Food borne illness usually arises from improper handling, preparation, or food storage where hygienic approach is not followed. There is potential for a wide range of food products to become contaminated with microorganisms. Most of the reported outbreaks have been associated with bacterial

contamination, particularly members of the Enterobacteriaceae. Of these, *Salmonella* and *Escherichia coli* are of particular concern.<sup>[2]</sup> Other bacteria commonly responsible for food spoilage are *Bacillus cereus*, *Staphylococcus aureus*, etc. Thus, considering all the above facts it is important to explore remedy to illness caused by food spoilage. It is also important because we are daily exposed to such infections in our lives. We consume such food that might be unhygienic or casually prepared. Ultimately we compromise with our health. Remedy to all such disorders is not far away. As soon as we realize that nature has entitled us with solution to all of our problems. There has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of antimicrobial chemical additives.

India is rich in biodiversity and has a wide spectrum of habitats from tropical rainforests to alpine vegetation and from temperate forests to coastal wetlands. About one third of the country's recorded flora is endemic and is concentrated mainly in the North-East, Western Ghats, and North-West Himalaya. Western Ghats of India are known for their valuable biodiversity and has been considered as one amongst the top most important eight hotspots in the world. This hotspot of biodiversity is a treasure house of genetic resources of many plant species. *Garcinia indica* (family- Clusiaceae) is one such tree species endemic to tropical rain forests of Western Ghats of India and is included under the list of endangered species of medicinal plant of Southern India.<sup>[3]</sup> Its fruits are a rich source of Hydroxycitric Acid (HCA), an important biologically active plant metabolite used as anti-obesity and anti-cholesterol drug. The fruits are also used to prepare a pleasant attractive beverage which has bilious action. The fat extracted from the seeds is used in cosmetics as emollient. A lot of work has been carried out on various aspects of extracts separated from fruit rinds of *G.indica*. Fruit rind extracts have shown good anti hyaluronidase and anti elastase properties.<sup>[4]</sup> Garcinol and Hydroxycitric Acid (HCA) present in *G.indica* have showed significant anti oxidant and anti hyperlipidemic activity. Fruit rinds of *G. indica* contain anthocyanins like cyanidin-3-glucoside and cyanidin-3-sambuboside.<sup>[5]</sup> Along with this, garcinol the yellow colored pigment and cambogiol present in the fruit rinds showed good antioxidant activity due to presence of phenolic group.<sup>[6]</sup> Fruit extract of *G.indica* showed antidandruff activity against *M. furfur*.<sup>[7]</sup> But there are very few reports on anti microbial activity of fractions separated from fruit rinds of *G.indica*. Taking this into consideration it was decided to screen various fractions of fruit rinds of *G.indica* for their antimicrobial activity against *E.coli* and *S.aureus*..

## MATERIALS AND METHODS

### 1. Plant Source

The ripe fruits of *G.indica* were collected from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra.

### 2. Microorganism used

The organisms used in this study were *E.coli* (ATCC 11775) & *S.aureus* (ATCC 35552) were procured from MTCC, Chandigarh, India.

### 3. Reagents and chemicals

This work was carried out in Research Laboratory of Department of Botany, K.V. Pendharkar College, Dombivli. The ripe fruits of *G.indica* were collected from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra.

### 4. Preparation of fruit extracts

**Preparation of Methanolic Extract:** The methanolic extract (ME) was prepared by immersing (20 gms) of dried fruit rinds of *G.indica* in 200 ml of acidified methanol (2% Concentrated HCl). The extract was poured in the evaporating dish and allowed to dry at room temperature to obtain 6 gm solid (ME).

**Separation of fractions:** One gram of ME was dissolved in 50 ml of D/W. To this 50 ml of ethyl acetate and 50 ml of chloroform was added and three fractions were allowed to separate in a separating funnel for at least one hour. Ethyl acetate fraction (EAF), chloroform (CF) and water fraction (WF) were separated. All the fractions were air dried to obtain 0.2 gm EAF, 0.3 gm CF and 0.5gm WF. These fractions were used to study antimicrobial activity.

### Antimicrobial test assay

Antimicrobial susceptibility test was carried out by the disc diffusion method. The petridish containing nutrient agar was plated with 0.1 ml culture of both bacterial strains (*E. coli*, and *S. aureus*). The plates inoculated with bacteria, were made in triplicate. The discs containing the fractions (10mg/ml and 25mg/ml) separated from fruit rinds were placed on the agar using sterile forceps. Gentamycin (30 mcg) was used as positive control. The plates were incubated at 37°C for about 24 to 48 hours. The diameter of resultant zone of inhibition was measured in millimeters.

## RESULTS AND DISCUSSION

Numerous studies have documented the antimicrobial potency of the crude extracts from genus *Garcinia* as well as that of some of their antimicrobial components.<sup>[8], [9]</sup> Although the *Garcinia* species are gaining much attention worldwide due to their potential bioactivities, the *Garcinia* species in the Western Ghats are least investigated for their bioactivities. *Garcinia indica* commonly known as Kokam plant has already gained a lot of attention due to its various anti-inflammatory, anti-oxidant, free radical scavenging as well as anti-dandruff activities. (-) Hydroxycitric acid from leaves and fruit rind is anti-obesity and anti-cholesterol drug. Fruits are rich source of Hydroxycitric Acid (HCA), an important biologically active plant metabolite used as anti-obesity and anti-cholesterol drug. The fruits are also used to prepare a pleasant attractive beverage which has bilious action. The fat extracted from the seeds is used as cosmetics as emollient.

Our studies showed that parent methanolic extract was not that effective against both the bacteria. Very less inhibition was observed. As compared to this, ethyl acetate fraction at 10mg/ml showed significant inhibition ( $17.34 \pm 0.37$  for *E.coli* &  $18.33 \pm 0.50$  for *S.aureus*) respectively and for 25mg/ml results were comparable with Gentamycin (**Table No. 1**). On the other hand water and chloroform fraction were not that effective against both the microorganisms.

**Table No. 1: Antimicrobial activity of various fractions of fruit rinds of *G. indica* against *E.coli* and *S.aureus*.**

Sr. No.	Drug used	Concentration	Diameter of zone of inhibition (mm)	
			<i>E. coli</i>	<i>S. aureus</i>
1	Methanolic Extract	10 mg/ ml	$7.00 \pm 0.57$	$9.00 \pm 0.57$
		25 mg/ ml	$12.33 \pm 0.37$	$14.22 \pm 0.22$
2	Ethyl Acetate Fraction	10 mg/ ml	$17.34 \pm 0.37$	$18.33 \pm 0.47$
		25mg/ ml	$22.45 \pm 0.37$	$27.33 \pm 1.22$
3	Water Fraction	10 mg/ ml	$8.33 \pm 0.37$	$10.22 \pm 0.50$
		25mg/ ml	$10.2 \pm 0.37$	$11.22 \pm 2.12$
4	Chloroform Fraction	10 mg/ ml	$9.22 \pm 0.37$	$9.45 \pm 0.44$
		25mg/ ml	$12.33 \pm 0.37$	$14.22 \pm 0.58$
5	Gentamycin	30mcg	$23.00 \pm 1.155$	$25.11 \pm 1.15$

## CONCLUSION

Food spoilage is often caused by the growth of many pathogenic bacterial strains. Prevention of food spoilage in food industry and food stuff is mainly based on the application of

chemical preservatives. The adverse effects of these chemical preservatives on human health increase the demand to search for potentially effective, healthy safer and natural food preservative. In this preliminary studies, ethyl acetate fraction of fruit extract of *G.indica* which proved to be potentially effective as (*E.coli and S.aureus*) can be used as natural alternative preventives to control food poisoning diseases. Synergistic effect of this fraction with other plant extracts could be further exploited.

### ACKNOWLEDGEMENT

The author thanks Principal and Head, Dept of Botany for providing the laboratory facilities.

### REFERENCES

1. Bhatia R and Narain J. P, The growing challenge of antimicrobial resistance in the South-East Asia Region - are we losing the battle? *Indian Journal of Medical Research*, 2010; 132(5): 482–486.
2. Boucher H.W., Talbot G.H., Bradley J.S. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America,” *Clinical Infectious Diseases*, 2009; 48(1): 1–12.
3. Rajasekharan PE. and Ganeshan S. Conservation of medicinal plant biodiversity – an Indian perspective. *J. Med. Arom. Plants*, 2002; 24: 132-134.
4. Sahasrabudhe A. and Deodhar M. Anti-hyaluronidase and anti-elastase activity of *Garcinia indica*. *Int. J. Bot*, 2010; 6(3): 299-303.
5. Krishnamurthy N, Lewis S, and Ravindranath B. On the structures of garcinol, isogarcinol and cambogiol, *Tetrahedron let.*, 1981; 22: 793-796.
6. Krishnamurthy N, Lewis S and Ravindranath B. Chemical constituents of Kokam fruit rind. *J. Food Sci, Technol.*, 1982; 19: 97-99.
7. Sahasrabudhe A. and Phanse M. Culturing of *Melassezia furfur* and its growth inhibiting activity of *Garcinia indica*. *Int. J. Pharm.* 2015; 2(8): 409-413.
8. Manandhar S, Luitel S, and Dahal R. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *J. Trop. Med.*, 2019: 1-5.
9. Lakshmi C, Akshaya Kumar, Dennis, T.J. and Sanath Kumar T.S. Antibacterial Activity of Polyphenols of *Garcinia Indica*. *Ind. J. Pharm Sci.* 2011; 73(4): 470–473.

## A SURVEY OF ETHNOMEDICINAL PLANTS TO MANAGED THE HUMAN FERTILITY FROM DHAMNI VILLAGE IN DHARAMPUR TALUKA, GUJARAT

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Received on: 17/11/2020

Revised on: 07/12/2020

Accepted on: 27/12/2020

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### ABSTRACT

India is a prosperous in its flora resources and a residence of different cultural as well as a enriching groups, which have developed their own individual strength care systems. The traditional and herbal medicines are frequently depended by the poor as well as people living in remote areas as those medicines had considered as efficient, secure, expenditure effective and reasonable than the people living in the urban areas. The survey was conducted in Dhamni village of Dharampur taluka situated in Valsad district. Thetribal's living in that area have a great potentiality in the profitable as well as in botanical point of view. For their sexual impotency; they rely more on plants of their area to overcome that tendency for recovering.

**KEYWORDS:** Ethnomedicine, Dhamni, Valsad & Tribals.

### INTRODUCTION

India is a rich source of flora and fauna where it had been recorded 7% of its world diversity. Ethnobotany is the branch which deals with the relationship of plants and the people. In India, Dr. S.K. Jain was considered as a pioneer of Ethnobotany. According to him, the plants were the source of medicine for the rural people staying in particular area. The rural people were in a deep and unique knowledge of the plants uses and their ailments to treat dreadful diseases as well as in discovery of new herbal sources for benefit the human-beings. India had occupied a top position in exporting plant drugs and its derivatives in the use of herbal drugs. In India, medicinal plants lead a history of 3,000 plants which had been listed. (Asolkar, et al., 1992). The local villagers and tribal staying in the vicinity of forest collect the medicinal plants which had been used as medicines and their source of income. To relief pain and discomfort, the ethnomedicine are used by human over many generations. The main source of drug therapy in ethnomedicine which had been passed on to the generation after generation, gathered by the ancestors. In rural and tribal population, the folk system of health traditions is widely spread. The knowledge of medicinal plants is considered as a chief uniqueness for curing their ailments by tribal's.

The medicinal plants have been beneficial in reproductive health of human body. The nature has given a boon to human life to cure the human body which is a gift from the god to propagate its progeny. To overcome

that incapability the medicinal plants have been used as remedies to overcome it. To treat gynecological health issues the study of Ethnobotanical plants is required to identify and document it.

### MATERIALS AND METHODS

The survey was conducted in Dhamni village for ethnomedicinal plants used as remedies to cure impotency in people of that area. To achieve relevant information regarding the local names, plant part and the method of its dosage; a continuous meets and expansive dialogues have been made among the knowledgeable local residential people. Photographs of those plants have been taken to keep a record as a document. Due to continuous meet and a good rapport among the people helps to know the relevant knowledge of indigenous plants in that area. All the seasons were covered during the field visits. The plants were enumerated in alphabetical order along with its Botanical name, Family, Local name and mode of its remedies. **Study Area:** In Gujarat state of Valsad district, Dhamni village is situated in Dharampur taluka; considered as a large area where 580 families were residing. Most of the village population is from Schedule tribes (ST) and has a lower literacy rate compared to the other villages of Dharampur taluka. Among the total population, Schedule tribes constitutes 99.64%. As tribal's are more in number in Dhamni village, they fully depend on the plants for their food, shelter and of medicinal uses.

**RESULT AND DISCUSSION**

The information of ethnomedicinal plants have been gathered from the area under study 24 species belonging to 23 genera and 19 families. The collected data based on Reproductive system of Local inhabitant. (Table 1.) shows importance of medicinal plants in Dhanu tribe

people.. It is observed that the dosages and duration of area; medicine generally depend on the intensity of the sexual potency of human being. To obtain maximum concentration of the active constituents, the tribal's harvest the medicinal plants at different stages of growth or season.

**Table 1: Ethnomedicinal plants survey and Its uses in tribal people Dahnu Gujarath.**

Botanical Name	Local name	Family	Part used	Uses
1. <i>Acacia nilotica</i> Linn.	Baval	Mimosae	Gum	For good health after delivery for pregnant women and her child. Acacia gum along with coconut fruit sugar is mixed with half teaspoon applied to body.
2. <i>Achranthes aspera</i> L.	Andhedi	Amaranthaceae	Leaves	In sexual debility, fresh leaves are taken on empty stomach.
3. <i>Bauhinia racemosa</i> Lam.	Raktakanchnar	Caesalpinaceae	Root	For easy delivery, root extract is used.
4. <i>Bryonia laciniata</i> Linn.	Shivlingi	Curcubitaceae	Leaf, seeds	The seeds 5-9 with milk are given to women, for conceived after the periods. The seed powder is used for increasing sperm count in males.
5. <i>Calotropis procera</i> (Wild) R. Br.	Vachhnag	Asclepiadaceae	Flower bud	For menstrual problems in women, along with betel nut, the flower bud is taken.
6. <i>Carica papaya</i> L.	Papaya	Caricaceae	Leaf, Root	Roots extraction is used to abort in early pregnancy.
7. <i>Curculigo orchioides</i> Gaertn.	Kali Musli	Amaryllidaceae	Root	Root powder in 5-10 grams with milk is taken to stimulate male hormone and also useful to reduce stress.
8. <i>Dendrocalamus strictus</i> (Roxb.) Nees	Vans	Gramineae	Leaves	For normal delivery, leaves are cut in small pieces and tied with cotton thread on the neck of the pregnant lady to occur normal delivery.
9. <i>Echinops echinatus</i> Roxb.	Utkantho	Asteraceae	Root	To easy delivery, root is tied on the cold temperature in the body.
10. <i>Flemingia tuberosa</i> Dalzell	Bhadeli	Fabaceae	Root	Due to high content of iron in roots, it is preferable eating.
11. <i>Hollarhena pubescens</i> Wall. ex G. Dun	Kadvo Indrajav	Apocynaceae	Seed	In women, the seeds are used for tone up the vaginal tissue after delivery. It also helps to promote milk in nursing mothers.
12. <i>Gloriossasuperba</i> L.	Dudhiyovachnag	Liliaceae	Root	For easy delivery, root extract is used.
13. <i>Ipomoea sepiaria</i> Koenig ex-Roxb.	Laxmana	Convolvulaceae	Root	Root decoction is given to women in gynecological disorder.
14. <i>Manikara hexandra</i> Roxb	Rayan	Sapotaceae	Stem	To abort the child, stem bark is crushed and given a half cup of it to the pregnant lady.
15. <i>Moringa concanensis</i> Nimmo.	Jangali Saragavo	Moringaceae	Leaves	In women for fertility. The juice of fresh leaves is to be taken internally.
16. <i>Moringa oleifera</i> Lam.	Saragvo	Moringaceae	Leaf, Pods	In pregnant women, cooked leaves are given in last trimester for easy delivery. It also helps in treating menstrual cramps. The seeds are

				used in treating male impotency.
17. <i>Mucuna pruriens</i> (L.) DC.	Kavach	Fabaceae	Seeds	To increase longer sex in both men and women, seeds are used.
18. <i>Nelumbo nucifera</i> Adans.	Kamal	Nelumbonaceae	Seeds	In male, the seeds are beneficial to treat weak sexuality. It also benefitted to women in leucorrhoea.
19. <i>Phaseolus vulgaris</i> L.	Udad	Fabaceae	Seeds	Pods are used in improving sexual power and boost in immunity in the body.
20. <i>Pueraria tuberosa</i> (Willd.) DC	Phagvelo	Fabaceae	Root	In human, it enhances and improves sexual desire
21. <i>Ruellia tuberosa</i> L.	Bandhukadi	Acanthaceae	Leaf	The leaf decoction is given to pregnant women to form a combed hair of the pregnant women and immediately to be removed after delivery.
22. <i>Saraca asoca</i> (Roxb.) de Wilde.	Ashok	Caesalpinaceae	Bark, Seed	The bark decoction is given to control irregularity in menstrual cycle.
23. <i>Trapa bispinosa</i> Roxb.	Shingoda	Trapaceae	Fruits	The fruit improves sexual potency in human being. It is also given to women suffering from imminent abortion.
24. <i>Woodfordia fruticosa</i> (L) Kurz	Dhavdo	Lythraceae	Seeds	The dried seed powder along with milk is taken to control menstrual disorder.

### SUMMARY AND CONCLUSION

The paper focus on some bright feature of local plants used as a medicine in various aspects of human reproductive system and ailments by the tribal people of Dhamni. It had been found that the local respondent still relay on plant resources for treating the sexual imbalance in the human body, from the survey. As it has been noticed that medicinal plants are getting diminished from the area due to the deforestation, grazing of cattle's in that area. The allopathic medicine is more applicable by young generation as traditional medicines are a slow process of recovering. For incoming future the people as to encourage growing varied medicinal plants species in the field and wild plants to be protected for upcoming generation.

### REFERENCE

- Rafik U. Shaikh et al., "Ethnobotanical Study of Folk Medicinal Plants used by Villagers in Nanded District of Maharashtra (India)". International Journal of Ayurvedic and Herbal Medicine, 2014; 4(5): 1585-1595.
- Ashok K Pandey & Y C Tripathi Ethnobotany and its relevance in contemporary research", Journal of Medicinal Plants Studies, 2017; 5(3): 123-1293.
- Chandrakant Laxman Marathe and v. V. Bhaskar "Traditional methods of healing practiced by warli tribes in Thane district of Maharashtra state", International Journal of Pharmacy & Life Sciences, 2011; 2(6): 884-893.
- Vishal H. Rao, et al., "Floristic study of Kaprada's hilly forest in South Gujarat", IJPS International Journal of Plant Sciences, 2013; 8(1): 100-102.
- M K Desale et al., "Medicinal plants used by the rural people of Taluka Purandhar, district Pune, Maharashtra". Indian Journal of Traditional Knowledge, 2013; 12(2): 334-338.
- C. Patel Dharmesh and B.L. Jat "An Ethnobotanical Survey of Medicinal Plants used by Traditional Healers of Kaprada Forest (Valsad District), Gujarat, India", International Journal of Current Microbiology and Applied Sciences, 2018; 7(07): 2034-2043.
- Mahadkar Shivprasad et al., "Documentation and Ethnobotanical Survey of Wild Edible Plants from Palghar District", Asian Journal of Pharmaceutical and Clinical Research, 2016; 9(2): 16-19.
- Shivangi Chaudhari et al; "Traditional Ethnomedicinal Plants used by the Tribal in Coastal Area of Dahanu Taluka, Palghar District, Maharashtra State, India", IJSR. International Journal of Scientific Research, 2017; 6(2): 748-750
- Sadale A.N. and Karadgen B A. "Survey on Ethno-Medicinal Plants of Ajara Tahsil, District Kolhapur, Maharashtra- (India)", TLS An International Peer – Reviewed Journal, 2013; 2(1): 21-25.

EFFECT OF BIOFERTILIZERS ON SEEDLING GROWTH OF PADDY (*ORYZA SATIVA* L.) CV. PANVEL 3.N. B. Pawar and N. S. Suryaawanshi<sup>2\*</sup><sup>1</sup>Department of Botany, Mahatma Phule A. S. C. College, Panvel, Dist Raigad M.S. India. 410206.<sup>2</sup>Research Laboratory, Department of Botany, DSPM, S K. V. Pendharkar College, of Art, Science and Commerce Dombivili €, Mumbai India. 421203.

Received on: 16/12/2020

Revised on: 06/01/2021

Accepted on: 26/01/2021

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## ABSTRACT

The present investigation was carried out in kharif season, during 2015 and 2016 at research farm, 'Rayat Shikshan Sanstha's, M.P.A.S.C. College Panvel, District- Raigad (Maharashtra), India. To observe the effect of different biofertilizers on growth and yield parameters on Paddy (*Oryza sativa* L. cv. Jaya). The experimental farm was geographically situated at 18° 59' 40" N latitude and 73° 06' 50" E longitude at an altitude of 28 meters above mean sea level. The experiment was laid out in RBD replicated thrice with twelve treatments i.e. (T0) Control (without fertilizer), (T1) Chemical fertilizer (19:19:19), (T2) Blue green algae, (T3) *Azospirillum brasilense*, (T4) *Bacillus megaterium*, (T5) *Trichoderma viride*, (T6) Mycorrhizae, (T7) *Pseudomonas aeruginosa*, (T8) T2+T7, (T9) T2+ T6, (T10) T3+T4, and (T11) T3+T4+T7. RDC fertilizer was applied in three splitted doses. The first dose, consisting of 1/3 the normal dose, was applied before transplantation; the second 1/3 at the time of tillering; and the last 1/3 at the panicle initiation phase. The study revealed the growth parameters like shoot length, root length, and dry matter production at various stages of growth in Paddy (*Oryza sativa* L.) cv. Jaya were favorably influenced by biofertilizers treatment. Overall results suggest that combine effect of Biofertilizers improves vegetative and reproductive growth of Paddy (*Oryza sativa* L. cv. Jaya)".

**KEYWORDS:** Biofertilizers, Growth and yield parameters, Paddy (*Oryza sativa* L. cv. Jaya).

## INTRODUCTION

Paddy (*Oryza sativa* L.) is most important staple food crop in the world and is grown under a broad range of environmental conditions. India is second largest producer and consumer of rice in the world after China. At national level, area under cultivation is 42.5 million hectares with the production of 152.6 million tones and average productivity is of 3.5 tones per hectares. At global level, paddy is cultivated under 158.4 million hectares area with annual production of around 697.2 million tones and average productivity of 2.85 tones per hectares (Sarvan et al., 2016).

Fertilizers come in two types - they are either chemical or biofertilizers. Increasingly high inputs of chemical fertilizers during last 15 decades have not only left soils degraded, polluted and less productive but have also posed severe health and environmental hazards. Organic farming methods (such as the use of biofertilizers) would solve these issues and make the ecosystem healthier. Biofertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen, both, in association with plant roots and without it, solubilise insoluble soil phosphates and produce plant growth

substances in the soil. They are in fact being promoted to harvest the naturally available, biological system of nutrient mobilization (Venkateshwarlu, 2008). The role and importance of biofertilizers in sustainable crop production has been reviewed by several authors (Biswas et al. 1985; Wani and Lee, 1995; Katyal et al. 1994).

Biofertilizers are becoming increasingly popular in many countries and for many crops. They are defined as products containing active or latent strains of soil microorganisms, either alone or with algae or fungi that increase plant availability and uptake of mineral nutrients (Vessey et al., 2006) 571-586. Bio-fertilizers containing beneficial bacteria and fungi improve soil chemical and biological characteristics, phosphate solutions and agricultural production (El-Habbasha et al., 2007; Yosefi et al., 2011). Microbiological fertilizers are important to environment friendly sustainable agricultural practices (Bloembergen et al., 2000). The Biofertilizer includes mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms (Goel et al., 1999).

## MATERIALS AND METHODS

### Collection of seeds and raising seedlings

Paddy (*Oryza sativa* L. cv. JAYA) seeds were collected from the Kharland research station Panvel, Dist Raigad. Jaya is a medium duration high yielding variety of rice. It is recommended for both crop seasons. The variety is known for its greater yield potential. The grains are long and white with good cooking quality.

**Transplanting of paddy seedlings:** Twenty one days old paddy seedlings were transplanted at 20 cm x 15 cm spacing during both the seasons with five seedlings per hill. Gap filling was carried out twelve DAT in order to ensure uniform plant population.

### Experimental site

This investigation was carried out at research farm of RayatShikshanSanstha's MahatmaPhuleA.S.C.College, Panvel, Dist.Raigad (Maharashtra). The experimental farm is geographically situated at 18°, 59' 40" N latitude and 73°, 06' 50" E longitude at an altitude of 28 meters above mean sea level. The experiment was conducted on the same site and layout during both the years. The study area is representative of the agro-ecological sub-region 19.3 covering north Konkan coastal zone of Maharashtra (Sehgal *et. al.*, 1992), which comprises of Thane & Raigad districts.

Experimental details-	
Type of Soil	Garden clay-loamy
Name of the Method:	Seed treatment
No .of Replications	3
No. of seeds sown	20
Size of pot	1x1 m <sup>2</sup>
Treatment details-	
Notation for treatment	T
T0	Control
T1	<i>Azospirillum brasilense</i>
T2	<i>Bacillus megaterium</i>
T3	<i>Azospirillum brasilense</i> + <i>Bacillus megaterium</i>
T4	

\*

### Collection of experimental data

#### a) Growth parameters-

vi) Straw yield (q./ha.)- The weight of the straw harvested from the net area in each treatment was recorded after five days sun drying in the field and then converted onq/ha.

**Statistical Analysis-** Pooled data was used for analysis. Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments (Gomez and Gomez, 1984).

**Table 1: Effect of bio fertilizers on root length at various stages of growth in Paddy (*Oryza sativa* L.) variety, Jaya. (Pooled data of two yrs.).**

Treatments	Root length (cm.)			
	30DAT	60 DAT	90 DAT	At harvesting
T0- Control	3.916	7.332	8.393	10.08
T1- Chemical fertilizer	6.15	8.855	9.828	11.985
T2- BGA	4.537	7.884	9.45	10.507
T3- <i>Azospirillum</i>	4.933	10.166	11.971	12.039
T4- <i>Bacillus</i>	4.809	10.431	11.9333	13.025
T5- <i>Trichoderma</i>	4.439	9.072	10.487	12.078
T6- Mycorrhizae	5.527	10.621	12.986	13.769
T7- <i>Pseudomonas</i>	5.274	9.894	12.881	13.781
T8- T2+ T7	6.129	11.863	14.186	15.099
T9- T2+ T6	6.964	13.794	15.435	16.057
T10- T3+T4	7.866	14.459	15.326	17.605
T11- T3+T4+T7	7.989	16.033	16.433	17.959
SE m ±	0.306	0.69	0.453	0.421
CD at 0.05 %	0.866	1.951	1.281	1.189
C.V.%	0.722	0.975	0.528	0.443

Values are the Mean of three replicates.

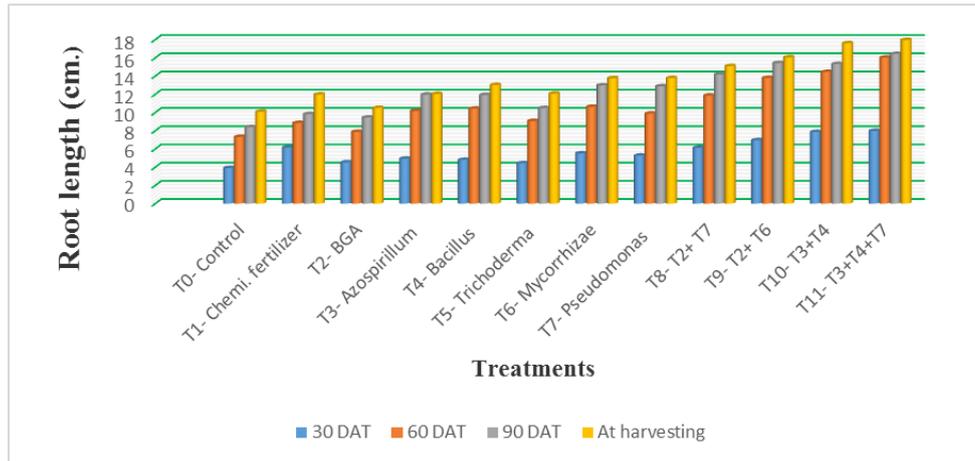


Fig. 1: Root length.

Table 2: Effect of bio fertilizers on shoot length at various stages of growth in Paddy (*Oryzasativa L.*) variety, Jaya. (Pooled data of two yrs.).

Treatments	Shoot length (cm.)			
	30DAT	60 DAT	90 DAT	At harvesting
T0- Control	16.647	32.541	47.496	49.161
T1- Chemical fertilizer	23.329	43.349	54.469	55.735
T2- BGA	17.728	36.971	49.192	52.693
T3- Azospirillum	19.043	46.38	56.619	58.749
T4- Bacillus	19.612	43.899	54.983	56.996
T5- Trichoderma	18.334	34.902	52.956	54.226
T6- Mycorrhizae	19.899	37.438	54.924	57.219
T7- Pseudomonas	21.197	45.04	56.913	59.978
T8- T2+ T7	23.173	47.023	58.369	61.773
T9- T2+ T6	23.817	50.931	58.136	61.556
T10- T3+T4	26.081	51.044	58.004	63.031
T11- T3+T4+T7	26.911	53.884	60.314	63.635
SE m ±	0.519	1.049	1.107	1.001
CD at 0.05 %	1.469	2.967	3.132	2.833
C.V.%	0.351	0.344	0.285	0.254

Values are the Mean of three replicates.

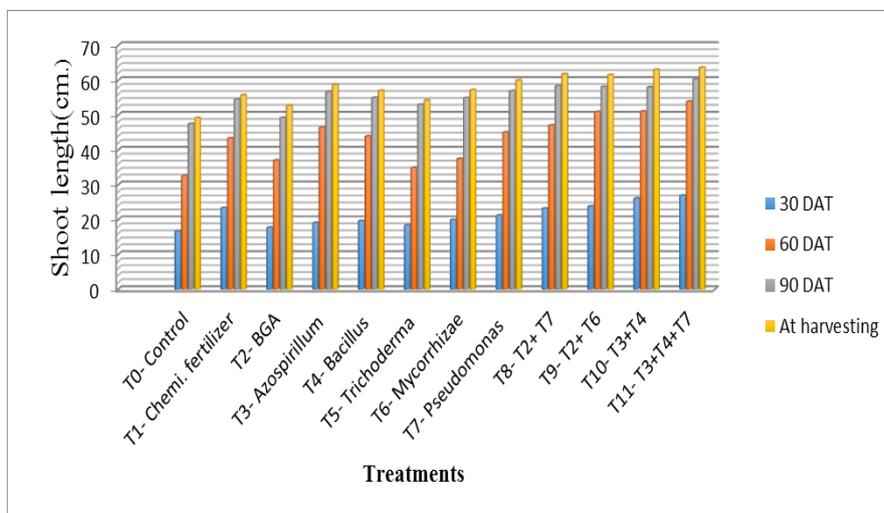
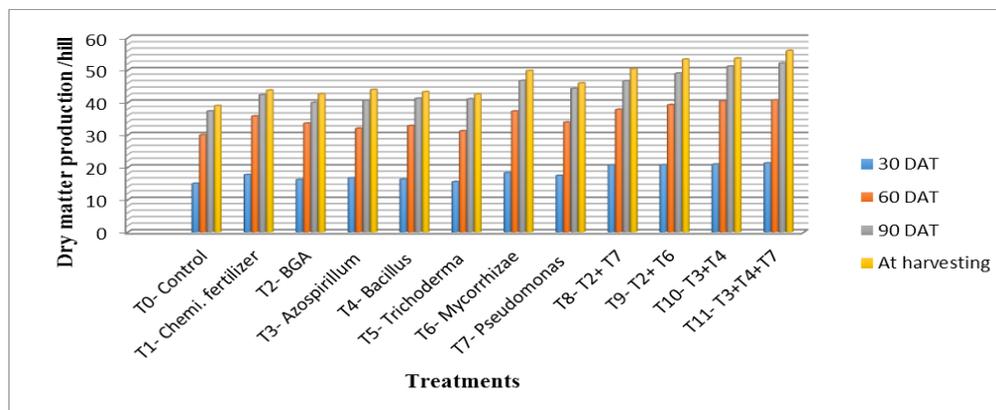


Fig. 2: Shoot length.

**Table 3: Effect of bio fertilizers on dry matter production at various stages of growth in Paddy (*Oryza sativa* L.) variety, Jaya.(Pooled data of two yrs.).**

Treatments	Dry matter production (gm./hill)			
	30DAT	60 DAT	90 DAT	At harvesting
T0- Control	14.982	30.012	37.321	39.012
T1- Chemical fertilizer	17.691	35.779	42.332	43.766
T2- BGA	16.156	33.563	39.975	42.605
T3- <i>Azospirillum</i>	16.675	32.001	40.6118	43.977
T4- <i>Bacillus</i>	16.321	32.827	41.2637	43.292
T5- <i>Trichoderma</i>	15.501	31.206	41.058	42.552
T6- Mycorrhizae	18.277	37.306	46.728	49.828
T7- <i>Pseudomonas</i>	17.442	33.899	44.319	45.986
T8- T2+ T7	20.68	37.826	46.626	50.339
T9- T2+ T6	20.732	39.286	48.994	53.328
T10- T3+T4	20.937	40.433	51.143	53.641
T11- T3+T4+T7	21.294	40.737	52.11	55.99
SE m ±	0.457	0.621	0.668	0.684
CD at 0.05 %	1.293	1.756	1.891	1.935
C.V.%	0.367	0.263	0.215	0.206

Values are the Mean of three replicates.

**Fig. 3: Dry matter production.****Table 4a: Effect of different bio fertilizers on length of panicle, weight of panicle and number of spikelet's/panicle of Paddy (*Oryza sativa* L.) variety, Jaya. (Pooled data of two yrs.).**

Treatments	Yield parameters		
	Length of Panicle (cm.)	Weight of panicle (gm.)	No. of Spikelet's Per panicle
T0- Control	20.344	2.191	10.836
T1- Chemi. fertilizer	23.395	2.395	11.823
T2- BGA	20.653	2.346	11.122
T3- <i>Azospirillum</i>	21.215	2.357	10.926
T4- <i>Bacillus</i>	22.238	2.435	11.218
T5- <i>Trichoderma</i>	21.444	2.344	11.265
T6- Mycorrhizae	23.601	2.541	12.343
T7- <i>Pseudomonas</i>	24.208	2.476	12.775
T8- T2+ T7	25.586	2.546	13.513
T9- T2+ T6	26.331	2.922	14.401
T10- T3+T4	26.461	3.114	14.992
T11- T3+T4+T7	27.088	3.153	15.498
SE m ±	0.293	0.09	0.296
CD at 0.05 %	0.832	0.257	0.837
C.V.%	0.183	0.511	0.334

Values are the Mean of three replicates.

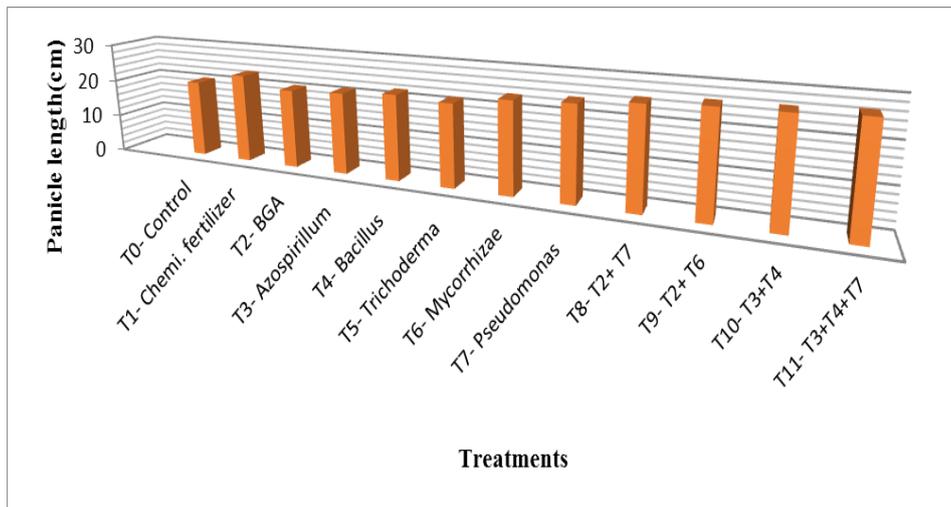


Fig. 4: Panicle length.

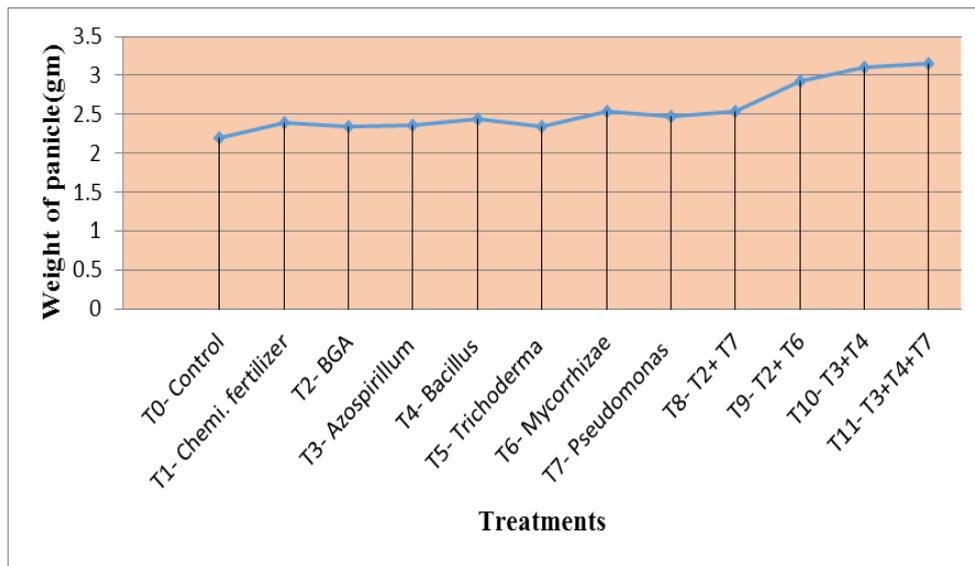


Fig. 5: Weight of Panicle.

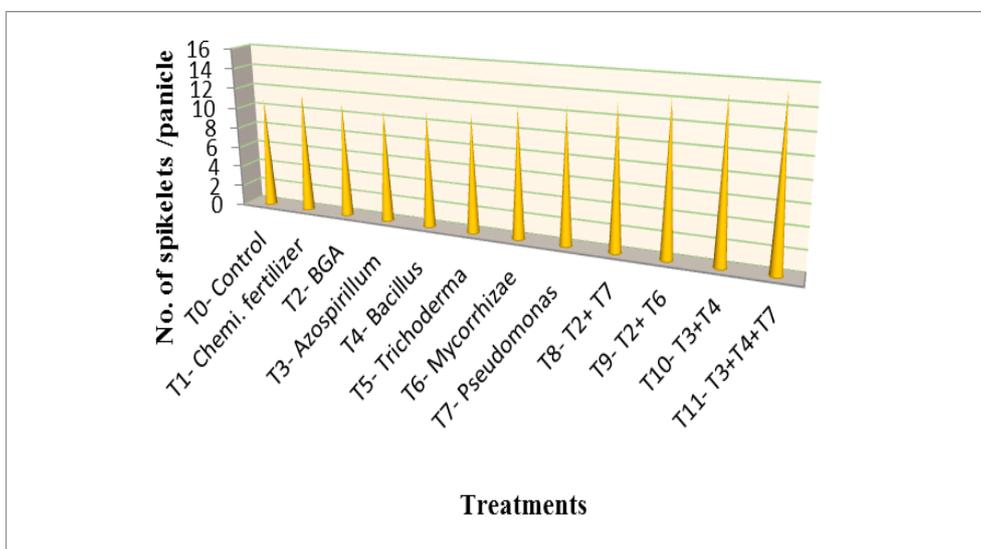
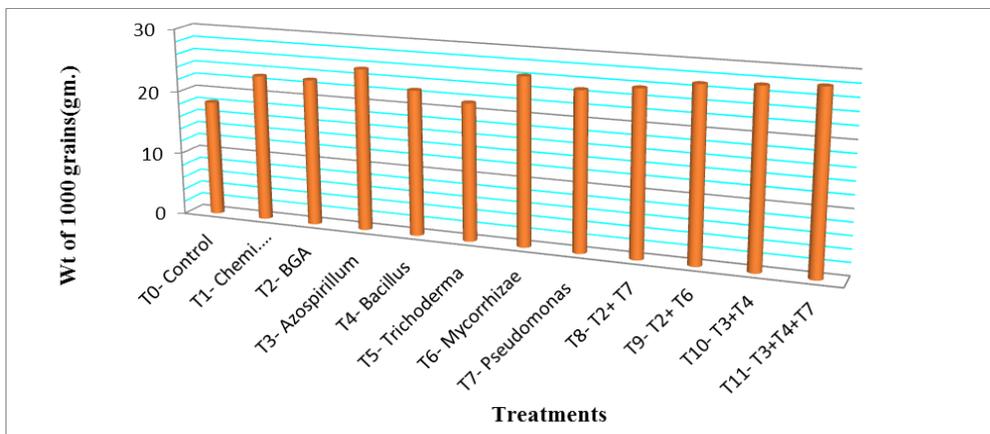


Fig. 6: No. of spikelet's/ panicle.

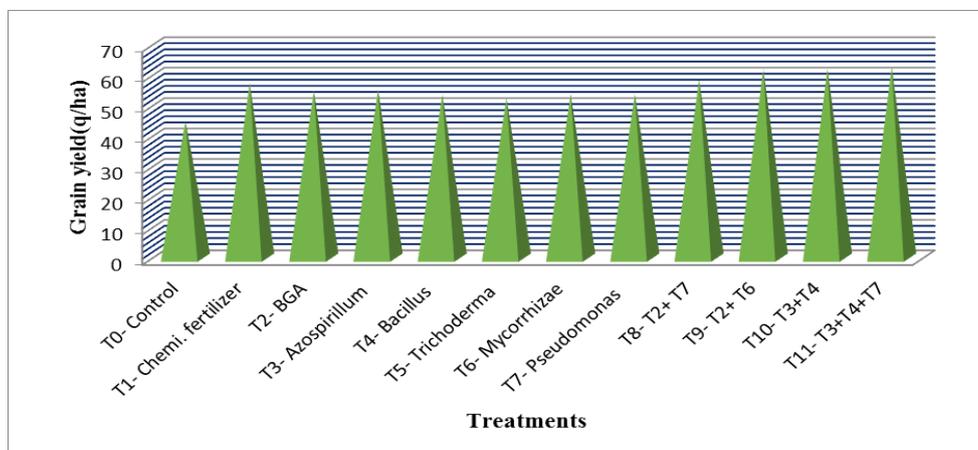
**Table 4b: Effect of different bio fertilizers on test weight, grain and straw yield of Paddy (*Oryza sativa* L.) variety, Jaya.(Pooled data of two yrs.).**

Treatments	Yield parameters		
	Wt. of 1000 Seeds(gm.)	Grain yield (q./ha.)	Straw yield (q./ha.)
T0- Control	18.265	44.905	67.941
T1- Chemi. fertilizer	23.113	57.291	74.648
T2- BGA	23.007	54.819	74.078
T3- <i>Azospirillum</i>	25.453	55.214	75.375
T4- <i>Bacillus</i>	22.808	53.667	73.781
T5- <i>Trichoderma</i>	21.527	52.385	72.908
T6- Mycorrhizae	26.244	53.675	74.363
T7- <i>Pseudomonas</i>	24.855	53.716	75.243
T8- T2+ T7	25.771	58.524	76.921
T9- T2+ T6	27.015	61.867	77.896
T10- T3+T4	27.467	62.043	77.923
T11- T3+T4+T7	27.973	62.685	78.715
SE m ±	0.463	1.153	1.679
CD at 0.05 %	1.301	3.261	4.726
C.V.%	0.274	0.297	0.324

Values are the Mean of three replicates.



**Fig. 7: Test weight of grains.**



**Fig. 8: Grain yield.**

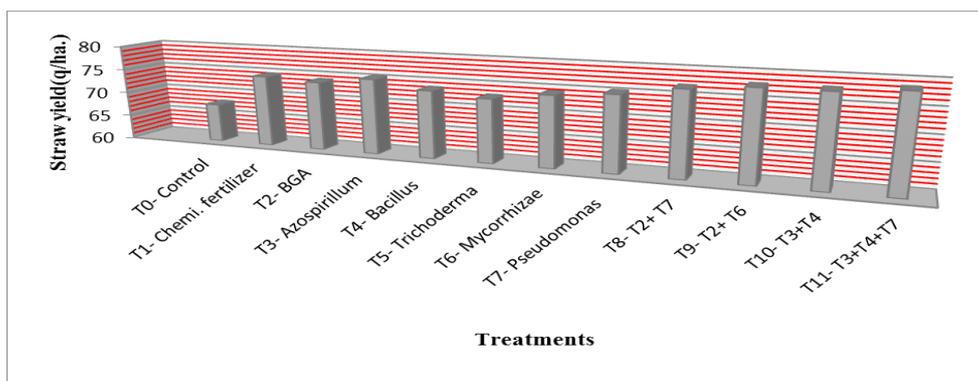


Fig. 9: Straw yield.

## RESULTS AND DISCUSSION

Data on mean values of growth parameters pertaining to different treatments are presented in **Table 1 to -4**. The good results were observed in biofertilizer treated plants in all respects and the results suggested that the treatment of biofertilizers in single, dual and multiple combination enhance the growth of paddy plants when compared to chemical fertilizer treated plants and control. The results on yield attributes such as Panicle length, weight of panicle, no. of Spikelet's per panicle, wt. of 1000 Seeds, Grain yield (q./ha.) and Straw yield (q./ha) showed a favorable influence during the entire study period (**Table 2**).

Combined application of *Azospirillum brasilense* + *Bacillus megaterium* + *Pseudomonas aeruginosa* recorded significantly higher growth parameters compared to single application. The results are in conformity with earlier reports (Nanda *et al.*, 2016). Growth parameters viz. plant height, number of tillers hill<sup>-1</sup> and dry matter production hill<sup>-1</sup> were significantly affected by bio-fertilizers. Combined application of *Azospirillum brasilense* + *Bacillus megaterium* + *Pseudomonas aeruginosa* recorded significantly higher growth parameters compared to single application. The results of the present experiment confirmed the findings of Murthy *et al.* (2015). Increase in yield components, grain and straw yield might be due to higher photosynthetic activity because of increased leaf area index, which ultimately promoted dry matter production resulted in higher grain and straw yield. These results confirmed the findings of Davari and Sharma (2010) and Singh *et al.* (2013).

## CONCLUSIONS

It can be seen from the above data that all the treatments were significantly higher than each other. The treatments T8 (*BGA* + *pseudomonas aeruginosa*), T9 (*BGA* + *Mycorrhizae*), T10 (*Azospirillum umbrasilense* + *Bacillus magisterium*) and T11 (*Azospirillum umbrasilense* + *Bacillus megaterium* + *Pseudomonas aeruginosa*) was significantly higher than all other treatments in growth and yield parameters. Based on these reports, it can be assumed that biofertilizers could offer an

opportunity for rice farmers to increase yields, productivity, and resource use efficiency.

## ACKNOWLEDGMENTS

The author is thankful to Prin.DR. G. A. Thakur M.P.A.S.C. College, Panvel, and Principal Dr. Mahajan I/C Principal Pendharkar College, Dombivli thanks are due to Research Laboratory, K V Pendharkar College, Dombivli for providing laboratory Facilities.

## REFERENCES

1. Biswas, B. C., Yadav, D. S., and S. Maheshwari Bio-fertilizers in Indian Agriculture. FertilizerNews, 1985; 30(10): 20-28.
2. Bloemberg G.V, Wijfijes A.H.M., Lamers G.E.M, Stuurman N and Lugtenberg BJJ Simultaneous imaging of *Pseudomonas fluorescens* WCS, 2000; 3655.
3. Gomez, K.A.; Gomez, A.A. Statistical Procedures for Agricultural Research, 2nd ed.; John Wiley & Sons: New York, NY, USA, 1984; 680.
4. Goel A.K., Laura R.D.S, Pathak G, Anuradha G and Goel A. Use of bio-fertilizers: potential, constraints and future strategies review. International Journal of Tropical Agriculture, 1999; 17: 1-18.
5. Davari, M. R., Sharma, S. N. Effect of different combinations of organic materials and biofertilizers on productivity, grain quality and economics in organic farming of basmati rice (*Oryza sativa*). Indian Journal of Aronomy, 2010; 55(4): 290-294.
6. Dawari. R and Sharma. N. Effect of organic manures on basmati rice (*Oryza sativa* L) under organic farming of rice - wheat cropping system. Int., Journal of Agricultural and crop sciences, 2011; 3(3): 76-84.
7. El-Habbasha SF, Hozayn M, Khalafallah MA Integration effect between phosphorus levels and biofertilizers on quality and quantity yield of faba bean (*Vicia faba* L.) in newly cultivated sandy soils. Research Journal of Agriculture and Biological Science, 2007; 3(6): 966-971.
8. Katyal, J.C., Das, S.K., Korwar, G.R. and Osman. M. Technology for Mitigation stresses: Alternated land uses. Stressed Ecosystems and Sustainable Agriculture eds, 1994; 291-305.

9. Murthy KMD, Rao AU, Vijay D, Sridhar TV. Effect of levels of nitrogen, phosphorus and potassium on performance of rice. *Indian Journal of Agriculture Research*, 2015; 49(1): 83-87.
10. Nanda, G., Sravan, U.S., Singh, A. and Singh, S.P. Effect of NPK Levels and Bio-Organics on Growth, Yield and Economics of Basmati Rice (*Oryza sativa* L.) cv. HUBR 10-9. *Environ. Ecol.*, 2016; 34: 1530-1534.
11. Sarvan, T., Jaiswal, H.K., Showkat, A.W. and Kumari, P. Heterosis for yield and yield attributes in rice (*Oryza sativa* L.). *Journal of Applied and Natural Science*, 2016; 8(2): 622- 625.
12. Venkateshwarlu, B. Role of bio-fertilizers in organic farming: Organic farming in rain fed agriculture: Central institute for dry land agriculture, Hyderabad, 2006; 85-95.
13. Vessey, J.K. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*, 2003; 255: 571–586.



## ETHNOMEDICINAL PLANTS USED FOR HAIR TREATMENT BY TRIBALS OF DHARAMPUR TALUKA, GUJARAT

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Article Received on  
17 Nov. 2020,  
Revised on 08 Dec. 2020,  
Accepted on 29 Dec. 2020  
DOI: 10.20959/wjpps20211-18127

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### ABSTRACT

Since time immemorial, Man has started using the plants to diminish sufferings and diseases. The present study attempts to investigate and document the medicinal plants used against hair care by the tribal and rural community of Dharampur area of district Valsad. During the survey from March to September 2019, a total of 24 different plant species belonging to equal number of genera and 20 different families were found to be used as effective remedies. The information regarding the treatment of hair ailments and hair care along with its formulation are recorded. In this paper, the botanical name, local name and family of these plant species along with their parts used and mode of its formulation are presented.

**KEYWORDS:** Ethnomedicine, Hair care, Dharampur, Valsad.

### INTRODUCTION

In human care system, Ethnomedicine has been playing a very important role since time immemorial. This practice of health care is based on belief and experience of the ethnic people, which is a part of their tradition and culture. In modern era, the traditional system of medicines and ethnobotanical studies has become a worldwide tendency in the different parts of globe. This application of health care is based on faith and knowledge of the ethnic inhabitants, which is a part of their ritual and traditions. There has been an increased requirement of herbal remedy in international trade because herbal drugs are cheap, more efficient, easily obtainable and without any side effects.

As hygienic necessity of hair care should be done as it protects the scalp and enhance the beauty of scalp and enhance the beauty of human. Due to inheritance, older age, deficiency in diet, cancer drugs (Chemotherapy), contagious (such as worms, lice, scabies, eczema, dandruff, etc.), using of synthetic products (soaps, shampoos and hair oils), etc. caused Hair disorder. Therefore, the utilization of herbal products is considered secure and free from allergic reaction. Thus, sources of natural plant drugs are required to be studied in detail.

Many workers have gathered and compiled data on the plant uses including medicinal properties. Silori & Rana have reported on indigenous medicinal plants from the tribal areas of Narayan Sarovar Sanctuary (NSS) district of Gujarat. Nirmal Kumar et al had mentioned the infectious diseases from tribal areas of Dangs forest, South Gujarat. Gavali & Diwakar studied most of the plants from the forests and agro-ecosystems of Gujarat, which is rich in biodiversity. Punjani & Kumar has laid more emphasis on certain less known and unrecorded uses of plants from Banaskantha and Sabarkantha districts, Gujarat state, India. Patil & Patil studied the ethnomedicinal plant species of Nashik district, Maharashtra.

To document the ethnomedicinal information from different talukas of Valsad district have been carried out from time to time, but the perusal of literature shows that no systematic study of locally available plants from ethno-medicinal point of view has been carried out in the area of investigation. Therefore, in the present study, an attempt has been made to document some medicinal plants used by the tribal and rural community of Dharampur area of Valsad district, Gujarat for curing various hair disorders.

## STUDY AREA

Dharampur taluka is situated in Valsad district in Gujarat state. Dharampur is located at 20.53°N 73.180E. It is sited on the bank of Swargavahiniriver and is bounded by the Sahyadri or Western Ghats range on the east, west and south. In these areas the tribal like Kholcha, Bhel, Nayakas, Koknas and Chaudhari are isolated in small pockets and they exclusively depend on ethnomedicines for the healing of different diseases. The tribal population is estimated around 91.92% in Dharampur region.

## MATERIAL AND METHODS

The study was conducted in the year 2018-2019. Many field trips were conducted in rural and tribal pockets of Dharampur area. The gathered the information on ethnomedicinal plants through interviews with local tribal women (15- 50 years old) having knowledge of herbal

medicine for hair disorders were recorded in the field book. The information was documented for ethnomedicinal species regarding the local names, mode of preparation and mixtures of other plants used as ingredients along with their vernacular names. The photographs were taken along with their natural habitats during the field work. By using the floristic literature pertained to Gujarat (Shah, 1978; Patel, 1971) and the neighboring Maharashtra State (Almeida, 2003), the collected plants samples were identified.

## EXXPERIMENTAL RESULTS

Owing to the side effects and toxicity of synthetic drugs, Natural product sources plays a major part in the human hair care system. The utilization of natural plant resources for hair care was gathered from tribal informants as well as from women community from different villages of Dharampur taluka. The information regarding the treatment of hair ailments and hair care along with its formulation are recorded. After survey and critical screening 24 plant species belonging to 20 families of ethnomedicinal interest are documented.

In the present investigation 24 species of medicinal plants belongs to 20 families, total 24 genera were used for the treatment of different diseases of hair problems. Out of the 24 families 2 were belongs to monocotyledons and 20 families were dicotyledons. The major plant families used by the tribals for their hair care are Fabaceae, Liliaceae, Asteraceae, and Euphorbiaceae (02species) followed by Malvaceae, Plantaginaceae, Apiaceae, Lamiaceae, Amaranthaceae, Meliaceae, Asclepiadaceae, Lythraceae, Sapotaceae, Sapindaceae, Verbenaceae, Anacardiaceae, Annonaceae, Caesalpiaceae, Dioscoraceae and Solanaceae. The plant species used by the tribals are mentioned in (Table .1) with their botanical & local names, family and plant parts used in various hair disorder.

**Table 1: Medicinal Plants used by tribal's for hair disorders.**

Sr.No.	Botanical Name/Family	Local name	Parts used	Mode of administration
1	<i>Abrus precatorius</i> L. /Fabaceae	Chanothi	Seeds	To promote hair growth which is loss due to alopecia, the paste of seeds is applied on bare scalp.
2	<i>Achyranthes aspera</i> L.. /Amaranthaceae	Anghedi	Roots	To cure dandruff and promotes hair growth, the paste of fresh roots is applied on scalp overnight.
3	<i>Allium cepa</i> L. / Liliaceae	Dungli	Bulb	To eradicate dandruff and kill lice, juice of bulb is applied on scalp.
4	<i>Aloe barbadensis</i> Mill. /Liliaceae	Kunwar	Leaves	To make hair silky and soft, the pulp from leaves is applied on hair for 3-4

				hours.
5	<i>Annona squamosa</i> L.. /Annonaceae	Seetaphal	Seeds	To eradicate lice and to dandruff from hair, paste of seeds is applied on scalp for six hours.
6	<i>Azadirachta indica</i> A.Juss/ Meliaceae	Limbdoo	Seeds, Leaves	To cure dandruff and remove lice, the crushed seeds and leaves are applied on the head.
7	<i>Bacopa nniari</i> /Plantaginaceae	Brahmi	Leaves	The leaves extract promotes hair growth as it activates protein.
8	<i>Centella asiatica</i> L.. /Apiaceae	Moti Brahmi	Leaves	Powder of leaves is soaked in oil and then boiled. This preparation is massaged well on head to prevent hair fall.
9	<i>Datura stramonium</i> L./ Solanaceae	Dhaturo	Seeds & Leaves	To stimulate hair on baldness, the extracted oil from seeds is applied on scalp. The leaf extract is used to control the dandruff.
10	<i>Delonix elata</i> L./ Caesalpiniaceae	Sandesaro	Leaves	To cure falling of hair, the leaves paste is applied on scalp.
11	<i>Dioscorea bulbifera</i> L.. /Dioscoraceae	Arithi	Tuber	To powder is used as a hair wash. enhance hair growth and remove dirt, the dried
12	<i>Eclipta prostrata</i> L. . /Asteraceae	Bhangro	Leaves	To prevent hair, fall and blacken hairs, the leaves extract is mixed with oil and applied on scalp.
13	<i>Emblica officinalis</i> Gaertn. /Euphorbiaceae	Ambla	Fruit	To prevent hair, fall and promotes hair growth, the fruit powder is mixed with sesame oil and apply on scalp.
14	<i>Hibiscus sinensis</i> Linn. /Malvaceae	Jasud	Flowers	To stimulate hair follicles and increase hair growth, paste of flowers along with coconut oil is massaged on head.
15	<i>Hemidesmus indicus</i> L./ Asclepiadaceae	Upalsari	Entire herb	For good hair growth, the herb is powdered and is used in the preparation of hair oil as hair tonic.
16	<i>Lawsonia inermis</i> L. / Lythraceae	Mendi	Leaves	To cure dandruff, the paste of the fresh leaves is spread in the form of thick layer and also used in preparation of hair oil and hair dyes.
17	<i>Madhucal longifolia</i> (Koenig) Macb.n/Sapotaceae	Mahudo	Petals	To promote hair growth, dried extract petal is massaged on head.
18	<i>Mangifera indica</i> L. / Anacardiaceae	Keri	Seeds	For dyeing the hair, Mango seed powder is mixed with henna powder in 500 ml of water (in iron pot)
19	<i>Ocimum sanctum</i> L.. /Lamiaceae	Tulsi	Leaves	The leaves extract is used to prevent hair loss caused by dandruff and itching as it has antifungal properties.
20	<i>Ricinus communis</i> Linn.	Arandi	Seeds	The oil extracted from seed is

	/Euphorbiaceae			massaged on scalp to prevent hair fall.
21	<i>Sapindusemarginatus</i> Vahl/ Sapindaceae	Aritha	Leaves, Stem, Fruits	The fruit powder is used in washing hair as it provides extra shine and removes lice from the hair.
22	<i>Tridaxprocumbens</i> Linn./Asteraceae	Pardeshibhangra	Leaves	Leaves crushed are applied on hair as it promotes hair growth.
23	<i>Trigonellafoenum-graecum</i> / Fabaceae	Methi	Seed	Seeds extract helps to increase hair volume and thickness as it enhances the hair growth.
24	<i>Vitexnegundo</i> L../Verbenaceae	Nargood	Leaves	The leaves are boiled in coconut oil and massage on scalp to prevent hair fall. The oil prepared from leaves is also used as a hair dye and kill the lice.

### SUMMARY AND CONCLUSION

As the study area is rich in diversity of medicinal plants and it plays a significant role in enhancing the beauty of hair. The effects of allopathic medicines are severe; therefore, the tribals and rural community people prefer medicinal plants for therapeutic hair problems faced by them. More investigation and preservation efforts should be focused on these assets of the area so that the future generation could benefit from these valuable plants that are actual gift to mankind and may proceed to progress of commercial products.

### REFERENCES

1. Arjit Sinha babu& Arpita Banerjee, "Ethno-botanical study of Medicinal Plants Used by Tribals of Bankura Districts, West Bengal, India". Journal of Medicinal Plant Studies, 2013; 1(3): 98-104.
2. Bhasker L. Punjani&Vimal Kumar, "Plants used in traditional phytotherapy for hair care by tribals in Sabarkantha district, Gujarat, India". Indian Journal of Traditional Knowledge, 2003; 2(1): 74-78.
3. D M. Sakarkar, U M Sakarkar, NM Sakarkar, VN Shrikhande, JV Vyas and RS Kale, "Medicinal plants used by the tribals for hair disorders in Melghat forest of Amravati district, Maharashtra". Natural Product Radiance, 2004; 3(5): 351-355.
4. Gavali, Deepa Sharma, Diwakar, "Traditional Knowledge and Biodiversity conservation in Gujarat". Indian Journal of Traditional Knowledge, 2004; 3(1): 51-58.
5. Silori, CS; Rana, AR, "Indigenous knowledge on medicinal plants and their use in Narayan Sarovar sanctuary, Kachchh". Ethnobotany, 2000; 12: 1-7.

6. K.D.Mitaliya, DC Bhatt, NK Patel & SK Dodia, "Herbal remedies used for hair disorders by tribals and rural folk in Gujarat".Indian Journal of Traditional Knowledge", 2003; 2(4): 389-392.
7. Nirmal Kumar, JI, S.Hiren & Rita N. Kumar, "Ethnobotanical Values of Certain plant species of Dangs Forest, Extreme Northern part of Western Ghats, South Gujarat, India.International Journal of Biosciences Reporter, 2004; 2(1): 63-74.
8. M V Patil& D A Patil, "Ethnomedicinal practices of Nashik District, Maharashtra".Indian Journal of traditional Knowledge, 2005; 4(3): 287-290
9. Shah G L. "Flora of Gujarat state. Part I & II." (Sardar Patel University, Vallabh Vidyanagar), 1978.



## CULTURAL AND MORPHOLOGICAL CHARACTERS OF *S. ROLFSII* CAUSING STEM ROT OF GROUNDNUT AND ITS MANAGEMENT USING BIOPESTICIDE

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Article Received on  
24 March 2021,

Revised on 14 April 2021,  
Accepted on 04 May 2021

DOI: 10.20959/wjpps20216-18985

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### ABSTRACT

Ten isolates of *Sclerotium rolfsii* Sacc. were isolated from stem rot of groundnut. It was collected from different parts of Maharashtra. Cultural and Morphological characters of *S. rolfsii* was studied using PDA medium. There were 10 isolates studied based on mycelial characters, colony colour, colony growth rate, appearance and also studied sclerotial formation and sclerotial arrangement viz. central, peripheral as well as scattered throughout colony, number of sclerotia per plate etc. Significant variations in mycelial and sclerotial characters of isolates were observed. All ten isolates were tested their sensitivity against Demold. It was observed that in all isolates *Sr1* was sensitive (MIC 20300 ppm) while *Sr 9* was tolerant (MIC 10300 ppm) to Demold.

**KEYWORDS:** Groundnut, stem rot, *Sclerotium rolfsii* and Demold.

### INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop, belongs to family Leguminosae (Sub-family- Fabaceae). It is also called as 'King' of oilseeds. It is origin in South America. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted and used as cooking oil. It also important because for its high oil ranges from 44 to 56 percent, total protein (16.2 to 36%). It is also a source of fibre, iron, magnesium, phosphorus, niacin, vitamin E and as well as phytoestrogen, flavones and other antioxidant compounds. The major groundnut producing countries in the world namely India, China, Nigeria, Senegal, Sudan, Burma and the United States of America. India is the second largest

groundnut producer in the world under 6.7 million cultivation. The major groundnut growing states are Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra which together account for about 80% of area and 81% of production in India (Reddy, 1992). In Maharashtra, it is mainly cultivated in Dhule, Kolhapur, Satara, Pune, Ahemadnagar, Nashik and Sangali. In 2019, over 187500 hectares of land in the state was used for groundnut production (Kharif 2019 survey).

Several factors are responsible for low productivity of groundnut in recent years among which the soil-borne fungal diseases like root rot, collar rot, stem rot and pod rot are very important. Stem rot is very essential and caused by *Sclerotium rolfsii* Sacc. has become a severe disease in groundnut growing regions. This disease causes severe damage and yield losses over 25% (Mayee and Datar, 1988). The symptoms of this disease are yellowing and wilting of branches, white mycelial growth at collar and formation of sclerotia which are like mustard seed (Asghari and Mayee, 1991). The pods, which are produced below the soil surface, come in contact with the *S. rolfsii* causing rotting of pods. This results in lowering of yield and quality of pods. Stem rot is also known as sclerotium blight.

## MATERIALS AND METHODS

### Collection and isolation of the pathogen

A survey was conducted in the year 2017 – 2018 in groundnut growing areas in Maharashtra. Infected plants of groundnut were collected from field and brought in Research Laboratory, K.V. Pendharkar. The pathogen, *S. rolfsii* was isolated from the stems of infected groundnut plants by tissue segment method (Rangaswami and Mahadevan, 1999) using potato dextrose agar (PDA) medium. Small pieces of infected tissue of about 0.5 to 1 cm from infected region were cut with sterile scalpel. The pieces were surface sterilized with 1% HgCl<sub>2</sub> solution for 30 sec. The tissue pieces were subsequently washed with SD water to eliminate excess HgCl<sub>2</sub> and then the pieces were transferred onto PDA medium in Petri dishes. Plates were incubated at 27 ± 2°C and observed periodically for growth of the pathogen. The isolates were further purified by using bit of mycelium from each colony on potato dextrose agar (PDA). The pathogen was identified as *Sclerotium rolfsii* based on its morphological and sclerotial characters (Barnett and Hunter, 1972).

### Cultural and Morphological characters of *S rolfsii*

A study was conducted to observe the variation among the 10 isolates of *S. rolfsii* in terms of cultural and morphological characters like colony colour, colony growth and appearance,

number of sclerotia per plate, colour and arrangement of sclerotia. To carry out this experiment, 20 ml of sterilized PDA medium was poured in each petriplate and allowed to solidify at room temperature. Later 5 mm mycelium disc was cut using sterilized cork borer from the periphery of actively growing seven days old cultures of the pathogen grown on PDA and transferred aseptically to the centre of each plate and replicated thrice. The inoculated plates were incubated at  $27 \pm 2^\circ\text{C}$  for 15 days. Visual observations on mycelial growth and sclerotial formation were recorded. A total of 7 cultural and morphological characters on the basis of mycelial (mycelial growth, colony colour and appearance) and sclerotial (sclerotial colour, shape, number of sclerotia and their formation pattern on PDA plate) were recorded at 7 and 15 days of incubation, respectively for each isolate.

### **Sensitivity of *S. rolfsii* against Demold (*In vitro*)**

*In vitro* sensitivity of all isolates of *Sclerotium rolfsii* against Demold were screened by food poisoned technique (Nene and Thapliyal, 1993). PDA medium was mixed with Demold solution of concentrations 2000 - 20000  $\mu\text{g/ml}$ . And without Demold served as control. A 5 mm mycelial disc of seven days old culture was inoculated at the centre and each treatment was replicated thrice and incubated at  $27 \pm 2^\circ\text{C}$  until full growth was observed in control. MIC and  $\text{ED}_{50}$  of all isolates were calculated on the basis of dose response curve.

## **RESULTS AND DISCUSSION**

Total of ten isolates of *S. rolfsii* were studied for cultural, morphological and sclerotial characters. Colony growth, colour and appearance were recorded and presented in (Table - 1 & plate 1). Aerial hyaline, septate, thin walled hyphae with profusely branched mycelium observed in microscopic study. From the results, it was evaluated that, there was significant difference between all isolates with reference to cultural and sclerotial characters. Isolate Sr1, Sr4, Sr5, Sr6 and Sr8 were showed fastest growth while Sr2, Sr3, Sr7 and Sr9 were showed moderate growth. Isolate Sr10 was showed very fast growth. All isolates were showed cottony white to extra white colour mycelium. Sr1, Sr5, Sr6 and Sr9 were fluffy and aggregated, Sr2, Sr2 and Sr10 were showed thin strand colonies while Sr3, Sr4 and Sr8 were compact. At the time of maturity of fungus, small white tufts were formed on mycelium which later give rise to small, mustard seed like called sclerotia which were light brown to dark brown in colour, shiny, hard and spherical to irregular in shape (Table-2). Light brown coloured sclerotia were produced in isolate Sr1 and Sr3. Most of isolates produced brown coloured sclerotia (Sr2, Sr6, Sr8 and Sr10), while Sr4 and Sr5 produced red brown sclerotia.

Sr7 and Sr9 produced dark brown sclerotia. In Sr1, Sr3, Sr4, Sr6, Sr7 and Sr10 sclerotia spread all over the plate, in isolate Sr2 and Sr 8 sclerotial bodies formed at periphery while it formed centrally in isolate Sr5 and Sr9. Number of sclerotia per plate varied from isolate to isolate. Similar, results were observed by Subramanian (1964), Barnett and Hunter (1972), Mahmood *et al.*, (1976), Sharma *et al.*, (2002), Rakholiya and Jadeja (2011), Kumar *et al.*, (2014) and Sekhar *et al.*, (2017). Sensitivity of *S. rolfsii* tested against Demold (*In vitro*). MIC of all isolates ranged from 10000  $\mu\text{g/ml}$  – 20300  $\mu\text{g/ml}$  while  $\text{ED}_{50}$  between 5400  $\mu\text{g/ml}$  to 11300  $\mu\text{g/ml}$ . Sr1 was sensitive (MIC 20300 ppm) while Sr 9 was tolerant (MIC 10300 ppm) to Demold revealed in (Table 3.).



Fig 1: Pure cultures of *Sclerotium rolfsii* Sacc.

**Table1: Morphological characters of *Sclerotium rolfsii* Sacc. on PDA media.**

Sr. No	Isolate	Location	Growth	Morphological and appearance
1	Sr1	Kothe	Fast	Cottony White Fluffy, aggregated
2	Sr2	Shirur	Moderate	Cottony white , thin strands
3	Sr3	Khanapur	Moderate	Cottony white, compact
4	Sr4	Sinnar	Fast	Cottony white, compact
5	Sr5	Khed	Fast	White, Fluffy
6	Sr6	Landewadi	Fast	White, Fluffy, aggregated
7	Sr7	Belapur	Moderate	Cottony White, thin strand
8	Sr8	Shrigonda	Fast	Cottony White, compact
9	Sr9	Nimdari	Moderate	Extra white Fluffy, aggregated
10	Sr10	Sangamner	Very Fast	Cottony White, thin strands

**Table 2: Sclerotial characters of different *Sclerotium rolfsii* Sacc. isolates On PDA media.**

Sr. No	Isolate	Colour	Shape	No per plate	Formation pattern
1	Sr1	Light brown	Spherical	160	Spread all over plate
2	Sr2	Brown	Irregular	288	Peripheral
3	Sr3	Light brown	Irregular	176	Spread all over plate
4	Sr4	Red Brown	Spherical	148	Spread all over plate
5	Sr5	Red Brown	Spherical	132	Central
6	Sr6	Brown	Spherical	92	Spread all over plate
7	Sr7	Dark brown	Spherical	224	Spread all over plate
8	Sr8	Brown	Irregular	252	Peripheral
9	Sr9	Dark brown	Irregular	296	Central
10	Sr10	Brown	Spherical	184	Spread all over plate

**Table 3: Sensitivity of *Sclerotium rolfsii* Sacc tested against Demold (*In-vitro*).**

Data characteristic to dose response curve ( <i>In-vitro</i> sensitivity of <i>S. rolfsii</i> Sacc.)						
Isolate No	Location	Regression constant	Regression coefficient	Correlation coefficient	ED <sub>50</sub> (µg/ml)	MIC (µg/ml)
<b>Sr1</b>	<b>Kothe</b>	<b>6.7759</b>	<b>-0.0046</b>	<b>-0.8947</b>	<b>5400</b>	<b>10300</b>
Sr2	Shirur	6.0969	-0.0043	-0.8440	5000	12600
Sr3	Khanapur	7.9033	-0.0048	-0.9881	6800	14700
Sr4	Sinnar	8.1466	-0.0046	-0.9954	7800	16100
Sr5	Khed	7.8436	-0.0044	-0.9833	8000	16300
Sr6	Landewadi	8.6111	-0.0041	-0.9956	10000	19300
Sr7	Belapur	9.4936	-0.0054	-0.9956	9200	17400
Sr8	Shrigonda	9.8968	-0.0050	-0.9974	10700	18200
<b>Sr9</b>	<b>Nimdari</b>	<b>9.5806</b>	<b>-0.0046</b>	<b>-0.9967</b>	<b>11300</b>	<b>20300</b>
Sr10	Sangamner	8.6427	-0.0053	-0.9773	6100	10900

- Values of three replicates.

**REFERENCES**

1. Asghari MA and Mayee CD. Comparative efficacy of management practices on stem and pod rots of groundnut. *Indian Phytopathology*, 1991; 44: 328-332.
2. Barnett HL and Hunter BB. *Illustrated Genera of Imperfect Fungi*. 3rd Edition, Burgess Publishing Co., Minneapolis, 1972; 241.
3. Damicone JP, Jackson KE. Factors affecting chemical control of Southern blight of peanut in Oklahoma. *Plant Dis.*, 1994; 78: 482-486.
4. Kharif-2019 Survey of Groundnut Crop Nene YL, Thapliyal PN. *Fungicides in plant disease control*, 3rd Edn. Oxford and IBH Publishing Company, New Delhi, 1993.
5. Mahmood M, Abu Mohammad, Gupta SK, Kumar S. Studies on root rot disease of groundnut caused by *Sclerotium rolfsii*. *Proc. Bihar Aca. Agri. Sci.*, 1976; 13: 157-158.
6. Mayee CD, Datar VV. Diseases of groundnut in the tropics. *Rev. Trop. Plant Pathol.*, 1988; 5: 85-118.
7. Rakholiya KB, Jadeja KB. Morphological diversity of *Sclerotium rolfsii* caused stem and pod rot of Groundnut. *J. Mycol. Plant Pathol.*, 2011; 41(4): 500-504.
8. Rangaswami G, Mahadevan A. *Diseases of crop plants in India*. Prentice Hall of India Pvt. Ltd., New Delhi, 1999; 6079.
9. Reddy PS. Groundnut situation in India, NRCG, Workshop cum Seminar on Groundnut. Production Technology, Advanced Centre of Training (Oilseeds), G. A. U., Junagadh, 1992.
10. Sarma B, Singh U and Singh K. Variability in Indian Isolates of *Sclerotium rolfsii*. *Mycologia*, 2002; 94: 1051-8. 10.2307/3761870.
11. Sekhar YC, Ahammed SK, Prasad TNVKV and Jayalakshmi DRS. Morphological and Pathogenic Variability of *Sclerotium rolfsii* Isolates Causing Stem Rot in Groundnut, *Int. J. Pure App. Biosci.*, 2017; 5(5): 478-487.
12. Subramanian KS. Studies on sclerotial root disease of groundnut (*Arachis hypogaea* L.) by *Sclerotium rolfsii* Sacc. *Madras Agri. J.*, 1964; 51: 367-378.



# NICKEL NANOPARTICLES DECORATED BIOGENIC CARBON NANO FIBERS FOR ENHANCING HYDROGEN STORAGE CAPACITY

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**Abstract:** Carbon nano-fibres (CNFs), which possess properties like large surface area, unique physical, mechanical properties, inherent high-aspect-ratio, hollow nano-geometry is studied for its hydrogen adsorption capacity. For enhancing the H-storage, CNF prepared from plants as precursor is decorated with Ni nanoparticles, as it offers hydrogen spill-over effect. The hydrogen adsorption isotherms were measured by static volumetric technique using Sievert's apparatus at ambient temperature. Ni NP decorated CNF showed not only increased surface area as compared to CNF (i.e. from 504.1 to 1080.8 m<sup>2</sup>g<sup>-1</sup>) and pore volume (from 0.2973 to 0.4106 cc/g) but also nearly doubled the hydrogen Wt% adsorption capacity i.e. from 3.25 Wt% to 6.05 Wt%.

**Index Terms - Carbon Nano Fibers, Hydrogen-adsorption, Plant derived CNF, Sievert's apparatus**

## I. INTRODUCTION

In recent years, energy shortage, caused by limited energy resources and environmental calamities, drives the need to find new efficient sources of energy. Hydrogen is an ideal substitute for energy converters due to its high efficiency and essential role in reducing air pollution. The combustion of hydrogen releases useful thermal energy, which can be used as an eco-friendly fuel that does not interfere with natural cycles, and is emerging as an important material in various fields of applied science [1-4]. Over the past few years, a number of different hydrogen storage technologies have been proposed viz. liquefied hydrogen, compressed hydrogen, metal hydrides and hydrogen physisorption on different substrates, including carbon nanomaterials (CNMs). The storage for liquid hydrogen present a risk of explosion at ambient temperature and is costly. Metal hydride alloys are capable of storing hydrogen but its heavy weight making heavy storage system and intrinsically low thermal conductivity makes system uneconomical. To resolve these issues hydrogen storage method using porous carbon materials has been proposed [5-9].

Carbon nano fibres (CNFs), which possess large surface area, unique physical, mechanical properties, inherent high-aspect-ratio, hollow nano-geometry [10] etc. recently attracted a lot of attention both in academic and industrial communities. Attachment or decoration of metal nano particles on CNFs enhances hydrogen storage capacity due to hydrogen spill-over effect [11-13]. Among transition metals for enhancing hydrogen storage capacity, nickel is particularly promising because it is abundant and inexpensive as compared to noble metals and known metal for hydrogen catalysis [14-15]. Edgar et. al. reported 1.2 to 2.0 wt% storage at atmospheric temperature and various pressure. [16] Geng et al. has reported 1.65 wt% of H<sub>2</sub> uptake capacity of corncob derived activated carbon at ambient temperature and upto 180 bar. [17] Jaybhaye et al. has also reported hydrogen storage capacity of semiconducting CNF 0.65 wt% and 3 wt% at two different pressure 11 kg/cm<sup>2</sup> and 135 kg/cm<sup>2</sup>. [18]. Juarez et al. has reported 6.8 wt% at 8 MPa at -196°C by alkali activated carbon synthesized from coal [19]. CNF is quite interesting material to study its hydrogen storage capacity.

In the present paper results of nickel nano particles decorated CNFs synthesized from plant fibre for hydrogen storage capacity is discussed.

## 2.0 EXPERIMENTAL

### 2.1 Synthesis of Carbon Nano-Fibers (CNF)

Cotton fiber was used as precursor for making CNF was collected from local market Mumbai, India. All the chemicals were AR grade and were used without further purification. The natural cotton was first cleaned. The synthesis of CNF was carried out by pyrolyzing cotton fibers in Lyndberg's horizontal furnace, at 650° C temperature for 4hours in presence of carrier gas Argon. The as-obtained CNF was then treated with 1N NaOH solution and was treated by two different methods viz. (1) as prepared CNF was annealed at 700°C for 2 hours in presence of CO<sub>2</sub> gas and named as ACNF. (2) CNFs was decorated with Nickel nano-particles during annealing at 700°C for 2 hours in presence of CO<sub>2</sub>. It was named as Ni-CNF. Both these CNFs were used for hydrogen adsorption study.

## 2.2 Measurements of Hydrogen adsorption by ACNF and Ni-CNF

Carbon sample (ACNF/Ni-CNF) weighing 10 grams each were used for the study. The hydrogen adsorption isotherms were measured by static volumetric technique using Sievert's apparatus at ambient temperature. [9, 18] The apparatus was loaded with the sample of carbon and the study was carried out at 6 MPa by method described by Mukherjee et al. [14]

**Table 1: Impact of Surface Area (as measured by BET) and Pore volume on Hydrogen Adsorption Capacity of ACNF and Ni-CNF as Measured using Sievert's Apparatus at ambient temperature.**

Sample	BET Surface area $\text{m}^2\text{g}^{-1}$	Pore Volume $\text{cc/g}$	Adsorption Wt%
ACNF	504.1	0.2973	3.25
Ni-CNF	1080.8	0.4106	6.05

## 3. Result and Discussion

### 3.1 Morphology of Ni-CNF

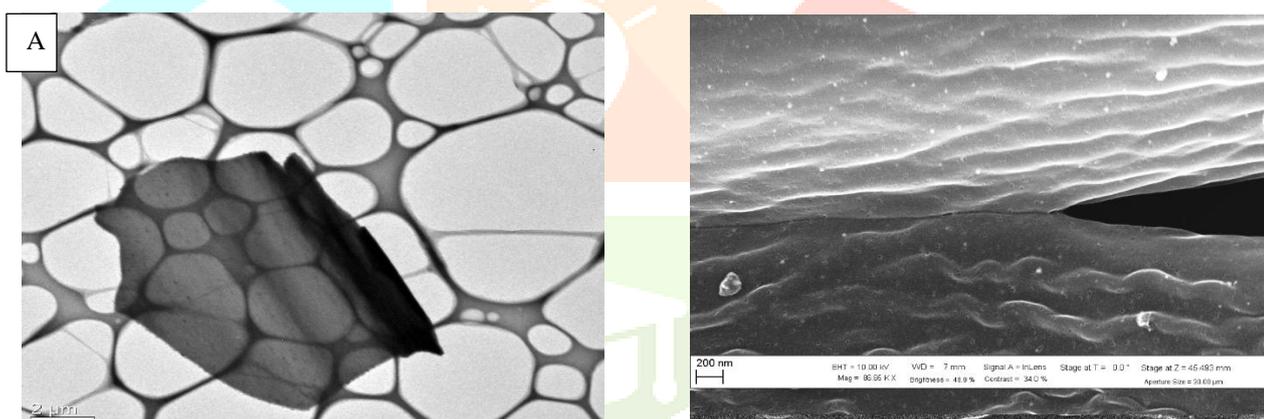
The scanning electron microscopy (SEM) micrograph (Fig. 1(A)) of CNFs shows inherent design on its surface along with uniform distribution of the Ni-nps having thickness in the range of 20-30 nm addition to usual aberrations. The transmission electron microscopy (TEM) micrograph [Fig. 1 (B)] of the sample shows multi-layered carbon particle and also confirming large surface area as detected by BET.

### 3.2 Characterisation of Ni-CNF

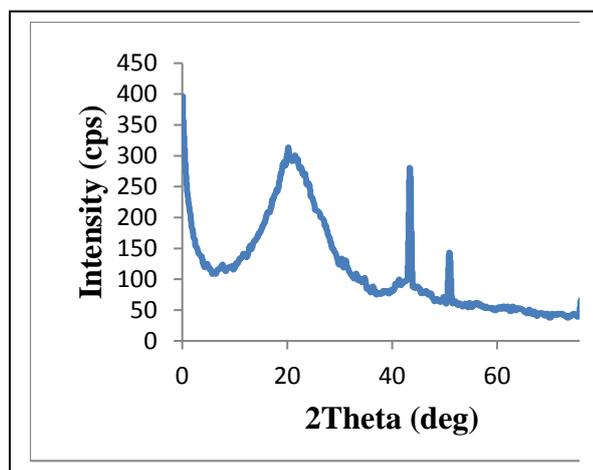
The CNFs obtained by pyrolysis is analyzed by X-Ray Diffraction (XRD). XRD of CNFs shows broad peak at  $2\theta = 26^\circ$  corresponding to (002) plane indicating partial graphitization of carbon materials and sharp peaks at  $2\theta = 43.9^\circ$ ,  $56^\circ$  and  $78^\circ$  shows presence of nickel nanoparticles (Ni-nps) which is co-related with JCPDS file number 04-850. [Fig. 2].

### 3.3 Raman Spectroscopy Analysis of Ni-CNF

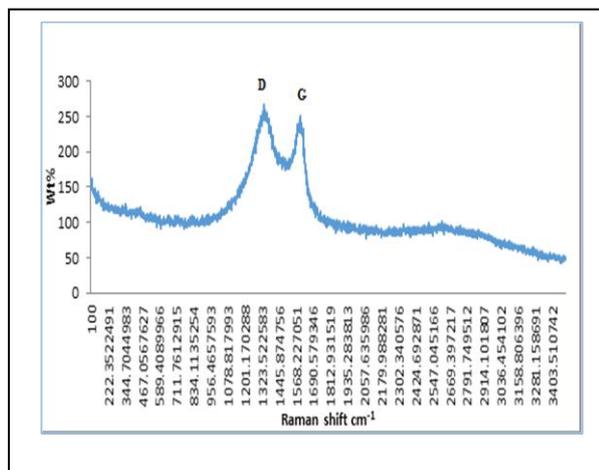
A Raman spectrum [Fig. 3] shows one peak at  $1580\text{ cm}^{-1}$  of G band and second at  $1360\text{ cm}^{-1}$  of D band and its  $I_g/I_d$  value is more than which are the characteristic for partial graphitic material containing some disorder structure as well as presence of crystalline graphene. This suggests that the sample is a mixture of amorphous and partial graphitic carbon materials [21-22].



**Fig. 1.** Carbon nano-fibers. (A) Transmission electron microscopy (TEM) micrograph of transparent single sheet type CNFs. (B) scanning electron microscopy (SEM) micrograph of Ni-CNF [in figure give A and B]



**Fig.2.** X-Ray Diffraction of Ni-CNFs



**Fig.3.** Raman spectra of Ni-CNFs

#### 4. Hydrogen Adsorption Studies

There are two samples of CNF are used to check the comparative study of the hydrogen adsorption capacity. Table 1 shows the hydrogen adsorption measurement of ACNF and NCNF at ambient temperature. Hydrogen adsorption values do not reflect their dependence on specific surface area. The increase in the extent of adsorption of hydrogen by Ni-CNF subscribe to the Spill-over theory. For instance, ACNF and Ni-CNF have hydrogen adsorption capacity of 3.25 wt% and 6.05 wt% respectively measured at ambient temperature and 6.0 MPa pressure. The Brunauer-Emmett-Teller (BET) surface area of CNF and Ni-CNF are 504.1 m<sup>2</sup>g<sup>-1</sup> and 1080.8 m<sup>2</sup>g<sup>-1</sup> also the pore volume of CNF and Ni-CNF are 0.2973 cc/g and 0.4106 cc/g respectively. Mukherjee *et. al.* and Zacharia *et. al.* have reported that incorporation of metal particles into carbon causes metal nanoparticles embedded into carbon sheets. Incorporation of metal particles into carbon surface is known to increase the hydrogen adsorption by increasing the surface area and porosity of carbon materials. [9, 21] These metal particles dissociate hydrogen thus enhancing the hydrogen adsorption capacity. Thus, the present work confirms enhancement of hydrogen storing capacity of sample Ni-CNF. It is found that specific surface is much higher than reported earlier [18] Moreover, the mass of CNFs used in the present work was 10 gm which is significantly larger quantity in comparison to papers reported earlier.

#### Conclusion

In summary, the hydrogen storage capacity of CNFs and NCNFs synthesized from cotton fibre was determined at ambient temperature using Sievert's apparatus. The hydrogen uptake capacity for NCNF at ambient temperature was measured to be 6.05 wt%. The results show considerable extent of hydrogen uptake capacity in the scale up process using 10g of CNF.

#### Acknowledgement

Authors acknowledge the financial support provided by the Ministry of New and Renewable Energy (MNRE Grant no. 103/225/2014-NT), New Delhi, India without which this work could not have been done.

#### References

- [1] Schallbach L., & Zuttel A. (2001). Hydrogen-storage materials for mobile applications. *Nature*, 414, 353-358. DOI: 10.1038/35104634
- [2] Liu C, F. Y. (1999). Hydrogen storage in single-walled carbon nanotubes at room temperature. *Science*, 286, 1127-1129. DOI: 10.1126/science.286.5442.1127
- [3] Rosi N L, E. J. (2003). Hydrogen storage in microporous metal-organic frameworks. *Science*, 300, 1127-1129. DOI: 10.1126/science.1083440
- [4] Xia K, H. J. (2014). Enhanced room-temperature hydrogen storage in super activated carbons: The role of porosity development by activation. *Applied Surface Science*, 315, 261-267. DOI: 10.1016/j.apsusc.2014.07.144
- [5] Fukuzumi S, S. T. (2013). Hydrogen storage and evolution catalysed by metal hybrid complexes. *Dalton Transactions*, 42, 18-28. DOI: 10.1039/C2DT31823G
- [6] Brooks K P, S. T. (2014). Slurry-based chemical hydrogen storage systems for automotive fuel cell applications. *Journal of Power Sources*, 268, 950-959. DOI: 10.1016/j.jpowsour.2014.05.145
- [7] Silambarasan D, S. V. (2013). Single walled carbon nanotube-metal oxide nanocomposites for reversible and reproducible storage of hydrogen. *ACS: Applied Materials and Interfaces*, 5, 11419-11426. DOI: 10.1021/am403662t
- [8] Jung M J, K. J. (2009). Nitrogen and hydrogen adsorption of activated carbon fibers modified by fluorination. *Journal of Industrial and Engineering Chemistry*, 15, 410-414. DOI: 10.1016/j.jiec.2008.11.001
- [9] Mukherjee B, K. G. (2013). Hydrogen storage by carbon fibers from cotton. *QScience Connect*, 45. DOI: 10.5339/connect.2013.45
- [10] Dresselhaus M S, Dresselhaus G, Avouris P. (2001) Carbon nanotubes: synthesis, structure, properties and applications. New York: Springer-Verlag.
- [11] Zhou H, L. X. (2014). Enhanced room-temperature hydrogen storage capacity in Pt-loaded graphene oxide/HKUST-1 composite. *International Journal of Hydrogen Energy*, 39, 2160-2167. DOI: 10.1016/j.ijhydene.2013.11.109
- [12] Rosalba J M, A. M. (2015). Theoretical analysis of hydrogen spillover mechanism on carbon nanotubes. *Frontiers in Chemistry*, 3, 2160-2167. DOI: 10.3389/fchem.2015.00002
- [13] Mortazvi S Z, P. P. (2013). Hydrogen storage property of laser induced Pd-nanoparticle decorated multi-walled carbon nanotubes. *Royal Society of Chemistry Advances*, 3, 1397-1409. DOI: 10.1039/c2ra22224h
- [14] Sharon Maheshwar, S. m. (2011). Hydrogen Storage by Carbon Fibers Synthesized by Pyrolysis of Cotton Fibers. *Carbon Letters*, 12 (1), 39-43. DOI: 10.5714/CL.2011.12.1.039
- [15] Mukherjee B T, S. M. (2016). Ambiguity in determining H<sub>2</sub> adsorption capacity of carbon fibre by pressure technique. *International Journal of Hydrogen Energy*, 41, 2671-2676. DOI: 10.1016/j.ijhydene.2015.12.110
- [16] McAllister M J, L. J. (2007). Single Sheet Functionalised Graphene by Oxidation and Thermal Expansion of Graphite. *ACS: Chemistry of Materials*, 19, 4396-4404. DOI: 10.1021/cm0630800

- [17] Edgar M, D.-D. D. (2014). Characterisation and hydrogen storage in multi-walled carbon nanotubes grown by aerosol-assisted CVD method. *Diamond and Related Materials* , 43, 66-71. DOI: 10.1016/j.diamond.2014.01.016
- [18] Geng Z, W. D. (2014). Spillover enhanced hydrogen uptake of Pt/Pd doped corn-cob-derived activated carbon with ultra-high surface area at high pressure. *International Journal of Hydrogen Energy* , 39, 13643-13649. DOI: 10.1016/j.ijhydene.2014.02.065
- [19] Jaybhaye S, S. M. (2007). Semiconducting Nanofibers and Hydrogen Storage, Synthesis and Reactivity in Inorganic. *Metal-Organic, and Nano-Metal Chemistry* , 37 (6), 473-476. DOI: 10.1080/15533170701471729
- [20] Tellez-Jua'rez M C, F. V.-H. (2014). Hydrogen storage in activated carbons produced from coals of different ranks: Effect of oxygen content. *International Journal of Hydrogen Energy* , 39, 4996-5002. DOI: 10.1016/j.ijhydene.2014.01.071
- [21] Mukherjee B T, S. S. (2017). Microwave absorption by CNM decorated with nickel nano particles. *International Journal of Engineering Science Invention* , 6 (9), 77-80.
- [22] Zacharia R, K. K. (2005). Enhancement of hydrogen storage capacity of carbon nanotubes via spill-over from vanadium and palladium nanoparticles. *Chemical Physics Letters* , 412 (4-6), 369-375. DOI: 10.1016/j.cplett.2005.07.020
- [23] Schaefer S, F. V. (2016). Physisorption, chemisorption and spill-over contributions to hydrogen storage. *International Journal of Hydrogen Energy* , 30, 1-11. DOI: 10.1016/j.ijhydene.2016.07.262





# HYDROGEN ADSORPTION STUDY OF METAL NANOPARTICLE DECORATED CARBON NANOFIBER

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**Abstract:** In this study, synthesis of carbon nanofibers (CNFs) from natural cotton fibers have been discussed. The synthesized CNFs were activated by KOH solution and Ni nanoparticles were decorated on to the surface of CNFs by thermal reduction method. The as obtained carbon material is used for studying hydrogen adsorption capacity by static volumetric techniques using Sievert's apparatus. Raman spectra and X-ray diffraction of carbon revealed the presence of combination of graphitic and amorphous nature. The CNF decorated with Ni nanoparticles have the higher Specific surface area (SSA) which is confirmed by the BET analysis. Due to the spillover effect, the Ni-CNFs exhibited the maximum hydrogen adsorption as 7.01%.

**Index Terms – Carbon nano fibers, hydrogen adsorption, metal decoration Sievert's apparatus.**

## I. INTRODUCTION

The decrease in fossil fuels has created a tremendous demand for renewable energies having higher energy efficiency. In addition, this has raised worldwide efforts to develop alternative fuels and technologies. (Schlapbach & Zuttel 2001) The pollution due to combustion of fossil fuels has added to the drastically changing climatic issues. Thus, the need of the hour is to develop eco-friendly clean fuels, which, unfortunately is very challenging. (Spyrou et al, 2013)

Hydrogen storage technologies play an important role in the transition of carbon-based energy economy. (DOE-USA) Hydrogen is considered to be one of the promising energy fuels for automobiles and its use can be further extended to smaller portable device like laptops and mobile phones etc. (Dimitrakakis et al, 2008) Hydrogen is efficient, renewable and eco-friendly source and has great potential to replace non-renewable fossil fuels. The present energy consumption for mobility and transport applications accounts for one third of the fossil fuel being used and therefore use of hydrogen will cause reduced carbon dioxide production as combustion of hydrogen will produce water as the by-product. (Spyrou et al. 2013, Dimitrakakis et al. 2008 and Satyapal et al. 2007) While at the same time, low volumetric energy density of hydrogen hinders the development of safe storage system. (Zuttel, 2004) Several ways of hydrogen storage have been investigated such as high-pressure gas, liquid hydrogen, metal hydrides and adsorption on nano-materials. (Schlapbach & Zuttel 2001 and Spyrou et al, 2013) Among all the methods currently adopted for hydrogen adsorption, physisorption on carbon material is quite promising as there is no chemical bond between hydrogen and carbon surface, thus giving completely reversible hydrogen uptake and release. (Sircar et al. 1996, Rzepka et al. 1998 and Benard et al. 2007) It is believed that adsorption at ambient conditions on porous material with weak Van der Waals interaction can fulfill the value that have been set by the U.S. Department of Energy (DOE). (DOE, Sumida et al. 2013 and Salam et al. 2013) Numerous materials with sufficient adsorption capacities have been investigated in recent years for hydrogen storage via physisorption and chemisorption. (Sharon et al. 2007 and Tellez-Juarez et al. 2014)

Over the years, porous nano materials have gained considerable attention, as an adsorbent. For example, adsorbent materials like nano-structured carbonaceous materials including carbon nanotube (CNT), graphite nanofibers, graphene and CNFs have been developed. (Tellez-Juarez et al. 2014, Schaefer et al. 2017 and Cheng et al. 2008) Carbon nanomaterials (CNM) due to their high SSA and porosity, in addition to its unique mechanical property, allow it to be an ideal substrate which can be modified through metal decoration for increasing hydrogen adsorption. (Wang et al. 2012, Rangel al. 2014 and Gao et al. 2008) Sheng et al. has reported 2 % hydrogen adsorption on decorated CNT (Sheng et al, 2014). Magnesium and Copper decorated CNMs have shown considerable hydrogen adsorption (Pandyan et al. 2014 and Prakash et al. 2016). Among transition metal for enhancing hydrogen

storage capacity, nickel is promising because it is abundant, inexpensive compared to other metals and can enhance hydrogen adsorption properties.

In this study, nickel nanoparticles were decorated onto the CNFs by thermal reduction method. The nickel on the CNF surfaces enhanced the hydrogen adsorption capacity of CNFs by hydrogen spill-over effect.

## II. EXPERIMENTAL

### Synthesis of carbon nano-fibers

The natural cotton fibers procured from local market were first cleaned and used for synthesis of CNF. The synthesis was carried out by pyrolysis technique using Lindberg's horizontal furnace at 600°C for 4 hours in presence of Argon. The as-obtained CNFs were then activated by 2M KOH solution. The activated CNFs were treated by two different method viz.

- (1) as-obtained CNFs were annealed at 700°C for two hours in presence of CO<sub>2</sub> gas and named as K-CNF
- (2) CNFs obtained were decorated with Ni nanoparticles during annealing period and named as Ni-CNF.

### Hydrogen Uptake Measurements

10 grams of each of the CNFs viz. K-CNF and Ni-CNF were used independently for the hydrogen uptake measurement. The hydrogen adsorption isotherms were measured by static volumetric technique using Sievert's apparatus at ambient temperature at 60 bars.

**Table 1: Impact of activation Temperature of carbon fiber on Hydrogen Adsorption Capacity of KCNF and NCNF as Measured using Sievert's Apparatus at ambient temperature**

Sample	BET m <sup>2</sup> g <sup>-1</sup>	Pore Volume Cc/g	Adsorption wt%
K-CNF	660.13	0.1842	3.81
Ni-CNF	1229.84	0.7521	7.01

## III. RESULT AND DISCUSSION

### Characterisation of Ni-CNF

The CNFs obtained by pyrolysis is analyzed by X-Ray Diffraction (XRD). XRD of CNFs shows broad peak at  $2\theta = 24.9^\circ$  corresponding to (002) plane of graphitic oxide and sharp peaks at  $2\theta = 43.9^\circ$ ,  $56^\circ$  and  $78^\circ$  shows presence of nickel nano-particles [Fig. 2]. It is observed that peak  $2\theta = 24.9^\circ$  low value which suggests sample was not crystallized well but is a mixture with amorphous carbon.

### Morphology of Ni-CNF

The scanning electron microscopy (SEM) micrograph of CNFs shows that, the peculiar morphology of cotton fibers has been retained in the CNFs formed with fairly uniform distribution of nickel nano particles [Fig. 1]. The CNFs were found to have thickness in the range of 20-30 nm.

### Raman Spectroscopy Analysis of Ni-CNF

A Raman spectrum shows [Fig. 3] one peak at 1565 cm<sup>-1</sup> of G band and second at 1380 cm<sup>-1</sup> of D band which are the characteristic for graphitic material containing some disorder structure as well as presence of crystalline graphene. This confirms that the sample is a mixture of amorphous and graphitic carbon materials (Mukherjee et al. 2013 and Zacharia et al. 2005).

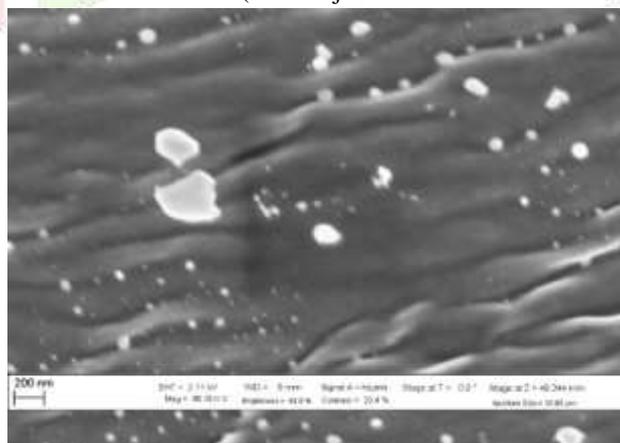


Fig 1. Scanning electron microscopy (SEM) micrograph of Ni-CNF

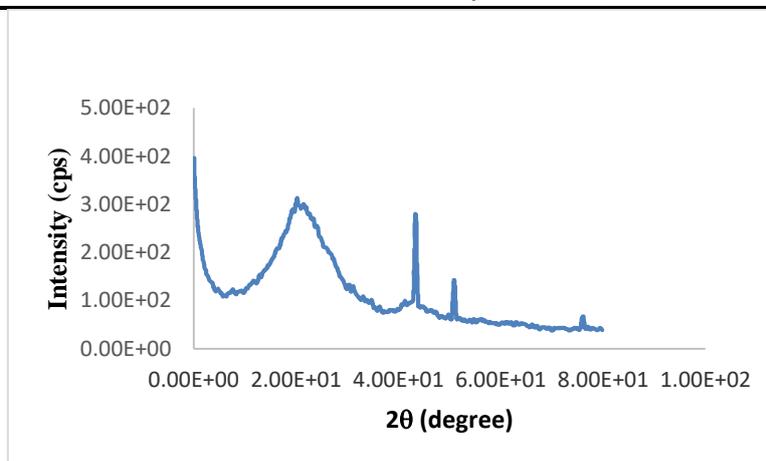


Fig 2. X-Ray Diffraction of Ni-CNFs

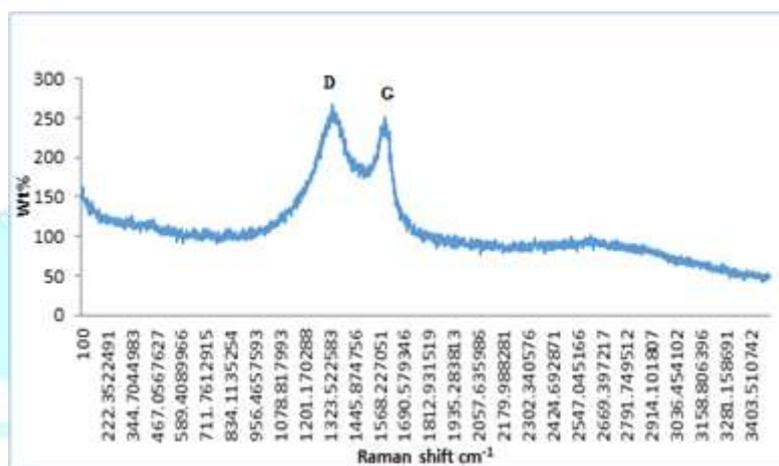


Fig. 3 Raman spectra of Ni-CNF

#### Hydrogen adsorption study of K-CNF and Ni-CNF

There were two samples of CNF used to study the hydrogen storage capacity. In the Table 1, the adsorption measurements of K-CNF and Ni-CNF mentioned are at ambient temperature. The hydrogen adsorption capacity of K-CNF and Ni-CNF were found to be 3.81 wt% and 7.01 wt% respectively at 60 bar pressure. The Brunauer-Emmett-Teller (BET) surface area of K-CNF and Ni-CNF are  $660.13 \text{ m}^2\text{g}^{-1}$  and  $1229.84 \text{ m}^2\text{g}^{-1}$  respectively. For the decoration of the CNF with metal nano particles, it is treated with metal ion solution. During the annealing of the metal ion treated CNF further chiseling effect on the carbon is observed resulting in increased SSA, this effect is not observed in untreated CNF. In addition, the pore volume of K-CNF and Ni-CNF are  $0.1842 \text{ cc/g}$  and  $0.7521 \text{ cc/g}$  respectively. Among K-CNF and Ni-CNF the adsorption is high in Ni-CNF. The hydrogen capacity of Nickel nanoparticle decorated CNM (Ni-CNF) is higher than that of untreated CNM i.e., K-CNF. This is due to the presence of nickel nanoparticles, which strongly enhances the hydrogen storage capacity of CNFs coupled with synergic effect of increased SSA. In general, hydrogen adsorption depends on specific surface area, pore volume and pore size of the adsorbent. In this case hydrogen adsorption is not consistent with the textural properties. Nickel nano particles act as hydrogen favorable site and play important role in hydrogen adsorption. In Nickel nanoparticles decorated material, charge transfer takes place between materials and nickel. A nickel nanoparticle carries a positive charge, polarizes the hydrogen molecules, and then binds the hydrogen atoms. The nickel nanoparticles of the adsorbents led to a spill-over effect of hydrogen molecules leading to physisorption. Nickel particles act as catalyst to hydrogen spill-over, causing enhancement in the hydrogen storage capacity (Ruse et al. 2016 and Geng et al. 2014). The metal particles dissociate hydrogen thus enhancing the hydrogen adsorption capacity. Thus, present work explains the enhancement of hydrogen storing capacity of sample Ni-CNF.

#### IV. CONCLUSION

In this investigation, Pyrolysis of cotton (utilizing Lindberg's horizontal furnace at  $600^\circ\text{C}$  for 4 hours in presence of Argon) is carried out and hydrogen adsorption is determined for it. It has been tracked down that because of the Ni-CNF there is an upgrade in hydrogen adsorption capacity as compared to K-CNF at 60 bar pressure. Raman spectra unmistakably expresses the combination of graphitic and amorphous nature of carbon. Additionally, CNFs obtained with decorated Ni nanoparticles have higher surface area and pore volume than K-CNF which are upheld by the outcomes obtained from BET and SEM analysis.

#### V. ACKNOWLEDGEMENT

Authors acknowledge the financial support provided by the Ministry of New and Renewable Energy (MNRE Grant no. 103/225/2014-NT), New Delhi, India without which this work could not have been done.

## VI. REFERENCE

- Banard, P., & Chahine, R. (2007). Storage of hydrogen by physisorption on carbon and nanostructured materials. *Scripta Materialia*, 56(10), 803–808. doi:10.1016/j.scriptamat.2007.01.008
- Cheng, H., Chen, L., Cooper, A. C., Sha, X., & Pez, G. P. (2008). Hydrogen spillover in the context of hydrogen storage using solid-state materials. *Energy & Environmental Science*, 1(3), 338. doi:10.1039/b807618a
- Dimitrakakis, G. K., Tylianakis, E., & Froudakis, G. E. (2008). Pillared Graphene: A New 3-D Network Nanostructure for Enhanced Hydrogen Storage. *Nano Letters*, 8(10), 3166–3170. doi:10.1021/nl801417w
- Gao, Y., Zhao, N., Li, J., Liu, E., He, C., & Shi, C. (2012). Hydrogen spillover storage on Ca-decorated graphene. *International Journal of Hydrogen Energy*, 37(16), 11835–11841. doi:10.1016/j.ijhydene.2012.05.029
- Geng, Z., Wang, D., Zhang, C., Zhou, X., Xin, H., Liu, X., & Cai, M. (2014). Spillover enhanced hydrogen uptake of Pt/Pd doped corn-cob-derived activated carbon with ultra-high surface area at high pressure. *International Journal of Hydrogen Energy*, 39(25), 13643–13649. doi:10.1016/j.ijhydene.2014.02.065
- Mukherjee B T, S. S. (2017). Microwave absorption by CNM decorated with nickel nano particles. *International Journal of Engineering Science Invention*, 6 (9), 77-8
- Pandyan, R. K., Seenithurai, S., Kumar, S. V., & Mahendran, M. (2014). Magnesium Hydride Doped on Single-Walled Carbon Nanotubes for Hydrogen Adsorption. *Fullerenes, Nanotubes and Carbon Nanostructures*, 23(2), 175–180. doi:10.1080/1536383x.2013.863766
- Prakash, J., Tripathi, B. M., Dasgupta, K., Chakravarty, J. K., Pai, M. R., Kumar, A., & R. Bharadwaj, S. (2016). Tuning of Hydrogen Storage Property of Multi-walled Carbon Nanotube by Decorating Ni, Cu and Fe Nanoparticles. *Current Nanomaterials*, 1(2), 124–131. doi:10.2174/2405461501666160808164652
- Rangel, E., & Sansores, E. (2014). Theoretical study of hydrogen adsorption on nitrogen doped graphene decorated with palladium clusters. *International Journal of Hydrogen Energy*, 39(12), 6558–6566. doi:10.1016/j.ijhydene.2014.02.062
- Ruse, E., Pevzner, S., Pri Bar, I., Nadiv, R., Skripnyuk, V. M., Rabkin, E., & Regev, O. (2016). Hydrogen storage and spillover kinetics in carbon nanotube-Mg composites. *International Journal of Hydrogen Energy*, 41(4), 2814–2819. doi:10.1016/j.ijhydene.2015.12.017
- Rzepka, M., Lamp, P., & de la Casa-Lillo, M. A. (1998). Physisorption of Hydrogen on Microporous Carbon and Carbon Nanotubes. *The Journal of Physical Chemistry B*, 102(52), 10894–10898. doi:10.1021/jp9829602
- Salam, M. A., Sufian, S., Lwin, Y., & Murugesan, T. (2013). Hydrogen Storage of a Fixed Bed of Nanocrystalline Mixed Oxides. *ISRN Nanomaterials*, 2013, 1–10. doi:10.1155/2013/539534
- Satyapal, S., Petrovic, J., Read, C., Thomas, G. and Ordaz, G. (2007) The U.S. Department of Energy's National Hydrogen Storage Project: Progress towards Meeting Hydrogen-Powered Vehicle Requirements. *Catalysis Today*, 120, 246-256. <http://dx.doi.org/10.1016/j.cattod.2006.09.022>
- Schaefer S, et al., Rice straw-based activated carbons doped with SiC for enhanced hydrogen adsorption, *International Journal of Hydrogen Energy* (2017), <http://dx.doi.org/10.1016/j.ijhydene.2017.02.043>
- Schlapbach, L., & Zuttel, A. (2001). Hydrogen-storage materials for mobile applications. *Nature*, 414(6861), 353–358. doi:10.1038/35104634
- Sharon M., Soga T., Afre R., Sathiyamoorthy D., Dasgupta K., Bhardwaj S., Jaybhaye S. (2007). Hydrogen storage by carbon materials synthesized from oil seeds and fibrous plant materials. *International Journal of Hydrogen Energy*, 32(17), 4238–4249. doi:10.1016/j.ijhydene.2007.05.038
- Sheng, Q., Wu, H., Wexler, D., & Liu, H. (2014). Effects of Reducing Temperatures on the Hydrogen Storage Capacity of Double-Walled Carbon Nanotubes with Pd Loading. *Journal of Nanoscience and Nanotechnology*, 14(6), 4706–4709. doi:10.1166/jnn.2014.8251
- Sircar, S., Golden, T. C., & Rao, M. B. (1996). Activated carbon for gas separation and storage. *Carbon*, 34(1), 1–12. doi:10.1016/0008-6223(95)00128-x
- Spyrou, K., Gournis, D., & Rudolf, P. (2013). Hydrogen Storage in Graphene-Based Materials: Efforts Towards Enhanced Hydrogen Absorption. *ECS Journal of Solid State Science and Technology*, 2(10), M3160–M3169. doi:10.1149/2.018310jss
- Sumida, K., Stuck, D., Mino, L., Chai, J.-D., Bloch, E. D., Zavorotynska, O., Long, J. R. (2013). Impact of Metal and Anion Substitutions on the Hydrogen Storage Properties of M-BTT Metal–Organic Frameworks. *Journal of the American Chemical Society*, 135(3), 1083–1091. doi:10.1021/ja310173e

Tellez-Juarez, M. C., Fierro, V., Zhao, W., Fernandez-Huerta, N., Izquierdo, M. T., Reguera, E., & Celzard, A. (2014). Hydrogen storage in activated carbons produced from coals of different ranks: Effect of oxygen content. *International Journal of Hydrogen Energy*, 39(10), 4996–5002. doi:10.1016/j.ijhydene.2014.01.071

Wang, T., Zhang, Q., Li, B., Chen, H., & Chen, L. (2012). Density functional study of hydrogen spillover on direct Pd-doped metal-organic frameworks IRMOF-1. *International Journal of Hydrogen Energy*, 37(6), 5081–5089. doi:10.1016/j.ijhydene.2011.12.065

[www.hydrogen.energy.gov/storage](http://www.hydrogen.energy.gov/storage) US-DOE (department of hydrogen energy).

Zacharia R, K. K. (2005). Enhancement of hydrogen storage capacity of carbon nanotubes via spill-over from vanadium and palladium nanoparticles. *Chemical Physics Letters* , 412 (4-6), 369-375. DOI: 10.1016/j.cplett.2005.07.020

Zuttel, A. (2004). Hydrogen storage methods. *Naturwissenschaften*, 91(4), 157–172. doi:10.1007/s00114-004-0516-x



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IMPACT FACTOR:4.197(IJIF)

ISSN: 2454-5503

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# CHRONICLE

## OF HUMANITIES AND CULTURAL STUDIES

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VOL. 6

SPECIAL ISSUE 1

JUNE 2020

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A Bimonthly Peer Reviewed International Journal

Special Issue On

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## Reading Frightening Experiences and Emotional Trauma During the Pandemic Covid-19

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**Abstract:-** *The outbreak of the corona virus named as Covid-19 has disrupted and devastated millions of people's lives across the globe. Its quick spread all over the world shadowed a holocaust of the fear and trauma worldwide. Human lives are turned pale because of this stunning unexpected deaths brought by this pandemic disease. A virus has caused the irreparable and irreversible loss of the treasures of beautiful lives blooming in the garden of the nation. This loss of lives is unforgettable and stunning which has agonized people intensely who have lost their near and dear one. The oceans of grieves are created which remained unfathomed and unsolicited. Numerous kinds of loss such as loss of bread and butter, employment, business, source of income, food, shelter and many more things pushed poor people, daily earners and laborers to die of hunger and starvation, compelled them to leave their small shelters as there remained no money to pay the rent and were turned bound to start their painful journey on their own feet causing mass reverse painful migration which again posed the great challenge to the industry and economy globally and locally as well. There seems no end of this painful experience till advances of science come out with solid medical output which would heal-up the wound of the humanity. With this hope I placed my sincere efforts to examine and analyze the impact of Covid-19 on humanity across the globe.*

**Key words:** pandemic, shadow, holocaust, treasure, stunning, unfathomed, unsolicited, migration, irreversible, irreparable.

"Life is a flow that goes on constantly without any pause, sometimes smoothly and uninterrupted and sometimes distracted and perturbed intensely like ripples in the ponds changing the facets of life itself."

The present paper demonstrates the fear of the human mind received from this pandemic Covid 19 which has created an intense fear in the mind of the humanity and devastated to the millions of lives not only in India but also in the hundreds of nations across the globe. Covid 19 comes as a threat to the survival of the humanity which has posed a great challenge to the intellectual humanity of different zones across the globe. People are frightened of the pandemic bitterly. They are scared of physical touch of even very near and dear one as it may lead to the infection of the virus. Doctor parents, nurses, paramedical staff, police

officers and other front Corona warriors who are the parents of the little angel kids are afraid of this pandemic in such a way that they avoid to pour their affection to their small kids by hugging and taking them in their laps. Everywhere is seen a kind of emotional gap and trauma and fear of the pandemic. This pandemic has been read as biological war by the ambitious nation to set up their hold and power over the states and nations across the world. America and China has opened a new cold war over this issue which has been intensifying the fright to the humanity at wider scale. This pandemic has devastated US, Britain, Germany, France, Italy, India, Pakistan China and many more countries in a very pathetic manner. The pain of the humanity is oozing of this pandemic. Humanity has been intensely suffering of the loss of their dear relatives and close fellows. The different nations have been studying the impacts and the implications of this pandemic over the human experiences. Academicians across the globe including Indian academics, have been investigating many traditional issues of the literary tops of this Covid 19, ranging from a reflection on morality and justice to the contagion and clinical features of the disease.

In particular, we academicians have been collectively putting our efforts and inviting the attentions of the people to focus behavioural responses of the humanity to this pandemic, showing the emergence of fear, irrationality, and selfishness in present civilized and modern society. By the early 20th century, epidemics were no longer considered divine punishments or supernatural events; 19th century bacteriologists had demonstrated that they are caused by germs that infect humans, and epidemiologists and public health experts had shed light on the mechanisms of disease transmission, including suggestions of general preventive measures to limit pandemics. Despite these scientific developments, however, the contemporary time across the different zones world-wide, the general public's fear of the world is very high. The millions of lives completely doomed and devastated. Millions of people are adversely affected with this pandemics; thousands and lakhs of people have lost their close near and dear one; they have lost their people, their jobs and are now clad with a sense of not only with this pandemic's threat but also with a sense of insecurity and instability in their lives. Economies across the globe have been imposed the great challenge to survive in the market. Slowdown has been scaring the people across the boundaries.

Public trust in science during the 21st century is marked very high as new innovations and experiments at advance level has assured the societies of their security of better survival and well-being across the world. However, the people are frightened by "this astonishing quickness

it inevitably has killed lakhs of people in all over the world ... From the moment of the first signs of it, a man would be dead in an hour. Some lasted for several hours. And many people varying of different age groups died within short span of time unbelievably and unexpectedly". At the ground reality lakhs of people in the corona affected nations are affected, infected and even died of this pandemic's illness;

*"The heart began to beat faster and the heat of the body to increase. Then comes pandemic's infection, spreading like wildfire over the millions of people world- wide. Most persons across the boundaries never noticed the increase in heat and heart-beat, and the first they came to know was the time when the Covid 19infection came out of the virus lab. The hurt of the disease remains incurable and its impact is very severe. ... People first look for testing of the Covid19 and once they are found positive, they fall under mental distress, emotional trauma, physical isolation in some puffed white chambers with randomly white walking shades around...The heels became numb first, then the legs, and hips, and when the numbness reached as high as their heart, they died."*

Mumbai and New York experiences the worst trauma of pandemic where lakhs of people are infected of the diseases and many of them are died leading a chaos to the humanity. Graveyardfell short to bury the dead bodies of their own people. Hooters and sirens are running continually in the ambulances coming to hospitals and then after from hospitals to graveyards. A very pathetic situation is being created where man has been fallen helpless to control the disease and tolerate the pain of losing the dearest relatives. Doctors and paramedical staff are posed with the great challenge of curing the people. Rapidburial of the corpsesagain appear a great challenge which has increased the risk of immediate release of billions of germs, accelerating the spread of the disease and causing problems for the front Corona warriors, scientists who are not able to quickly find the specific treatment. By the time it appears a challenge to stop the epidemic for the community of researchers and the scientists. Medicine and scientific progress are falling short and insufficient to protect the citizens. Doctors, police officers, and other front pillars of the fighter against the disease are defeated and infected by this pandemic. Mass relocations and migration of the labours has posed the financial burdens to the government agencies and crisis to the industries at intense level which have been interrupting to the motion of the production and the economies.

There is hardly any escape from this crisis. Virus has been still spreading very fast in an uncontrolled way. The mechanism developed to fight with the pandemic is not in position to stop it, and the world is in a state of sheer pain and fear never experienced before. People started

been turned panic while reacting to the outbreak of the pandemic. People just want to keep them away from the infection of the disease. For it most of us have tried to isolate ourselves and even many of us pretend to fly away to avoid the contagion but that seems impossible as movement is restricted. Hospitals' mortuaries are overloaded and have been turned short to store the corpses properly. Patients are seen bound to remain in isolated covid ward with dead bodies lying on the nearby beds. Lives are contaminated and human hearts have been shivering of the fear, trauma and frightening. Everywhere appears fright... fright of getting infected, fright of being isolated and fright of quick death lurking towards the lives... and many more frights bursting up in the human heart.

People across the globe have been dying and the entire globe is being devastated by virus;

*"The virus infection is filled in the air, the heaven is darkened; the day is turned as a gloomy twilight, and even the fear is seen in the shifts of wind, sometimes in the sun shines through the dimly and dull red orbit. It looks like some horrible pangs of destructions and devastations. People fear of their own death; the cities are being destroyed by the virus; the people have been fleeing away in the state of hysteria. This immense panic felling has been frightening the people in an unprecedented way, experiencing them a hopeless sign of death all around. The brutality of the death is marked everywhere across the world, somewhere more and somewhere less. The apocalyptic scenario illustrates a common fear of epidemic across the globe."*

Now a day, despite the development of several antibiotics and life-saving drugs, infectious diseases, germs and virus continue to generate fear and trauma as recently demonstrated by Covid-19 worldwide. Several studies have been conducted to analyse and hypothesize about the emotional, cognitive, and behavioural responses of the humanities to the epidemic so that certain policies would be framed to develop the safety measures for the public. No doubt it has been going to change the public perception and behaviour in the aftermath of biological disaster like deadly epidemic. A recent studies and surveys conducted by several agencies analysed post pandemic perceptions of collectives implicated outbreak and found that physicians and researchers are considered "heroes" of the present pandemic. We can come to conclusion that the public trusts are mainly in scientists rather than any agencies and authorities of the states. But in case of Indian state it is well observed that public has demonstrated full faith and extended whole hearted support to honourable Prime Minister Shri Narendra Modiji and his administrative officers and diplomats in strategic fighting of the Covid-19.

On the other hand, private corporations in particular the pharmaceutical industry too, have tried their best to felicitate the mass

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production of the medicines, testing kits and other required equipment to exploit the pandemic season in to an opportunity. However, recent outbreaks have demonstrated that even the scientific community falls short in managing infectious disease in present context. During this pandemic, human emotion are affected but at the same many heroic deeds are too, performed by scientists and health care workers. Devotion to professional duty resulted in a high level of camaraderie, cohesion, and encouragement in hospitals. However, the haunting fear of acquiring and spreading the disease to families, friends, and colleagues may also lead to understandable selfishness and cowardice in health providers. But the cordial sympathetic face of the Indian police at different place across the country is seen as they helped the needy people, provided foods from their own salaries to the worst victims of unemployed and poverty ridden labours re-migrating to their native places during the pandemic. Over all collective unity among the public was seen to avoid and escape from the deadly influences of the Corona disease.

Work of media too, inspires us greatly as their works reflect tireless efforts of media to provide ground report of the infection to be informed to the public of the country. This helped a lot to the citizens to read the message of the government authorities which was aired live time to time and established a kind of emotional communication between the heads of the states and the public. Appeals and requests made by them through the media acted as an alarm which alerted the public in general regarding the way pandemic spread and the precautions public needed to take. Such communications with the Prime Minister, Chief Ministers and other political delegates, social agencies and other machineries working to fight against the pandemic infection. T.V., Newspapers, internet, mobiles, and phone calls and Setu App developed by the health department of Indian government are the tools for obtaining information on epidemic spread. The camera men and their team along with other mercenary working for the news are actually admirable for their bold report they provided to the nation. It is their efforts which reduced the intensity of the panic situation.

"The men, who sent this news, the wireless operators, were alone with their instrument on the top of many lofty buildings to ensure the smooth live airing of the ground reports which helped people immensely to remain disciplined and alert which ultimately turned very much helpful to control the disease's expansion. They too, are the real heroes of this crucial time." If today, the main sources of information on pandemics are widely available including the mass media, such as television, radio, and print media such as magazines and newspapers, then there is drenching efforts of these men who are behind the curtain but whose role is very much important in alerting and alarming the public

about the pandemic. Sometimes, the role of media may seem to be accused of exaggerating the risks of the pandemic and contributing to public misunderstandings on public health research evidences. But over all media played an excellent role in informing the public as media coverage can directly affect public risk perceptions and have had a positive influence on disease perception. It stood as a useful resource in controlling epidemic fear, enabling a bridge to be created between government/science and public opinion. It is clearly visible that there is created a kind of intense painful situation all over the world due to this pandemic which has resulted in to fear and trauma to the millions of people all over the world. The economies are collapsed, the cities are turned in to the graveyard counting the dead bodies consistently arriving and people are stunned to see such heart melting view of the humanity. No words can express the exact pain and the trauma, humanity has received through this Covid-19. The wound this pandemic has given is very deep which even wouldn't be able to be healed up by the time itself. It would be a very horrible memory that definitely would be preserved in the pages of the past.

**References:**

1. Watts SJ. Epidemics and history: Disease, power, and imperialism. London: Yale University Press; 1997
2. Beidler PG. The plague and Chaucer's Pardoner. *Chaucer Rev.* 1982;16:257-69
3. Grigsby BL. Pestilence in Medieval and early modern English literature. London: Routledge; 2004
4. Steel D. Plague writing: from Boccaccio to Camus. *J Eur Stud.* 1981;11:88-110
5. Watts SJ. Epidemics and history: disease, power, and imperialism. London: Yale University Press; 1997





## CORTISOL HORMONE VARIATIONS DUE TO SOUND STRESS: PLEASANT AND UNPLEASANT SOUND

### Endocrinology

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### ABSTRACT

The current study concentrates on the severity of noise pollution on public health. During this study cortisol hormone analysis was done for confirmation of stress in animals when exposed to high decibel noise like traffic noise and temple bell clapping. During this experiment, the two-Test sets of animals (T1) set exposed to traffic noise and (T2) set to temple bell clapping were used and a standard set of animal (C) who were not exposed to any loud noise but kept in the silent zone (below 55 dB). During the experiment, it became evident that loud noise did cause a surge of cortisol in the animal in the T1 group but no cortisol was present in the T2 group as well as in the Control group of animals. The presence of cortisol in an animal exposed to traffic noise implies stress in those animals and the absence of cortisol in the T2 group indicates the animals were not stressed even though they were exposed at high decibels noise. Also, the control group display absence of cortisol hormone in their blood. This concludes that sound stress is a result of exposure to pleasant or unpleasant sound irrespective of high decibel sound. This could be the reason why loud music is liked by people without causing any harm but is noise for others. It is a sheer matter of perspective of the individual which cause him/her to be stressed of loud noise.

### KEYWORDS

Cortisol, Stress, Temple bell, Noise pollution

#### INTRODUCTION:

Hormones play a vital role in the life of an organism. They are the messenger molecules of the body that control and co-ordinates various responses and activities of an organism. Hormones are highly specific chemical substances which attach to their target organs only.

Cortisol hormone is a stress hormone which is glucocorticoid class of hormone released from the adrenal gland which brings in glycogenesis or gluconeogenesis. The function of this hormone is to co-ordinate with the hippocampus in the brain and reduces stress, basically a negative feedback mechanism in stress situations. When an animal faces stress it immediately finds an escape from the situation for which it requires strength and navigation skills which is the preparation for "fight or flight reflex". The signal for this situation is the release of cortisol hormone. It is released through the hypothalamus-pituitary-adrenal axis. The stimulus of stress signal from the surrounding like an unpleasant noise from the non-specific auditory pathway triggers the hypothalamus to release corticotropin release hormone (CRH) which induces the pituitary gland to release adrenocorticotropic hormone (ACTH) which in turn induces the adrenal gland to release cortisol hormone. This cortisol hormone manages the stress within the organisms by fight or flight reflexes initiating glycogenolysis or gluconeogenesis. (Sapolsky, R.M., Romero, M., et.al).

The glucocorticoid hormone cortisol is considered a marker hormone for stress since it forms the negative feedback system for stress. (Sapolsky, R.M., Romero, M., et. al) The presence of this hormone in the blood triggers alertness in the individual and causes all the sensory system to collect maximum information from its surrounding to act on 'fight or flight' reflex against stress. (Lucassen, P.J, Pruessner, J., et.al) Cortisol is a glucocorticoid hormone. It is a steroid hormone which is released by the zona fasciculata of the adrenal cortex. This hormone has a major role in the physiological preparation of the organism to protect and preserve itself from crucial situations by increasing blood sugar level through gluconeogenesis which indirectly supports the increased ATP molecule formation in an organism's body (McEwen, B.S.), (Lucassen, P.J, Pruessner, J., et.al), (Sapolsky, R.M., Romero, M., et.al). The present study has a trending towards the fact that cortisol was released in blood collected from test rats exposed to high decibel traffic noise. While the control rats did not show any cortisol in their blood sample.

#### MATERIAL AND METHOD:

##### MATERIAL:

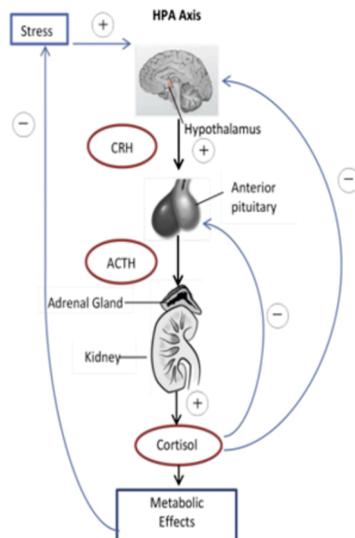
Exposure chamber, Decibel metre, Blood serum sample vacutainer, white albino rats, sound i.e. speaker, HPLC machine, cortisol control, loading buffers, etc.

The animals were exposed to noise pollution in the exposure chamber specifically designed for the experiment by the researcher. The chamber was made of wood and glass. Wood was utilized because it is the best sound-absorber and glass promotes visibility of the animal during the experimentation. The exposure chamber dimensions are 3feet\*2 feet.

#### METHODOLOGY:

This experimentation was undertaken with 7 albino Wistar rats. They were segregated into two basic groups –test (4 no.) and control (3 no.) animals. The experiment was performed based on the experimentation consisted of two test group –Test 1 group of animals exposed to traffic noise, Test 2 animals exposed to temple bell clapping and control groups of animals. The control group of animals exposed to no loud noise but were kept in the silent zone with sound below 55 decibels. The hormone analysis of the control and experimental animals were performed by the HPLC instrument.

The sample collection i.e. blood was collected from the retro-orbital sinus near the eyes of the animal by insertion of the capillaries through the sides of the eyes and collected in vacutainer. The collection of a blood sample for all animals were done on the same i.e. the last day of exposure at the same time i.e. 4:30 PM (Indian Time). This was done to keep in-check on the cortisol state of the animal since it is released in the normal condition through the active state i.e. awake state of the



**Fig. 1, Cortisol release through the HPA axis activation as a stress response (source: <https://www.sciencedirect.com/>)**

animal and since the animal under consideration here are rats who are nocturnal they cannot have cortisol during this hour.

The total duration of the experiment was for 20 days. Out of the 20 days, the Test 1 animals (4 no.) were exposed to traffic noise for 15 days and thereafter exposed to temple bell for 5 days which became Test 2 group. The control animals (3 no.) were not exposed to any sound as they always kept in the silent zone with sound level below 55 dB.

The sound stressor was applied only on the test group while the control group was not exposed to high decibel sound but was kept strictly in the area below 55 dB sound.

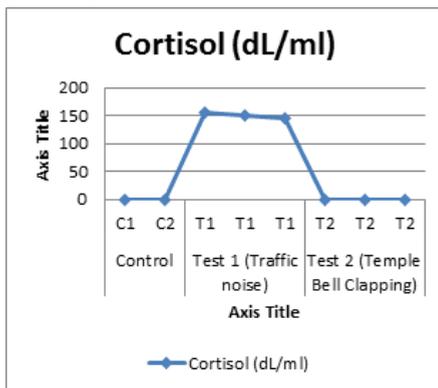
The high decibel sound exposed to the test groups were between 90-110 dB sounds. The test animals were exposed to two sets of high decibel sound, one at a time. The first set of high decibel sound exposure was for first 15 days of experimentation Test 1 group which was a high decibel traffic noise (sound stressor) for 30 minutes daily whereas the second exposure of high decibel sound was of temple bell (therapy) for 3-5 mins daily for 5 days to the same set of stressed animals Test 2 group.

**RESULT:**

**OBSERVATION AND RESULTS:**

	Cortisol (dL/ml)	
Control	C1	-
	C2	-
Test 1 (Traffic noise)	T1	155
	T1	150
	T1	145
Test 2 (Temple Bell Clapping)	T2	-
	T2	-
	T2	-

**Tab. 1, Cortisol reports**



**Chart. 1, Representation of cortisol**

As per the observation table, it is found that the control animals and Test 2 animal's (who were exposed to temple bell clapping) blood sample did not contain cortisol, but, the Test 1 animals had cortisol hormones present in the blood sample which indicates stress in Test 1 animal.

**DISCUSSION:**

The present research has thrust on the levels of cortisol released due to noise stress. It was observed that cortisol levels are none in the control animals as they are not exposed to any of the traffic sounds. They also ate normal food and water regularly. But in the test group which was exposed to traffic noise, the presences of cortisol levels were observed. These observations are parallel to the finding of Phillips, L.J., McGorry, P.D., Garner, B., Thompson, K.N., Pantelis, K., Wood, S.J., Berger, G., 2006 wherein they studied the relationship between psychological stress, HPA axis functioning and the hippocampus leading to the presence of cortisol in the stressed animal. Similarly, the current studies are also in response to the findings made by Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000 in which they have emphasised the secretion of glucocorticoid in response to stress. And also as seen in the work done by Sundareswaran Loganathan, Sheeladevi Rathinasamy, 2016 who has monitored the corticosterone level in response to the therapeutic plant extract *Scoparia dulcis*.

At the same time, a very interesting finding was the presence of no

cortisol in animals exposed to the sound of temple bell clapping at the same high decibel as traffic noise. This leads us to a very important aspect of the sound study of mindfulness. But mindfulness concentrates on low and smooth sound, not loud sound as high as 100-110 dB sound.

This has to be understood as the loud sound or smooth sound is analysed by the brain as pleasant and unpleasant by the brain and respond to sound in accordingly Chanda, M.L., Levitin, D.J., 2013.

**ACKNOWLEDGEMENT:**

I want thank my organization The Institute of Science, Fort, Mumbai and my PhD guide Dr. Varsha Andhare for allowing to do this work in the Zoology department and for their support and guidance for the accomplishment of this work.

**Funding:**

Since this was part of the PhD work it was completely self-funded by the PhD student Nisha Velayudhan.

**REFERENCE:**

- Chanda, M.L., Levitin, D.J., (2013), The Neurochemistry of Music, Trends in Cognitive Sciences, 17(4), 179-193.
- Harpreet Kour, Rajashree, R., Gougar, S.S., An experimental study to evaluate the effect of instrumental Indian classical and Western music therapy on learning and memory in stress induced young rats. IOSR Journal of Pharmacy, ISSN:2250-3013, Volume 2 Issue 4, July – August 2012, pp 29-32.
- Jamir, L., Nongkynrih, B., Gupta, S.K. (2014). Community noise pollution in urban India: Need for public health action. Indian Journal of Community Medicine, 39(1), 8-12.
- Khalfa, S., Dalla Bella, S., Roy, M., Peretz, I., Lupien, S.J., Effect of relaxing music on salivary cortisol level after Psychological stress. New York Academy of Science 999 (2003) pp :374 –376
- Knight, C.R., Swaddle, J.P., (2011), How do Glucocorticoids influence stress response? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Review, 2(1), 55-89.
- Knight, C.R., Swaddle, J.P., (2011). How and why environmental noise impacts animals: an integrative, mechanistic review, Pubmed NCBI, 14(10) 1052-1061.
- Lucassen, P.J, Pruessner, J., Sousa, N., Almeida, O.F.X., Van Dam, A.M., Rajkowska, J., Swaab & D.F., Czéh, B. (2014). Neuropathology of stress. Acta Neuropathol, 127,109-135.
- McEwen, B.S. (2000).The neurobiology of stress: from serendipity to clinical relevance. Brain Research, 886, 172-189.
- Passchier-Vermeer, W., Passchier, W.F. (2000). Noise Exposure and Public Health. Environmental health perspectives, 108(1), 123-131.
- Phillips, L.J., McGorry, P.D., Garner, B., Thompson, K.N., Pantelis, K., Wood, S.J., Berger, G., (2006). Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders. Australian and New Zealand Journal of Psychiatry, 40, 725-741.
- Reardon, G.E., Caldarella, A.M., Canalis, E., Determination of serum cortisol and 11-Deoxycortisol by Liquid Chromatography. Clinical Chemistry, Vol. 25, No.1, 1979, pp 122-126
- Salamon, E., Kim, M., Beaulieu, J., Stefano, G.B., (2003), Sound therapy induced relaxation: down regulating stress processes and pathologies, Med Sci Monit, 9(5), RA116-121.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, The endocrine society, 21(1), 55-89.
- Spiers, J.G., Chen, H.J.C., Sernia, C., Lavidis, N.A. (2015). Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress. Frontiers of Neuroscience, 8(456),
- Stansfeld, S.A., Berglund, B., Clark, C., Lopez-Barrio, Fischer, P., Ohrstrom, E., Haines, M.M., Head, J., Hygge, S., Kamp, I., Berry, B.F., (2005). Aircraft and road traffic noise and children's cognition and health: a cross-national study, The Lancet, Vol. 365, 1942-49.
- Sundareswaran Loganathan, Sheeladevi Rathinasamy, 2016, Alteration in memory and Electroencephalogram waves with sub-acute noise stress in Albino rats and safeguarded by Scoparia dulci, Pharmacognosy Magazine, Vol 12(45), S7-S13.
- Turner, J.G., Parrish, J.L., Hughes, L.F., Toth, L.A., Caspary, D.M. (2005). Hearing in Laboratory Animals: Strain Differences and Non-auditory Effects of Noise. National Institute of Health, 55(1), 12-23.
- Uno, H., Tarara, R., Else, J.G., Suleman, M.A., Sapolsky, R.M. (1989). Hippocampal Damage Associated with Prolonged and Fatal Stress in Primates. The Journal of Neuroscience, 9(5), 1705-1711.
- Westman, J.C., Walter, J.R., (1981). Noise and Stress: A comprehensive approach, Environmental health perspectives, Vol 41, 291- 309.

2020-21



ISSN 2249-9598 (Online)  
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## Effect of Melphalan on Ovarian cell and its hormone on female albino rat

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### Abstract

It is more difficult to determine how chemotherapy affects female reproductive function as there is no direct way of monitoring toxic effect on the ovaries. Gonadal damage is often manifest by amenorrhea, low estrogen levels, and increased concentrations of FSH and LH, which resemble the hormonal changes seen at menopause. As in men, alkylating agents appear to be the most toxic. Primary ovarian failure has been reported with both melphalan. Present study designed to understand, effects of melphalan (Alkeran) on ovary and their correlations with hormones and related physiological and reproductive functions. It is more difficult to determine how chemotherapy affects female reproductive function as there is no direct way of monitoring toxic effect on the ovaries.

**KEYWORDS:** Ovaries, alkylating agent, menopause, melphalan.

**Introduction** Melphalan (Alkeran) , L-Phenylalanine mustard is a alkylating agent used in the treatment of ovarian carcinoma (Dominique et.al. 1992). Studies on the biological alterations of the result suggest that non nitrogen mustard is the active alkylating agent.it is well absorbed orally and have effect on cell cycle phase non-specific. These drugs remain active in blood for approximately 6- hours in human blood. Early toxicological study indicate that it has a effect on gastrointestinal tract with anorexia, nausea and vomiting in the patient (Spitzer et.al., 1986). It has been generally used for the treatment in ovarian carcinoma, multiple myeloma, breast carcinoma, testicular seminoma and in the malignant melanoma (Sutton, 1994).

It is more difficult to determine how chemotherapy affects female reproductive function as there is no direct way of monitoring toxic effect on the ovaries. Gonadal damage is often manifest by amenorrhea, low estrogen levels, and increased concentrations of FSH and LH, which resemble the hormonal changes seen at menopause. As in men, alkylating agents appear to be the most toxic. Primary ovarian failure has been reported with both melphalan . Present study designed to understand, effects of melphalan (Alkeran) on histology of ovary and their correlations with hormones and related physiological and reproductive functions.

**Materials and methods** In the present study sexually matured healthy albino virgin female rats of 180 + 05 of body weight were used for present experiments. All animals acclimatized in the laboratory for 10 days prior to the experiment. Animal maintenance and experimental procedure strictly followed by "Principles of laboratory animal care (NIH)" and also as per the local "Ethical regulations". Melphalan (Alkeran) of 100% purity, chemist purchased from local chemist as which is marketed by Wellcome pharmaceutical S A, from Glaxo group. Substrate and enzyme kits were obtained from commercial manufactures. Most of the fine chemicals used in the present study were obtained from M/S Sigma chemicals, U



Research Article

# Finding the forgotten gems: revisiting the butterflies of Matheran after 125 years with introduction to novel colour barcode for depicting seasons and activity of the Indian butterflies

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Academic editor: Martin Wiemers

Received: 15 May 2020 | Accepted: 31 Jul 2020 | Published: 07 Aug 2020

Citation: Sawant M, Sarang S, Modak N (2020) Finding the forgotten gems: revisiting the butterflies of Matheran after 125 years with introduction to novel colour barcode for depicting seasons and activity of the Indian butterflies. Biodiversity Data Journal 8: e54333. <https://doi.org/10.3897/BDJ.8.e54333>

## Abstract

We present here an updated checklist for the butterflies of Matheran, Maharashtra, India, an eco-sensitive zone, with identification remarks for locally rare or very rare butterflies. This is the first dedicated checklist for butterflies of Matheran after 125 years. A total of 140 species of butterflies were recorded belonging to six families. Amongst them, 15 species were either listed under Schedule I, II or IV of the Indian Wildlife (Protection) Act, 1972. We also list the habitats of the species along with the data for their activity at the time of recording the observation. We propose a uniform colour code system for representing season and activity for the Indian butterflies. Examples of colour barcodes are provided with the images of rare and very rare butterflies. The lack of abundance data is a limitation of the study for which we propose long term monitoring with dedicated efforts.

## Keywords

Lepidoptera, Eco-sensitive zone, biodiversity hotspot, colour barcode

## Introduction

Butterflies are an ideal taxonomic group for ecological studies of landscapes (Thomas and Malorie 1985) and their value as indicators of biotope quality is being increasingly recognised because of their sensitivity to minor changes in micro-habitat, particularly to the luminosity (Kremen 1992). Further, the butterflies are good biological indicators of habitat quality, as well as for the general health of the environment (Larsen 1988; Kocher and Williams 2000; Sawchik et al. 2005). Long-term diversity studies could, therefore, indicate the health of the habitat and ecosystems therein.

Here, we provide a checklist for butterflies of Matheran surveyed between the years 2011 and 2019. Ours is the first dedicated checklist for the butterflies of Matheran after Betham (1894). He listed 78 species of butterflies, combining the list of sixty butterflies provided by Smith (1882) and the list of butterflies recorded by him between April and May 1892. Padhye et al. (2013) provided a list of 27 butterflies from Matheran, while compiling the checklists for the butterflies of Northern Western Ghats, which was far from complete when compared to that given by Betham (1894). Further, the data on the habitat and seasonal turnover for butterflies of Matheran are particularly lacking from all these studies. Our checklist is accompanied with data on habitat, seasonal turnover and behavioural observations taken at the time of recording the species. We provide a novel coloured barcode approach for indicating the season/s and types of behaviour which could be used for all Indian butterflies. Representative colour barcodes are provided with the images of rare and scheduled species.

## Materials and Methods

### Study Area

Matheran (18.9866°N 73.2679°E, 772 m a.s.l., WGS 84) is a small hill station located in Karjat Tehsil of Raigad District in the Indian State of Maharashtra (Fig. 1). It is spread over an area of 7 sq. km. Matheran literally means forest on the top of the mountains. Geologically, it is a basaltic mesa separated from the main escarpment of Western Ghats by the low lying plains of Konkan and is an example of regressive erosion (Pascal 1988). Matheran gained the status of an Eco-Sensitive Zone (ESZ) in 2003 from the Ministry of Environment, Forest and Climate Change, Government of India [S. O. 133 (E)]. The ESZ of the Matheran comprises an area of 214.73 sq. km. All types of industrial, developmental and vehicular activities are restricted by this governmental order, making Matheran unique amongst hill stations of Asia. It experiences a cooler climate throughout the year (23.2°C mean annual temperature) compared to the surrounding low lying area and experiences

heavy rainfall during the monsoon (4073 mm mean annual rainfall). The landscapes of Matheran are represented by open or forested laterite plateaus, hill-slopes, dense valley forests, non-perennial streams, manmade lakes, clearings near forest paths and human habitation. The flora of Matheran is represented by tree species found in mid elevation type wet evergreen forest (Ramesh et al. 1997), dominated by *Memecylon umbellatum*, *Syzygium cumini* and *Actinodaphne lanceolata* (Birdwood 1886, Ramesh et al. 1997). The plateau also hosts species like *Carallia integerrima*, *Glochidion lanceolarium*, *Olea dioica*, *Garcinia indica* and *Carissa carandas* (Birdwood 1886). The area also shows the presence of many endemic species of orchids, grasses and other herbaceous plants (Kothari and Moorthy 1993).

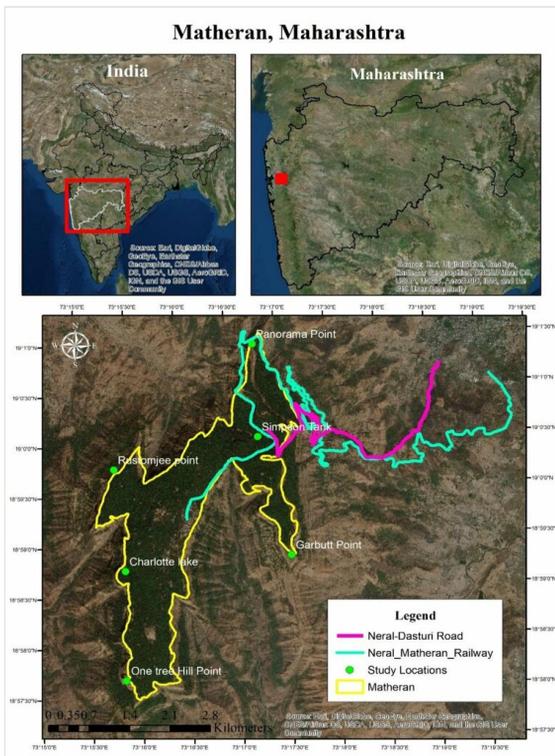


Figure 1. [doi](#)

Study area with its location in Maharashtra, India. Sampling sites are shown in green filled circles. Additionally, the survey was conducted on two trails, Neral-Dasturi Road (pink line) and Neral-Matheran Railway (green line).

## Field Survey and Data Collection

The area was visited in all the three seasons, namely summer (Feb-May), monsoon (Jun-Sept) and winter (Oct-Jan) throughout the year from September 2011 to March 2019. Intermittent observations were taken between 06.00 hrs and 17.00 hrs for around three days a month. The butterflies were observed in all possible habitats at six localities and on

two trails in and around Matheran (Table 1). A total of 22833 observations were made during nine years of the study (<https://indiabiodiversity.org/dataTable/show/1755286>) which are available as a data table on the India Biodiversity Portal (Vattakaven et al. 2016). To ascertain the identity of butterflies, photographs were taken and identifications were made with the keys provided by Evans (1932), Wynter-Blyth (1957), Kunte (2000), Kehimkar (2008), Kehimkar (2016) and Bhakare and Ogale (2018). The classification and nomenclature follows Kehimkar (2008), Van Gasse (2013) and Varshney and Smetacek (2015). The local status of the butterflies was decided, based on the number of records as very rare ( $\leq 5$  records), rare (between 5 and 10), not common (between 10 and 20), common (between 20 and 50) and very common ( $> 50$ ). This status does not correlate to the entire geographical distribution status of a corresponding species. The habitat, occurrence and behaviour of butterflies were noted and photo documented. The photo documentation was made with Nikon d500, d3200 and Cannon EOS 70d, Sony HX 100v digital cameras. The species were noted along with the date and location.

Table 1.

Survey sites in and around Matheran, India with their geographical, climatic and vegetation characteristics.

Site code	Study area	Characteristics
1	Simpson Tank	Small water barrage built on fast flowing stream surrounded by dense forest. Low canopy cover immediately over the barrage.
2	Charlotte Lake	Large artificial barrage enclosing artificial lake. Surrounded by dense forest.
3	Panorama Point	Mixed vegetation containing semi-evergreen forested patches and grasslands. High ambient moisture during monsoon accompanied by high wind currents.
4	Garbett Point	A small plateau associated with Matheran. Mixed vegetation containing semi-evergreen forested patches and grasslands. A small hamlet sustaining a human population prevalently that of the 'Dhangar' (Shepherd) tribe.
5	Rustumjee Point	Thick semi-evergreen vegetation. High ambient moisture during monsoon accompanied by high wind currents.
6	One tree hill point	Gradual hill slopes and edge of the valley. Thick semi-evergreen vegetation. High ambient moisture during monsoon accompanied by high wind currents. A torrential stream flows near this area.
7	Neral-Matheran Rail Route	Various types of vegetation elements with patches of wet evergreen, semi-evergreen forests and grasslands. Entire trail has valleys on one side and cliffs on the other. Many torrential streams intersect this area at various points during the monsoon. Cliffs seep with a thin film of water during the monsoon and early winter months. Gutters made for drainage of water hold it until late winter. Shutting down of railway transport during the monsoon leave this area more or less undisturbed from human interference for around four months.
8	Neral-Matheran Road way	Heavily-disturbed area with human interference holding patches of evergreen, semi-evergreen forests, monoculture of <i>Acacia auriculiformis</i> and grasslands. Entire trail has valleys on one side and cliffs on the other. Many torrential streams intersect this area at various points during the monsoon. Cliffs seep with a thin film of water during the monsoon and early winter months. Gutters made for drainage of water hold it until late winter and early summer.

## Data Analysis

Based on the occurrence data, a species accumulation curve (SAC) was prepared in R (R Core Team 2020) using the SpecAccum function in vegan (Oksanen et al. 2019). Expected (mean) species richness was calculated using the data collected from eight sites (Table 1). Further, the occurrence data of the species were analysed for calculating Similarity-Richness difference-Species replacement simplex (SDR Simplex) using SDRSimplex (a stand-alone computer programme) (Podani and Schmera 2011). Ternary plots were plotted using NonHier platform of SYNTAX 2000 (Podani 2001). The number or percentage of the species recorded per family, during each season, at each site was calculated in Microsoft Excel 2007 and visualised using pie and bar charts.

## Preparation of Colour codes

The colour codes (Table 2) were prepared for easy and uniform representation of seasons and various behavioural activities of the Indian butterflies. Summer, monsoon and winter were given basic red, green and indigo colours in the CMYK scheme. These colours also correspond to temperature shifts in the seasons from hotter to cooler weather conditions. For combination of seasons, the corresponding combination of colours was used. Colours were mixed online through Color Mixer platform of Color Designer (<https://colordesigner.io/color-mixer>). Grey colour represents the occurrence of the species in all seasons. All other colours were selected from the RGB scheme for it provides a wider range of colours. These colours were selected in such a way that they represent the corresponding activity, for example, brown for mud puddling, honey colour (orange palette) for nectaring, amber colour for tree sap feeding etc., except basking which is represented by magenta.

Table 2.

Colour scheme for colour barcodes with CMYK and RGB ratios and HEX numbers.

		Colour	CMYK Ratio (C:M:Y:K)	RGB Ratio (R:G:B)	HEX	Colour Name
Seasons	Summer		0:100:100:0	227:30:36	#E31E24	Red
	Monsoon		100:0:100:0	0:152:70	#009846	Green
	Winter		100:100:0:0	57:49:133	#393185	Indigo
	Summer+Monsoon		9:24:100:46	151:126:22	#977E16	Tan
	Summer+Winter		24:100:2:13	175:0:113	#AF0071	Purple
	Monsoon+Winter		86:36:9:20	0:115:162	#0073A2	Teal
	Summer+Monsoon+Winter		47:38:38:24	128:128:128	#808080	Grey (50% Black)
	Mud Puddling		19:52:85:37	153:102:51	#996633	Brown
	Basking		57:100:0:0	153:0:153	#990099	Magenta

		Colour	CMYK Ratio (C:M:Y:K)	RGB Ratio (R:G:B)	HEX	Colour Name
Feeding	Nectaring		0:45:10:4	235:150:5	#EB9605	Honey (Orange)
	Tree Sap		0:28:98:0	255:191:0	#FFBF00	Amber
	Animal Carcass		11:99:100:50	121:06:04	#790604	Kryon Cherry Red
	Animal Waste		3:0:93:0	255:255:0	#FFFF00	Yellow
	Bird Droppings		95:95:45:95	0:00:00	#000000	Black
	Rotten fruits		17:56:48:12	193:123:113	#C17B71	Rose Brown

## Results

### Species Richness

The SAC gained a plateau and standard deviation for species richness declined from  $97.75 \pm 17.07$  to  $141.0 \pm 0.0$  as the number of sights increased from one to eight, predicting sufficient efforts to record all the species found in the area (Asym = 146.42, xmid = 0.58, slope = 3.60) (Fig. 2). A total of 140 species belonging to six families have been observed and identified during the entire period of the study (Fig. 3, Table 3). The family Lycaenidae with 46 species (32.86%), followed by Nymphalidae with 43 species (31.43%), were amongst the most species-rich families in the area. Species belonging to the family Hesperidae (25 species), Pieridae (14 species) and Papilionidae (10 species) were amongst other common species found in the area. The range of *Cheritra freja* (Common Imperial) which was earlier recorded from Amboli, Sindhudurga, Maharashtra ( $15.9647^{\circ}\text{N}$ ,  $74.0036^{\circ}\text{E}$ ) (Saji and Ogale 2020) is extended further north around 345 km linear distance (calculated on <https://www.nhc.noaa.gov/gccalc.shtml>). The family Riodinidae was represented by only one species namely, *Abisara bifasciata* (Double Banded Judy).

Table 3.

List of butterflies of Matheran. Numeric codes of sites correspond to Table 1. Colour codes of season/s correspond to Table 2. VC- Very Common, C- Common, NC - Not Common, R - Rare, VR - Very Rare. Presence = 1; Absence = 0.

Common Name	Scientific Name	Season	Local Status	Study Sites							
				1	2	3	4	5	6	7	8
<b>Family: Hesperidae (N = 25)</b>											
Vindhyan Bob	<i>Ametta vindhiana</i>	All	VC	1	1	1	1	1	1	1	1
Brown Awl	<i>Badamia exclamationis</i>	All	C	1	1	1	1	1	1	1	1
Orange-Tailed Awlet	<i>Bibasis sena</i>	Monsoon	VR	0	0	1	0	1	1	0	0

Common Name	Scientific Name	Season	Local Status	Study Sites							
				1	2	3	4	5	6	7	8
Orange Awlet	<i>Burara jaina</i>	Monsoon	VR	0	1	1	0	0	1	0	0
Blank Swift	<i>Caltoris kumara</i>	Monsoon	VC	0	1	1	1	1	1	1	1
Golden Angle	<i>Caprona ransonnetii</i>	All	C	1	0	1	1	1	0	1	0
Malabar Flat	<i>Celaenorrhinus ambareesa</i>	All	VC	1	1	1	1	1	1	1	1
Common Spotted Flat	<i>Celaenorrhinus leucocera</i>	All	VC	1	1	1	1	1	1	1	1
Tamil Spotted Flat	<i>Celaenorrhinus ruficornis</i>	Monsoon	VR	0	0	0	1	0	1	0	0
Tricolor Pied Flat	<i>Coladenia indrani</i>	Monsoon+Winter	VC	1	0	1	1	1	1	1	1
Common Awl	<i>Hasora badra</i>	Winter	NC	0	1	1	1	1	1	0	0
Common Banded Awl	<i>Hasora chromus</i>	All	VC	0	1	1	1	1	1	1	1
Plain Banded Awl	<i>Hasora vitta</i>	Monsoon	VR	0	1	1	0	1	0	0	0
Chestnut Bob	<i>Iambrix salsala</i>	All	VC	1	1	1	1	1	1	1	1
Common Redeye	<i>Matapa aria</i>	Monsoon+Winter	R	1	0	1	1	0	1	0	0
Conjoined Swift	<i>Pelopidas conjuncta</i>	Monsoon	VC	1	1	1	1	0	1	1	1
Variable Swift	<i>Pelopidas mathias</i>	Monsoon+Winter	C	1	0	1	1	0	1	0	0
Common Small Flat	<i>Sarangesa dasahara</i>	All	VC	1	1	1	1	1	1	1	1
Spotted Small Flat	<i>Sarangesa purendra</i>	All	VC	1	1	1	1	1	1	1	1
Indian Skipper	<i>Spialia galba</i>	Monsoon	C	0	0	1	0	0	1	1	1
Indian Palm Bob	<i>Suastus gremius</i>	Winter	C	0	1	1	1	1	1	0	0
Black Angle	<i>Tapena thwaitesi</i>	Monsoon+Winter	C	1	0	1	1	1	1	1	0
Tamil Grass Dart	<i>Taractrocera ceramas</i>	Summer+Monsoon	VC	0	1	1	1	1	1	1	1
Dark Palm Dart	<i>Telicota bambusae</i>	All	C	1	1	1	1	1	1	1	1
Grass Demon	<i>Udaspes folus</i>	Monsoon+Winter	C	1	0	1	1	0	1	1	0
<b>Family: Lycaenidae (N = 46)</b>											
Common Hedge Blue	<i>Acytolepis puspa</i>	All	VC	1	1	1	1	1	1	0	1
Purple Leaf Blue	<i>Amblypodia anita</i>	Summer+Winter	C	1	1	1	1	1	0	0	0
Pointed Ciliate Blue	<i>Anthene lycaenina</i>	All	VC	1	1	1	1	1	0	0	1
Large Oakblue	<i>Arhopala amantes</i>	Winter	VR	1	0	0	1	1	0	0	0
Centaur Oakblue	<i>Arhopala centaurus</i>	Winter	VR	1	0	1	0	1	0	0	0
Angled Pierrot	<i>Caleta decidia</i>	All	VC	1	0	1	1	1	0	0	1
Common Pierrot	<i>Castalius rosimon</i>	All	VC	1	1	1	1	1	1	0	1
Forgetmenot	<i>Catochrysops strabo</i>	All	VC	1	1	1	1	1	1	1	1
Common Imperial	<i>Cheritra freja</i>	Monsoon+Winter	VR	0	0	0	1	1	1	1	0
Lime Blue	<i>Chilades lajus</i>	Summer+Winter	NC	1	0	0	1	1	0	0	0
Orchid Tit	<i>Chliaria othona</i>	Winter	VR	1	0	0	0	0	0	0	0
Angled Sunbeam	<i>Curetis dentata</i>	Summer+Winter	C	1	0	0	1	1	0	1	0

Common Name	Scientific Name	Season	Local Status	Study Sites							
				1	2	3	4	5	6	7	8
Indian Sunbeam	<i>Curetis thetis</i>	Monsoon+Winter	C	1	0	0	0	1	0	0	0
Cornelian	<i>Deudorix epijarbas</i>	All	C	0	0	1	1	1	1	1	1
Gram Blue	<i>Euchrysops cnejus</i>	Summer+Winter	C	1	1	1	1	1	0	0	0
Indian Cupid	<i>Everes lacturnus</i>	Summer+Winter	NC	1	0	1	1	0	0	0	0
Small Grass Jewel	<i>Freyeria putli</i>	Summer+Winter	C	0	1	1	1	0	0	1	1
Silverstreak Blue	<i>Iraota timoleon</i>	Summer+Winter	VC	1	0	1	1	1	1	0	0
Dark Cerulean	<i>Jamides bochus</i>	All	VC	1	1	1	1	1	1	1	1
Common Cerulean	<i>Jamides celeno</i>	All	VC	1	1	1	1	1	1	1	1
Peablue	<i>Lampides boeticus</i>	Winter	C	1	0	1	1	1	1	0	1
Zebra Blue	<i>Leptotes plinius</i>	Summer+Winter	C	1	0	1	1	1	0	1	1
Yamfly	<i>Loxura atymnus</i>	Monsoon+Winter	NC	0	1	1	1	1	1	0	0
Plains Cupid	<i>Luthrodes pandava</i>	Winter	C	1	1	0	1	1	0	0	0
Malayan	<i>Megisba malaya</i>	Winter	C	1	0	0	0	1	1	0	1
Opaque Six Lineblue	<i>Nacaduba beroe</i>	Summer+Winter	VC	1	0	0	0	1	1	0	0
Transparent Six Lineblue	<i>Nacaduba kurava</i>	Summer+Winter	VC	1	0	0	0	1	1	0	0
Dingy Lineblue	<i>Petrelaea dana</i>	Winter	C	1	0	0	0	1	0	0	0
Tailless Lineblue	<i>Prosotas dubiosa</i>	Summer+Winter	VC	1	1	1	1	1	1	0	1
Common Lineblue	<i>Prosotas nora</i>	Summer+Winter	VC	1	1	1	1	1	1	0	1
Common Red Flash	<i>Rapala iarbus</i>	Summer+Winter	C	1	1	1	1	1	0	0	1
Slate Flash	<i>Rapala manea</i>	Summer+Winter	VC	1	1	1	1	1	1	0	1
Indigo Flash	<i>Rapala varuna</i>	Summer+Winter	VC	1	0	0	0	1	0	0	0
Monkey Puzzle	<i>Rathinda amor</i>	All	VC	0	1	1	1	1	1	1	0
Common Apefly	<i>Spalgis epius</i>	Winter	VR	1	0	0	0	0	0	0	0
Long Banded Silverline	<i>Spindasis lohita</i>	Winter	NC	1	0	1	1	1	0	0	0
Common Silverline	<i>Spindasis vulcanus</i>	Summer	VR	0	1	1	1	1	0	0	0
Common Acacia Blue	<i>Surendra quercetorum</i>	Monsoon	NC	0	0	1	1	1	1	1	0
Peacock Royal	<i>Tajuria cippus</i>	Winter	C	1	0	0	0	1	1	0	0
Red Pierrot	<i>Talicauda nyseus</i>	Summer+Winter	C	0	0	1	1	1	0	0	1
Dark Pierrot	<i>Tarucus ananda</i>	Winter	VR	0	0	0	0	1	0	0	0
Common Guava Blue	<i>Virachola isocrates</i>	All	C	1	0	0	0	1	1	0	0
Large Guava Blue	<i>Virachola perse</i>	All	VC	1	0	0	0	1	1	0	0
Dark Grass Blue	<i>Zizeeria karsandra</i>	All	VC	1	1	1	1	1	1	1	1
Lesser Grass Blue	<i>Zizina otis</i>	All	VC	1	1	1	1	1	1	1	1
Tiny Grass Blue	<i>Zizula hylax</i>	Summer+Winter	VC	1	1	1	1	1	1	1	1

**Family: Nymphalidae (N = 44)**





Common Name	Scientific Name	Season	Local Status	Study Sites							
				1	2	3	4	5	6	7	8
<b>Family: Riodinidae (N = 1)</b>											
Double Banded Judy	<i>Abisara bifasciata</i>	Monsoon+Winter	C	1	0	1	1	1	1	0	0

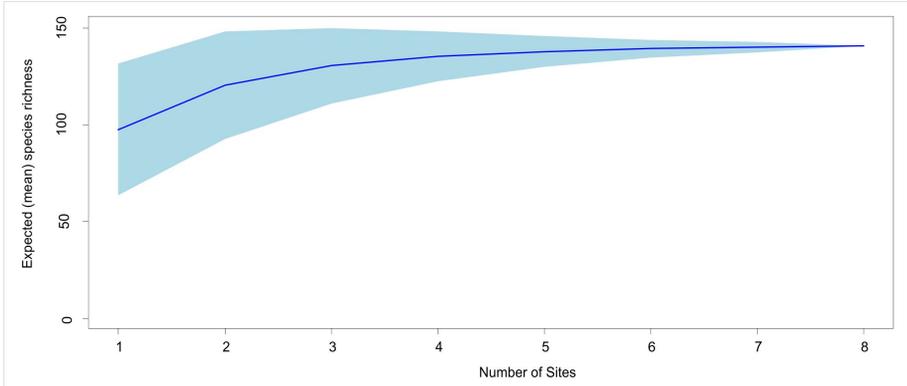


Figure 2. [doi](#)

Species Accumulation Curve (SAC) with asymptote model. Dark blue line indicates the expected (mean) species richness; shaded area denotes the standard deviation (Asym = 146.42, xmid = 0.58, slope = 3.60).

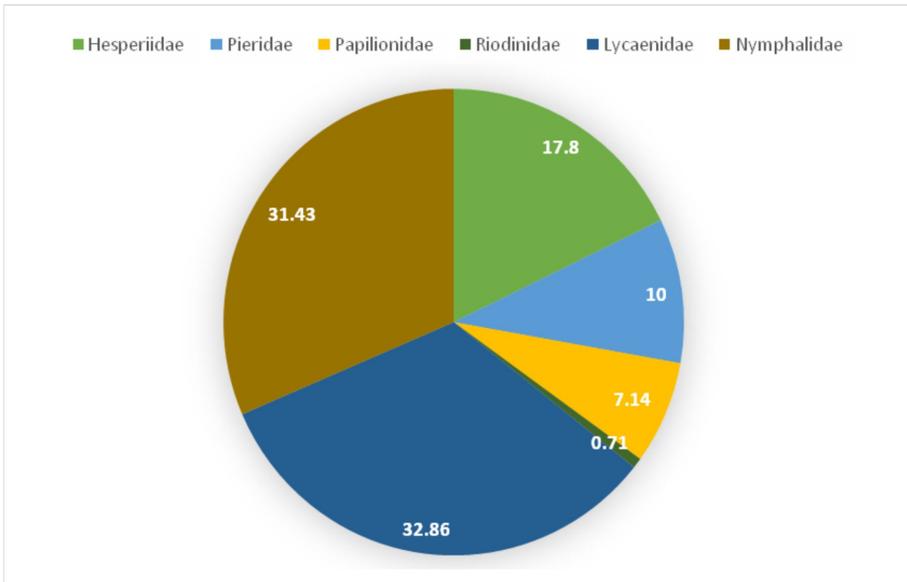


Figure 3. [doi](#)

Family-wise species composition pie of butterflies of Matheran.

### Seasonal turnover

The maximum numbers of species (N = 125) were recorded during winter, while minimum numbers of species (N = 80) were recorded during the monsoon (Fig. 4). Maximum numbers of species for all the families were recorded during winter, except the family Hesperidae for which the maximum numbers of species (N = 23) were recorded during the monsoon (Fig. 5). The species of the family Lycaenidae dominated the local butterfly species richness during the months of summer and winter with 36.05% (N = 31) and 34.40% (N = 43) of total species of butterflies recorded during respective seasons (Fig. 6). Members of the family Nymphalidae shared fairly equal percentages during all seasons. The percentage of the papilionids was the lowest during all seasons.

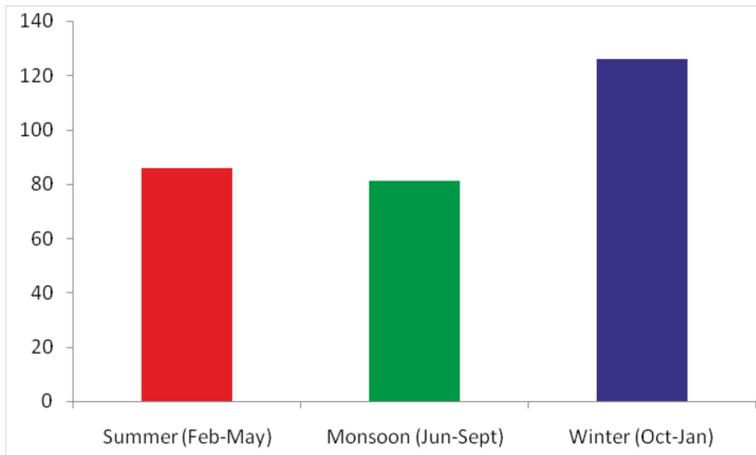


Figure 4. [doi](#)  
Seasonal variations in species richness.

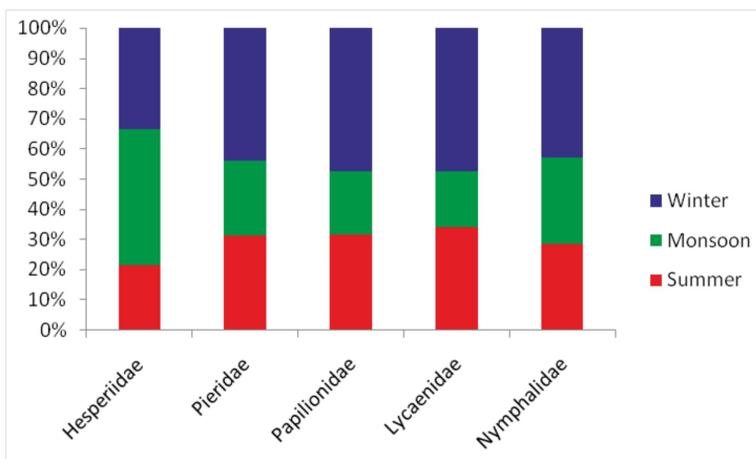
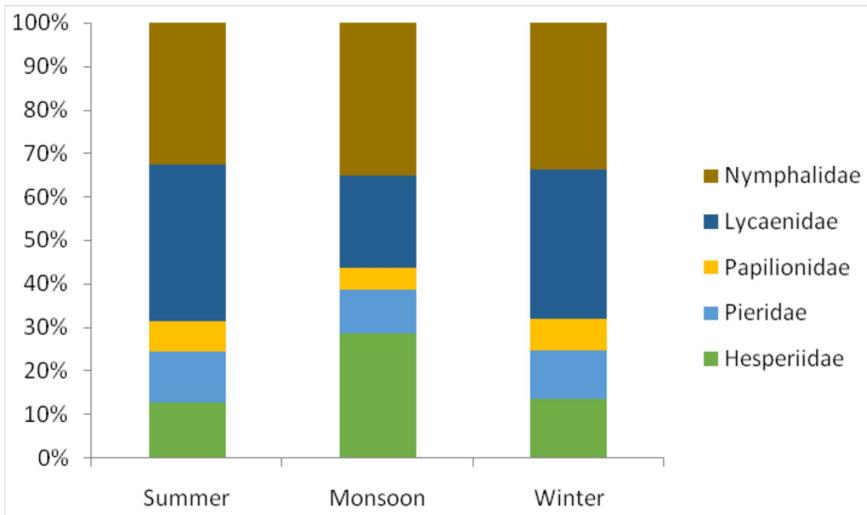


Figure 5. [doi](#)  
Family-wise percent species richness per season.

Figure 6. [doi](#)

Season-wise percent species richness per family.

## Spatial turnover

Members of the family Nymphalidae and Lycaenidae dominated the species diversity at all the sites studied in and around Matheran. Members of the family Lycaenidae were particularly present in higher numbers at Charlotte Lake while those of Hesperidae were particularly present in higher numbers at Garbett Point (Fig. 7). The Similarity-Richness difference-Species replacement simplex for all the families indicated high similarity, although with different patterns tending towards perfect nestedness (Fig. 8a-e, Suppl. material 1). Similarity was the highest for the family Nymphalidae (70.58%) with 78.22% of relativised strict nestedness (nestedness without considering the effect of species replacement) and lowest relativised beta diversity of 29.42%. Relativised strict nestedness was the highest (85.67%) for the family Hesperidae with a similarity of 65.91% and beta diversity of 34.10%, while relativised nestedness (nestedness considering the effect of species replacement) was the highest (93.56%) for the family Pieridae. Similarity of species composition between the sites was the lowest (49.10%) for the family Lycaenidae with the highest relativised richness difference (31.99%) indicating more site specific species composition for the members of the family Lycaenidae, unlike the members of other families.

## Activity of butterflies

No seasonal activity pattern could be observed (Table 3, Table 4). Most of the species were observed while mud puddling, basking or feeding on the nectar. Other common activities included feeding on bird droppings, tree sap, animal waste (other than that of birds) and/or animal carcasses.

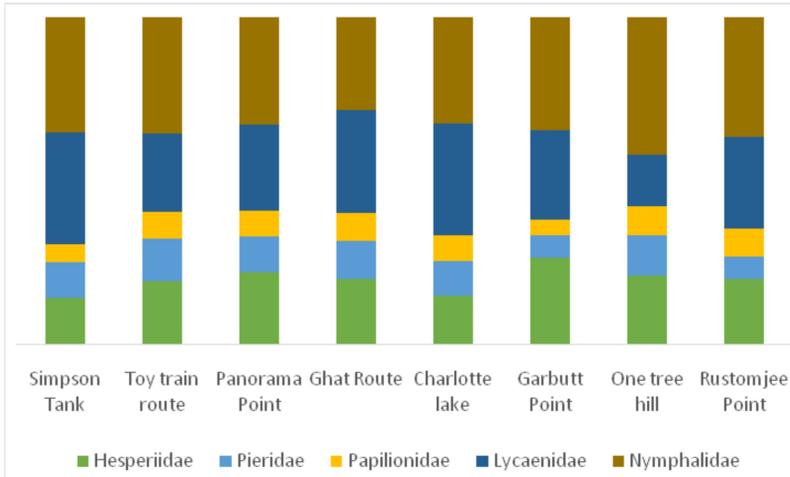


Figure 7. [doi](#)

Site-wise percent species richness for each family

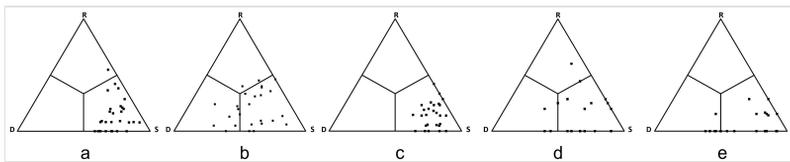


Figure 8. [doi](#)

Similarity-Richness difference-Species replacement simplex plot for a. Hesperiiidae; b. Lycaenidae; c. Nymphalidae; d. Papilionidae; e. Pieridae. S - Species Shared (Similarity); D - Richness difference; R - Species replacement. Squares indicate true simplex scores for each pairs of sites (N = 28 for 8 sites).

Table 4.

Activity chart for butterflies of Matheran observed during the survey. Colour codes correspond to Table 2.

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<b>Family Hesperiiidae</b>								
<i>Arnetta vindhiana</i>	+	+	+					+
<i>Badamia exclamationis</i>	+		+		+			

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<i>Bibasis sena</i>			+					
<i>Burara jaina</i>	+		+					+
<i>Caltores kumara</i>			+					
<i>Caprona ransonnetti</i>	+	+	+					+
<i>Celaenorrhinus ambareesa</i>	+	+	+					+
<i>Celaenorrhinus leucocera</i>		+	+					
<i>Celaenorrhinus ruficornis</i>		+	+					
<i>Coladenia indrani</i>	+	+	+					+
<i>Hasora badra</i>			+		+			
<i>Hasora chromus</i>	+		+					+
<i>Hasora vitta</i>			+					
<i>Iambrix salsala</i>		+	+					
<i>Matapa aria</i>	+		+					
<i>Pelopidas conjuncta</i>	+	+	+					
<i>Pelopidas mathias</i>	+	+	+					
<i>Sarangesa dasahara</i>	+	+	+					+
<i>Sarangesa purendra</i>	+	+	+					+
<i>Spialia galba</i>		+	+					
<i>Suastus gremius</i>			+					
<i>Tapena thwaitesi</i>	+	+	+		+	+		+
<i>Taractrocera ceramas</i>		+	+					

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<i>Telicota bambusae</i>	+	+	+					
<i>Udaspes folus</i>	+	+	+		+			+
<b>Family Lycaenidae</b>								
<i>Acytolepis puspa</i>	+	+	+		+	+		+
<i>Amblypodia anita</i>	+	+	+		+	+		
<i>Anthene lycaenina</i>	+	+	+					
<i>Arhopala amantes</i>	+	+						+
<i>Arhopala centaurus</i>	+	+						+
<i>Caleta decidia</i>	+		+	+	+	+		
<i>Castalius rosimon</i>	+	+	+	+	+	+		
<i>Catochrysops strabo</i>	+	+	+		+	+		+
<i>Cheritra freja</i>		+	+					
<i>Chilades lajus</i>	+	+	+					
<i>Chliaria othona</i>	+	+	+					
<i>Curetis dentata</i>	+	+						+
<i>Curetis thetis</i>	+	+						+
<i>Deudorix epijarbas</i>	+	+	+					+
<i>Euchrysops cnejus</i>	+		+					
<i>Everes lacturnus</i>	+		+			+		
<i>Freyeria putli</i>	+	+	+					
<i>Iraota timoleon</i>	+	+	+		+	+		+
<i>Jamides bochus</i>	+		+			+		

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<i>Jamides celeno</i>	+		+			+		
<i>Lampides boeticus</i>	+	+	+		+	+		+
<i>Leptotes plinius</i>	+	+	+			+		+
<i>Loxura atymnus</i>		+						+
<i>Luthrodes pandava</i>	+		+					
<i>Megisba malaya</i>	+		+			+		
<i>Nacaduba beroe</i>	+					+		
<i>Nacaduba kurava</i>	+					+		
<i>Petrelaea dana</i>	+					+		
<i>Prosotas dubiosa</i>	+		+			+		
<i>Prosotas nora</i>	+		+			+		
<i>Rapala iarbus</i>	+	+	+					
<i>Rapala manea</i>	+	+						
<i>Rapala varuna</i>	+	+			+			
<i>Rathinda amor</i>		+	+					+
<i>Spalgis epius</i>	+							
<i>Spindasis lohita</i>	+	+	+		+			
<i>Spindasis vulcanus</i>	+	+	+		+			
<i>Surendra quercetorum</i>	+		+		+			
<i>Tajuria cippus</i>	+	+			+	+		
<i>Talicauda nyseus</i>	+	+	+					
<i>Tarucus ananda</i>	+							
<i>Virachola isocrates</i>	+	+	+		+	+		+

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<i>Virachola perse</i>	+	+	+		+	+		+
<i>Zizeeria karsandra</i>	+	+	+			+		
<i>Zizina otis</i>	+	+	+			+		
<i>Zizula hylax</i>	+	+	+			+		
<b>Family Nymphalidae</b>								
<i>Ariadne ariadne</i>	+	+	+					
<i>Ariadne merione</i>	+	+	+					
<i>Athyma inara</i>	+	+						
<i>Athyma perius</i>	+	+						
<i>Charaxes psaphon</i>	+	+		+	+	+		+
<i>Charaxes solon</i>	+	+		+	+	+		+
<i>Cupha erymanthis</i>	+	+	+					
<i>Cyrestis thyodamas</i>	+	+			+			
<i>Danaus chrysippus</i>	+	+	+					
<i>Danaus genutia</i>	+	+	+					
<i>Euploea core</i>	+	+	+					
<i>Euploea klugii</i>	+	+	+					
<i>Euploea sylvester</i>	+	+	+					
<i>Euthalia aconthea</i>	+	+	+	+	+	+		+
<i>Euthalia lubentina</i>	+	+	+	+	+	+		+
<i>Hypolimnas bolina</i>	+	+	+		+	+		+

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<i>Hypolimnas misippus</i>	+	+	+		+	+		+
<i>Junonia almana</i>	+	+	+					+
<i>Junonia atlites</i>	+	+	+					
<i>Junonia iphita</i>	+	+	+		+			+
<i>Junonia lemonias</i>	+	+	+					+
<i>Kallima horsfieldii</i>	+	+		+	+	+		+
<i>Lethe europa</i>					+	+		+
<i>Lethe rohria</i>					+	+		+
<i>Libythea myrrha</i>	+	+						
<i>Melanitis leda</i>					+	+		+
<i>Moduza procris</i>	+	+	+		+	+		+
<i>Mycalesis mineus</i>	+	+	+		+			+
<i>Mycalesis perseus</i>	+	+	+		+			+
<i>Mycalesis visala</i>	+	+	+		+			+
<i>Neptis hylas</i>	+	+	+		+			+
<i>Neptis jumbah</i>	+	+	+		+			+
<i>Parantica aglea</i>	+	+	+					
<i>Phaedyma columella</i>	+	+	+		+			+
<i>Phalanta phalantha</i>	+	+	+		+	+		+
<i>Polyura bharata</i>	+	+		+	+	+		+
<i>Rohana parisatis</i>	+	+				+		+
<i>Symphaedra nais</i>	+	+	+		+			+

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<i>Tanaecia lepidea</i>	+	+		+	+	+		+
<i>Tirumala limniace</i>	+	+	+					
<i>Tirumala septentrionis</i>	+	+	+					
<i>Vanessa cardui</i>	+	+	+					+
<i>Ypthima baldus</i>		+	+					+
<i>Ypthima huebneri</i>		+	+					+
<b>Family Papilionidae</b>								
<i>Graphium agamemnon</i>	+	+	+		+	+		
<i>Graphium doson</i>	+		+		+	+		
<i>Graphium teredon</i>	+		+		+	+		
<i>Pachliopta aristolochiae</i>		+	+					
<i>Pachliopta hector</i>		+	+					
<i>Papilio clytia</i>	+		+					
<i>Papilio demoleus</i>	+	+	+					
<i>Papilio helenus</i>	+	+	+					
<i>Papilio polymnestor</i>	+	+	+					
<i>Papilio polytes</i>	+	+	+					
<b>Family Pieridae</b>								
<i>Appias albina</i>	+	+	+					
<i>Appias indra</i>	+	+	+					
<i>Appias libythea</i>	+	+	+					
<i>Catopsilia pomona</i>	+		+					

Scientific Name	Mud Puddling	Basking	Feeding					
			Nectaring	Tree Sap	Carcass	Animal Waste (other than that of birds)	Bird Droppings	Rotten Fruits
<i>Catopsilia pyranthe</i>	+		+					
<i>Cepora nerissa</i>	+	+	+					
<i>Delias eucharis</i>		+	+					
<i>Eurema hecabe</i>			+			+		
<i>Eurema laeta</i>			+			+		
<i>Hebomoia glaucippe</i>	+	+	+					
<i>Ixias marianne</i>		+	+					
<i>Ixias pyrene</i>		+	+					
<i>Leptosia nina</i>			+					
<i>Pareronia hippia</i>	+	+	+					
<b>Family Riodinidae</b>								
<i>Abisara bifasciata</i>	+	+						

### Locally rare and scheduled species

Our list contains 15 such species which are scheduled under the Wildlife (Protection) Act, 1972 of India (Table 5). Out of these, seven species were found rarely during the survey. Additionally, 20 species, which are not scheduled under the act, were observed rarely or very rarely during the survey (Figs 9, 10, 11, 12, 13)

Table 5.

List of scheduled species under the Wildlife (Protection) Act, 1972, India.

S r. No.	Common Name	Scientific Name	Schedule (Part)
1	Orange-tailed awlet	<i>Bibasis sena</i>	2 (2)
2	Plain Banded Awl	<i>Hasora vitta</i>	4
3	Striped Albatross	<i>Appias libythea</i>	4
4	Plain Puffin	<i>Appias indra</i>	2 (2)
5	Crimson Rose	<i>Pachliopta hector</i>	1 (4)
6	Long Banded Silverline	<i>Spindasis lohita</i>	2 (2)

S r. No.	Common Name	Scientific Name	Schedule (Part)
7	Dark Pierrot	<i>Tarucus ananda</i>	4
8	Gram Blue	<i>Euchrysops cnejus</i>	2 (2)
9	Lime blue	<i>Chilades lajus</i>	2
10	Peacock Royal	<i>Tajuria cippus</i>	2 (2)
11	Orchid Tit	<i>Chliaria othona</i>	1 (4)
12	Indigo Flash	<i>Rapala varuna</i>	2 (2)
13	Gaudy Baron	<i>Euthalia lubentina</i>	4
14	Grey Count	<i>Tanaecia lepidea</i>	2 (2)
15	Danaid Eggfly	<i>Hypolimnas misippus</i>	1

### Identification remarks for locally rare or very rare butterflies

Abbreviations: FW-Forewing, HW-Hindwing, UN-Underside, UNF-Underside of Forewing, UNH-Underside of Hindwing, UP- Upperside, UPF-Upperside of Forewing, UPH-Upperside of Hindwing

### Family Hesperiiidae Latreille, 1809

#### Genus *Bibasis* Moore, 1881

*Bibasis sena* (Moore, 1865) (Fig. 9a).

Common name: Orange-tailed awlet.

Identification remarks: Bright orange fringe on HW and on the tip of the abdomen. Broad, pure white, outwardly diffused, central band on UN. Wingspan 42–50 mm.

Season: Monsoon.

Habitat and activity: The species was observed in forested patches while nectaring.

#### Genus *Burara* Swinhoe, 1893

*Burara jaina* (Moore, 1865) (Fig. 9b).

Common name: Orange awlet.

Identification remarks: UN pale brown. UNH with orange stripes along veins and has orange fringe. UNF purplish. Wingspan 60–70 mm.

Season: Monsoon.

Habitat and activity: The species was observed in forested patches while nectaring.

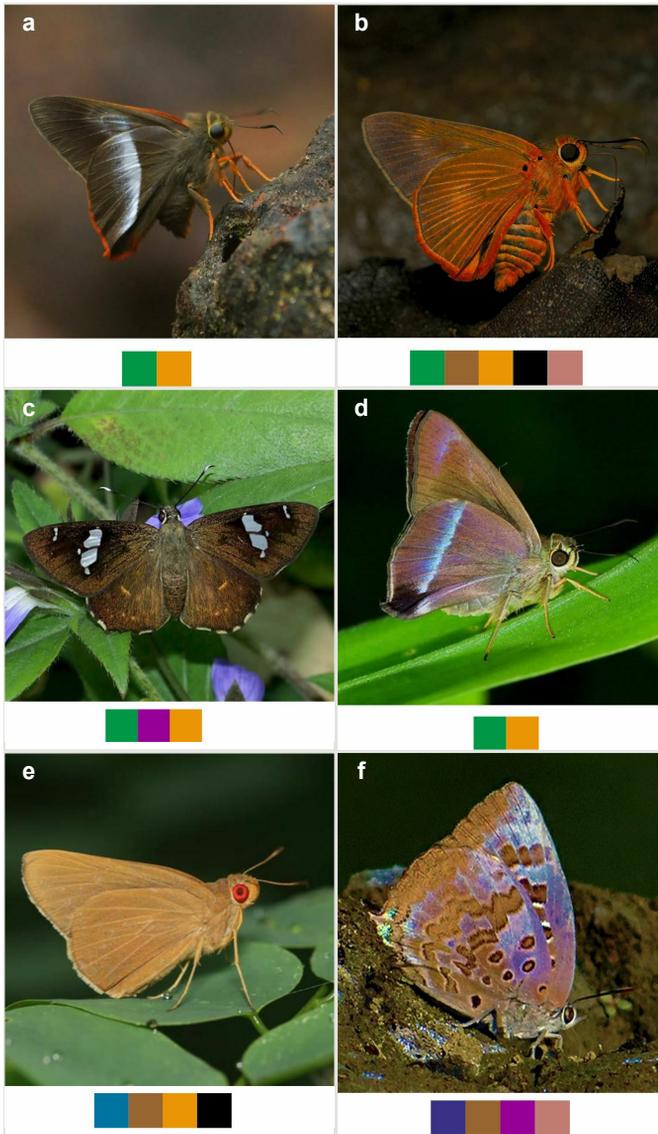


Figure 9.

Family HesperIIDae (a-e) and Family LycaenIDae (f). Colour barcodes depict season and activity of the species. Colour codes correspond to Table 2. Photo Credits: Gargi Geedh (a); Mandar Sawant & Sagar Sarang (b-f).

- a: *Bibasis sena* [doi](#)  
 b: *Burara jaina* [doi](#)  
 c: *Celenorrhinus ruficornis* [doi](#)  
 d: *Hasora vitta* (inverted image) [doi](#)  
 e: *Matapa aria* [doi](#)  
 f: *Arhopala amantes* [doi](#)

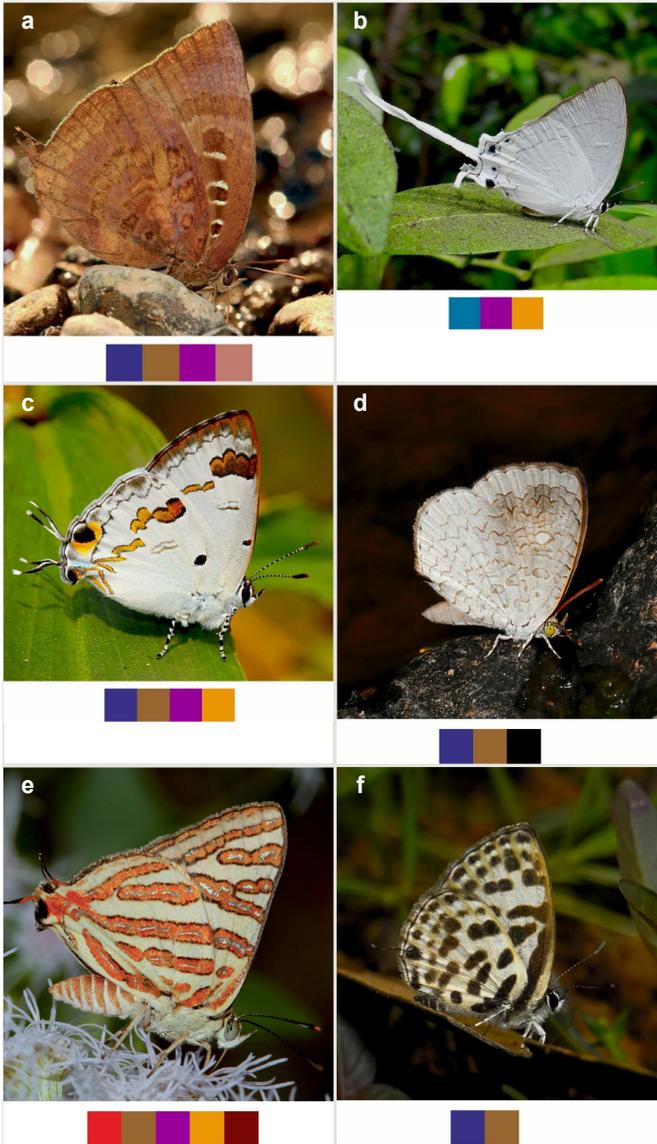


Figure 10.

Family Lycaenidae. Colour barcodes depict season and activity of the species. Colour codes correspond to Table 2. Photo Credits: Mandar Sawant & Sagar Sarang.

a: *Arhopala centaurus* [doi](#)

b: *Cheritra freja* [doi](#)

c: *Chliaria othona* [doi](#)

d: *Spalgis epius* [doi](#)

e: *Spindasis vulcanus* [doi](#)

f: *Tarucus ananda* [doi](#)

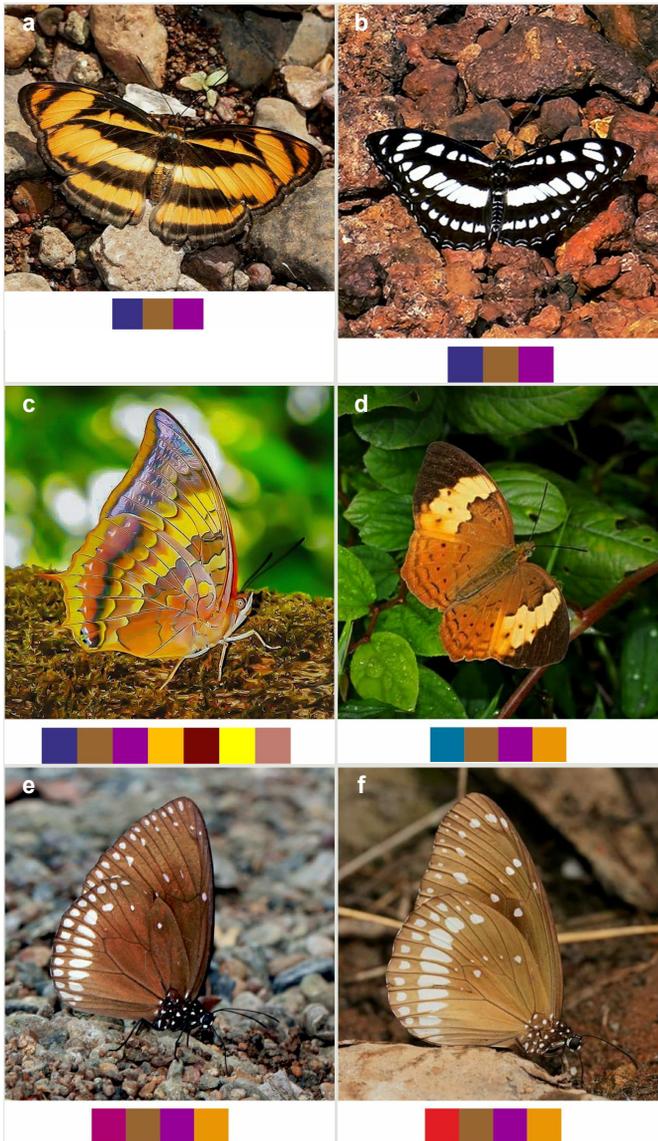


Figure 11.

Family Nymphalidae. Colour barcodes depict season and activity of the species. Colour codes correspond to Table 2. Photo credits: Mandar Sawant & Sagar Sarang.

a: *Athyma inara* [doi](#)

b: *Athyma perius* [doi](#)

c: *Charaxes psaphon* [doi](#)

d: *Cupha erymanthis* [doi](#)

e: *Euploea klugii* [doi](#)

f: *Euploea sylvester* [doi](#)



Figure 12.

Family Nymphalidae. Colour barcodes depict season and activity of the species. Colour codes correspond to Table 2. Photo credits: Mandar Sawant & Sagar Sarang.

a: *Polyura bharata* [doi](#)

b: *Tanaecia lepidea* [doi](#)

c: *Tirumala septentrionis* [doi](#)

### Genus *Celaenorrhinus* Hübner, 1819

*Celaenorrhinus ruficornis* Hampson, 1889 (Fig. 9c).

Common name: Tamil spotted flat.

Identification remarks: Similar to common spotted flat, but UPF has semi-transparent white spots separated from each other. Markings on UPH indistinct or absent. Antennae chequered, club white in male, white at base only in female. Wingspan 45–50 mm.

Season: Monsoon.

Habitat and activity: The species was observed in forested patches while nectaring.

### Genus *Hasora* Moore, 1881

*Hasora vitta* (Butler, 1870) (Fig. 9d).

Common name: Plain banded awl.

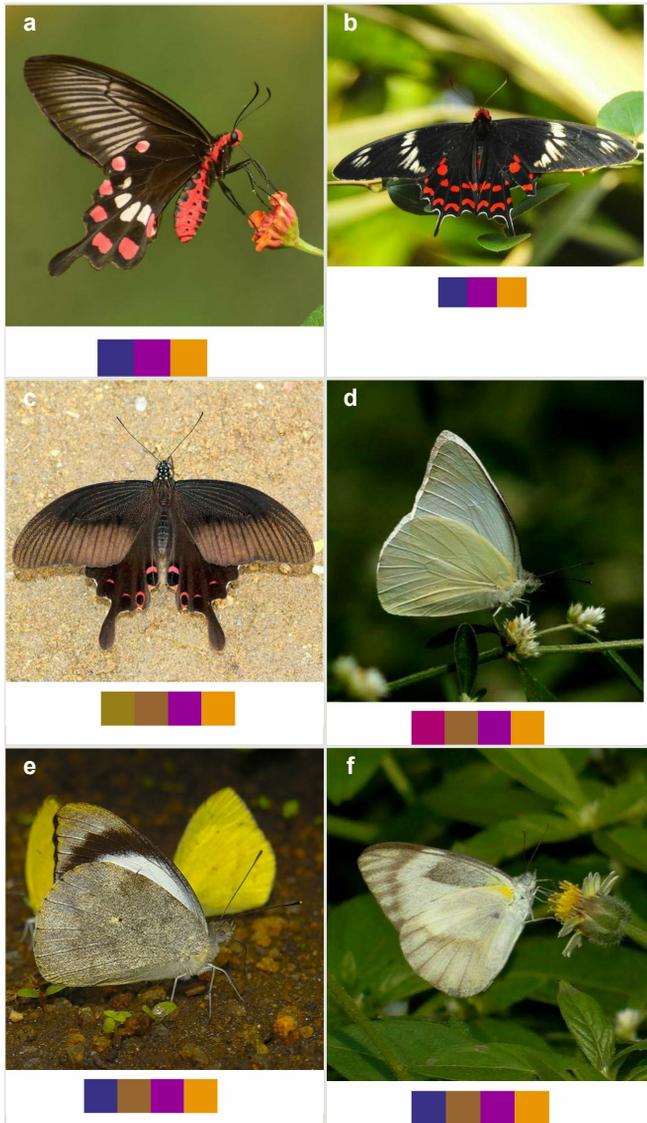


Figure 13.

Family Papilionidae (a-c) and Family Pieridae (d-f); (a) *Pachliopta aristolochiae* (Photo credit: Tejas Mehendale); (b) *Pachliopta hector* (Photo credit: Abhinav Nair); (c) *Papilio helenus*; (d) *Appias albina*; (e) *Appias indra*; (f) *Appias libythea*. Colour barcodes depict season and activity of the species. Colour codes correspond to Table 2. Photo Credits: Tejas Mehendale (a); Abhinav Nair (b); Mandar Sawant & Sagar Sarang (c-f).

**a:** *Pachliopta aristolochiae* [doi](#)

**b:** *Pachliopta hector* [doi](#)

**c:** *Papilio helenus* [doi](#)

**d:** *Appias albina* [doi](#)

**e:** *Appias indra* [doi](#)

**f:** *Appias libythea* [doi](#)

Identification remarks: Outwardly diffused broad white or bluish-white band on UNH. Female has an additional spot on UPF. UN paler, inner half has greenish gloss. Wingspan 45–55 mm.

Season: Monsoon.

Habitat and activity: The species was observed in forested patches while nectaring.

### **Genus *Matapa* Moore, 1881**

*Matapa aria* (Moore, 1865) (Fig. 9e).

Common name: Common Redeye.

Identification remarks: Dark buff-brown with no markings on UP. HW has greyish fringe tinged with pale yellow. UN more yellowish orange-brown. Indistinct black band on UPF of male. Wingspan 40–55 mm.

Season: Monsoon and winter.

Habitat and activity: The species was observed in forested patches while nectaring.

### **Family Lycaenidae Leach, 1815**

#### **Genus *Arhopala* Boisduval, 1832**

*Arhopala amantes* (Hewitson, 1862) (Fig. 9f).

Common name: Large oakblue.

Identification remarks: Tailed with lobe. UNH has central squarish spots in spaces 4 and 5 at right angles. Metallic scales at UNH lower tip. Wingspan 45–57 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while mud puddling, basking or feeding on rotten fruits.

*Arhopala centaurus* (Fabricius, 1775) (Fig. 10a).

Common name: Centaur oakblue.

Identification remarks: HW tailed. No HW lobe. Metallic scaling on UNH faint or absent. UNF band continuous and curved. UNF cell spots outlined by silver lines. Male UP brilliant violet-blue, narrow dark borders. Females UP paler blue, broad wing borders. Wingspan 53–62 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while mud puddling, basking or feeding on rotten fruits.

**Genus *Cheritra* Moore, 1881**

*Cheritra freja* (Fabricius 1793) (Fig. 10b).

Common name: Common Imperial.

Identification remarks: Two tails. UN of both sexes white to pale brown; faint bars at cell-ends. Narrow dark outer central line on UNF. UNH with outer central and marginal lines and black spots crowned with metallic scales at lower tip. Wingspan 38–42 mm.

Season: Monsoon and winter.

Habitat and activity: The species was observed in forested patches while basking or nectaring.

**Genus *Chliaria* Moore, 1884**

*Chliaria othona* (Hewitson, 1865) (Fig. 10c).

Common name: Orchid Tit.

Identification remarks: Two tails. UN white, faint cell-end bars, black-edged brown markings. UNF band upper part wider than the lower part. UNH central band broken twice; prominent black spot near base. Wingspan 24–27 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while mud puddling, basking or nectaring.

**Genus *Spalgis* Moore, 1879**

*Spalgis epius* (Westwood, 1851).

Common name: Apefly (Fig. 10d).

Identification remarks: HW Tailless. UN with several fine wavy vertical lines. Male FW has acute apex and straight outer edge. Female has rounded outer edge. Caterpillars feed on mealy bugs. Wingspan 20–30 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while feeding on bird droppings.

**Genus *Spindasis* Donzel, 1847**

*Spindasis vulcanus* (Fabricius, 1775) (Fig. 10e).

Common name: Common silverline.

Identification remarks: Two tails, one lobe on HW. UN light yellow, black or brown bordered brilliant reddish bands with central silver lines. Separate spots at base of UNH and outer basal band of spots does not extend downwards to first costal vein. Orange-crowned black spot on UNH lobe. Female larger than male and with more rounded FW. Wingspan 26–34 mm.

Season: Summer.

Habitat and activity: The species was observed in plains and undulating terrains while either mud puddling, basking, nectaring or feeding on carcass.

**Genus *Tarucus* Moore, 1881**

*Tarucus ananda* (de Nicéville, 1884) (Fig. 10f).

Common name: Dark Pierrot.

Identification remarks: HW Tailed. Resembles Assam Pierrot, differs in having the central spot in space 5 joined to the band of spots near margin on UN. Wingspan 22–28 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while mud puddling.

**Family Nymphalidae Rafinesque, 1815****Genus *Athyma* Westwood, 1850**

*Athyma inara* Westwood, 1850 (Fig. 11a).

Common name: Colour sergeant.

Identification remarks: UP dark brown with very broad orange bands. In male, UP velvety black with a white band and orange markings. UPF white band continues on UPH. Orange markings on UPF apex. UPH with orange band near outer edge. Wingspan 55–70 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while mud puddling or basking.

*Athyma perius* (Linnaeus, 1758) (Fig. 11b)

Common name: Common sergeant.

Identification remarks: A prominent row of black spots always towards the inner edge of the white band on both sides of HW. UPF white cell streak divided into four parts. Wingspan 60–70 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while mud puddling or basking.

### **Genus *Charaxes* Ochseneimer, 1816**

*Charaxes psaphon* Westwood, 1847 (Fig. 11c).

Common name: Plain Tawny Rajah.

Identification remarks: Male UN tawny with purple gloss. UPF tawny, broad black terminal border. UPH black terminal broad near apex. Female UN tawny with broad pale central band. UPH tawny with broad black terminal border and central white band. Wingspan 85–110 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while mud puddling or basking, feeding on nectar, animal waste or carcasses.

### **Genus *Cupha* Billberg, 1820**

*Cupha erymanthis* (Drury, 1773) (Fig. 11d).

Common name: Rustic.

Identification remarks: Basal area of UPF reddish-brown, a broad yellow or white central band and broad black apex. Two darker marginal lines of crescents on UPH. Sexes similar. Wingspan 50–60 mm.

Season: Monsoon and winter.

Habitat and activity: The species was observed in forested patches while mud puddling, basking or nectaring.

### **Genus *Euploea* Fabricius, 1807**

*Euploea klugii* Moore, 1858 (Fig. 11e).

Common name: Brown king crow.

Identification remarks: Similar to Common Crow, but UN of either wing has no spots. All wings bordered with series of marginal and sub-marginal white spots. Male has a short, oval, dark band on UPF. UPH has greyish scales on apical half and pale-yellow scent scales patch. Wingspan 85–100 mm.

Season: Summer and winter.

Habitat and activity: The species was observed in forested patches while mud puddling, basking or nectaring.

*Euploea sylvester* (Fabricius, 1793) (Fig. 11f).

Common name: Double branded crow.

Identification remarks: Similar to Common Crow, but male has two parallel brands on UPF; female has two similar faint streaks near inner edge on UPF. Wingspan 95–105 mm.

Season: Summer.

Habitat: The species was observed in forested patches while mud puddling, basking or nectaring.

### **Genus *Polyura***

*Polyura bharata* Drury, 1773.

Common name: Cryptic Nawab (Fig. 12a).

Identification remarks: Pale greenish-yellow, wide central band on both sides. Large pale green spot near FW apex on both sides. Wingspan 60–75 mm.

Season: Winter.

Habitat: The species was observed in forested patches while mud puddling or basking, feeding on tree sap, animal waste or carcasses.

### **Genus *Tanaecia* Butler, 1869**

*Tanaecia lepidea* (Butler, 1868) (Fig. 12b).

Common name: Grey Count.

Identification remarks: UP dark brown with pale grey border. Border broad on HW and narrow on FW, ending before apex. FW apex produced and outer edge incurved. Female, larger and duller coloured than male, with extra pale brown markings. Wingspan 65–85 mm.

Season: Monsoon and winter.

Habitat and activity: The species was found at forest edges while mud puddling or basking or feeding on tree sap, carcasses, animal waste, bird droppings or rotten fruits.

### **Genus *Tirumala* Moore, 1880**

*Tirumala septentrionis* (Butler, 1874) (Fig. 12c).

Common name: Dark Blue Tiger.

Identification remarks: Similar to Blue Tiger, but markings narrower and darker. UNH has a long V-shaped pale blue marking in the cell. UN darker than Blue Tiger. Male UNH has scent scales pouch. Wingspan 75–95 mm.

Season: Summer and winter.

Habitat and activity: The species was observed in forested patches while mud puddling, basking or nectaring.

## Family Papilionidae Latreille, 1802

### Genus *Pachliopta* Reakirt, 1865

*Pachliopta aristolochiae* (Fabricius, 1775) (Fig. 13a).

Common name: Common Rose.

Identification remarks: HW tailed. UNF black with pale greyish stripes between veins. UNH has large white patch of five elongate spots around end-cell, series of bright red or brownish-red spots on outer edge. Body red. Wingspan 80–110 mm.

Season: Winter.

Habitat and activity: The species was observed at forests edges, scrubs and in grasslands while nectaring.

*Pachliopta hector* (Linnaeus, 1758) (Fig. 13b).

Common name: Crimson rose.

Identification remarks: HW tailed. Markings on both sides similar. Body bright crimson. Female duller, with larger crimson crescents and spots on HW. Wingspan 90–110 mm.

Season: Winter.

Habitat and activity: The species was observed at forests edges, scrubs and in grasslands while nectaring.

### Genus *Papilio* Linnaeus, 1758

*Papilio helenus* Linnaeus, 1758 (Fig. 13c).

Common name: Red Helen.

Identification remarks: UPH with patch of three creamy white spots. UPH may have marginal series of indistinct red crescents. Wingspan 110–130 mm.

Season: Summer and monsoon.

Habitat and activity: The species was observed in forested patches while nectaring.

## Family Pieridae Swainson, 1820

### Genus *Appias* Hübner, 1819

*Appias albina* (Boisduval, 1836) (Fig. 13d).

Common name: Common Albatross.

Identification remarks: Male UPF with dark dusting in apical area and along outer edge, but may be absent. No dark spot on UPF. Pale dull yellow UNH unmarked. Seasonal variation seen in both sexes. In female, UPF apex, leading edge and outer edge bordered with black with four to five white spots near apex. No cell spot. UPH has toothed black border. Wingspan 60–75 mm.

Season: Monsoon and winter.

Habitat and activity: The species was observed in forested patches while nectaring.

*Appias indra* Moore, 1857 (Fig. 13e)

Common name: Plain Puffin

Identification remarks: Male UPF white with apical, outer and leading (half) edges black with two to five apical white spots. Males of northern population have complete row of four or five apical spots on UPF. UPF has black area along outer edge which extends inwards. In female, UPF black, with central white patch and two white spots at apex. UPH with black outer half and dusky grey or white basal half. UNF with broad dark band from leading edge to outer edge. UNH variable. Wingspan 60–70 mm.

Season: Winter.

Habitat and activity: The species was observed in forested patches while nectaring.

*Appias libythea* Fabricius, 1775 (Fig. 13f).

Common name: Striped Albatross.

Identification remarks: Female DSF white, UPF apex and outer edge broadly black and unspotted, leading edge broadly blackened from base to bar at end-cell. UPH with black spots along outer edge. Female WSF much darker, UN white with diffused greyish-brown markings.

Season: Winter.

Habitat and activity: The species was observed at forests edges, scrubs and in grasslands while nectaring.

## Discussion

### Species Richness

Betham (1894) had hoped that someone from Bombay (= Mumbai) would add to his list of 78 butterflies, quoting the fact that there must be many species which still could be obtained from Matheran. It is our honour to fulfil his wish and almost double the list of available butterflies at Matheran 125 years after his publication. Sixty three species of those recorded by us are common to the checklists of Smith (1882), Betham (1894) and Padhye et al. (2013) (Table 6). All the other 77 species are recorded for the first time from the region. Fifteen species recorded by Smith (1882) and three species recorded by Betham (1894) were not recorded during this study (Table 6). Seventeen species were recorded by Smith (1882) and us, but not by Betham (1894), while the same numbers of species were recorded by Betham (1894) and us, but not by Smith (1882). Our list contains all the species recorded by Padhye et al. (2013). Five specific names from Smith (1882) and Betham (1894) could not be traced and are mentioned as 'Not Found' in Table 6.

Table 6.

List of the butterfly species of Matheran common between Smith (1882), Betham (1894), Padhye et al. (2013) and the current study.

Accepted Name	Smith (1882)	Betham (1894)	Padhye et al. (2013)	Our list	Remarks
<i>Abisara echerius</i>	–	<i>Abisara suffusa</i>	–	–	
<i>Acytolepis puspa</i>	–	<i>Cyaniris puspa</i>	–	<i>Acytolepis puspa</i>	
<i>Anthene lycaenina</i>	–	–	<i>Anthene lycaenina</i>	<i>Anthene lycaenina</i>	
<i>Appias albina</i>	<i>Huphina albina</i>	–	–	<i>Appias albina</i>	A doubtful generic allocation by Smith (1882)
<i>Appias paulina</i>	<i>Catophaga paulina</i>	–	–	–	
<i>Ariadne ariadne</i>	<i>Ergolis ariadne</i>	<i>Ergolis ariadne</i>	–	<i>Ariadne ariadne</i>	
<i>Ariadne merione</i>	–	–	<i>Ariadne merione</i>	<i>Ariadne merione</i>	
<i>Athyma perius</i>	<i>Athyma perius</i>	<i>Athyma perius</i>	–	<i>Athyma perius</i>	
<i>Badamia exclamationis</i>	–	<i>Badamia exclamationis</i>	–	<i>Badamia exclamationis</i>	
<i>Belenois aurota</i>	<i>Belenois mesentina</i>	–	–	–	
<i>Bibasis sena</i>	–	<i>Bibasis sena</i>	–	<i>Bibasis sena</i>	

Accepted Name	Smith (1882)	Betham (1894)	Padhye et al. (2013)	Our list	Remarks
<i>Byblia ilithyia</i>	<i>Byblia ilithyia</i>	-	-	-	
<i>Caleta roxus</i>	<i>Castalius roxus</i>	-	-	-	
<i>Castalius rosimon</i>	<i>Castalius rosimon</i>	<i>Castalius rosimon</i>	-	<i>Castalius rosimon</i>	
<i>Catopsilia pomona</i>	<i>Catopsilia hilaria</i>	<i>Catopsilia catilia</i>	<i>Catopsilia pomona</i>	<i>Catopsilia pomona</i>	
<i>Catopsilia pyranthe</i>	<i>Catopsilia philippina</i>	-	<i>Catopsilia pyranthe</i>	<i>Catopsilia pyranthe</i>	
<i>Celaenorrhinus ambareesa</i>	-	<i>Celenorrhinus ambareesa</i>	-	<i>Celaenorrhinus ambareesa</i>	
<i>Cepora nerissa</i>	<i>Huphina phryne</i>	<i>Huphina phryne</i>	-	<i>Cepora nerissa</i>	
<i>Charaxes psaphon</i>	-	<i>Charaxes imna</i>	-	<i>Charaxes psaphon</i>	
<i>Cyrestis thyodamas</i>	<i>Cyrestis</i>	-	-	<i>Cyrestis thyodamas</i>	Smith (1882) mentions only generic name. Possibly <i>Cyrestis thyodamas</i>
<i>Danaus chrysippus</i>	<i>Danais chrysippus</i>	<i>Danais chrysippus</i>	-	<i>Danaus chryssipus</i>	Erroneous generic name by Smith (1882) and Betham (1894)
<i>Danaus genutia</i>	<i>Danais genutia</i>	<i>Danais genutia</i>	<i>Danaus genutia</i>	<i>Danaus genutia</i>	Erroneous generic name by Smith (1882) and Betham (1894)
<i>Delias eucharis</i>	-	<i>Delias eucharis</i>	-	<i>Delias eucharis</i>	
<i>Deudorix epijarbas</i>	-	<i>Deudorix epijarbas</i>	-	<i>Deudorix epijarbas</i>	
<i>Euchrysops cnejus</i>	<i>Catochrysops cnejus</i>	<i>Catochrysops cnejus</i>	-	<i>Euchrysops cnejus</i>	
<i>Euploea core</i>	-	<i>Euploea core</i>	-	<i>Euploea core</i>	
<i>Eurema brigitta</i>	-	-	<i>Eurema brigitta</i>	-	
<i>Eurema hecabe</i>	<i>Terias hecabe</i>	-	<i>Eurema hecabe</i>	<i>Eurema hecabe</i>	
<i>Graphium agamemnon</i>	<i>Papilio agamemnon</i>	-	<i>Graphium agamemnon</i>	<i>Graphium agamemnon</i>	
<i>Graphium teredon</i>	<i>Papilio sarpedon</i>	-	<i>Graphium sarpedon</i>	<i>Graphium teredon</i>	
<i>Hasora chromus</i>	-	<i>Parata chromus</i>	-	<i>Hasora chromus</i>	
<i>Hebomoia glaucippe</i>	<i>Hebomia glaucippe</i>	-	-	<i>Hebomoia glaucippe</i>	Erroneous generic name by Smith (1882)

Accepted Name	Smith (1882)	Betham (1894)	Padhye et al. (2013)	Our list	Remarks
<i>Hypolimnas bolina</i>	–	<i>Hypolimnas bolina</i>	<i>Hypolimnas bolina</i>	<i>Hypolimnas bolina</i>	
<i>Hypolimnas misippus</i>	<i>Hypolimnas misippus</i>	<i>Hypolimnas misippus</i>	<i>Hypolimnas misippus</i>	<i>Hypolimnas misippus</i>	
<i>Iraota timoleon</i>	<i>Iraota mecenias</i>	–	–	<i>Iraota timoleon</i>	
<i>Jamides bochus</i>	–	–	<i>Jamides bochus</i>	<i>Jamides bochus</i>	
<i>Jamides celeno</i>	–	–	<i>Jamides celeno</i>	<i>Jamides celeno</i>	
<i>Junonia almana</i>	–	<i>Junonia almana</i> , v. <i>asterie</i>	<i>Junonia almana</i>	<i>Junonia almana</i>	
<i>Junonia iphita</i>	<i>Precis iphita</i>	–	<i>Junonia iphita</i>	<i>Junonia iphita</i>	
<i>Junonia lemonias</i>	<i>Junonia lemonias</i>	<i>Junonia lemonias</i>	<i>Junonia lemonias</i>	<i>Junonia lemonias</i>	
<i>Junonia oenone</i>	<i>Junonia oenone</i>	<i>Junonia oenone</i>	–	–	
<i>Junonia orithyia</i>	<i>Junonia orithyia</i>	–	–	–	
<i>Kallima horsfieldii</i>	<i>Kallima horsefieldii</i>	<i>Kallima horsefieldii</i>	–	<i>Kallima horsfieldii</i>	Erroneous specific name in Smith (1882) and Betham (1894)
<i>Leptosia nina</i>	–	<i>Leptosia xiphia</i>	–	<i>Leptosia nina</i>	
<i>Leptotes plinius</i>	<i>Tarucus plinius</i>	<i>Tarucus plinius</i>	–	<i>Leptotes plinius</i>	
<i>Lethe rohria</i>	–	<i>Lethe nilgheriensis</i>	–	<i>Lethe rohria</i>	
<i>Luthrodes pandava</i>	–	–	<i>Chilades pandava</i>	<i>Luthrodes pandava</i>	
<i>Matapa aria</i>	<i>Matapa aria</i>	–	–	<i>Matapa aria</i>	
<i>Melanitis leda</i>	<i>Melanitis leda</i>	–	<i>Melanitis leda</i>	<i>Melanitis leda</i>	
<i>Melanitis leda</i>	<i>Melanitis ismene</i>	<i>Melanitis ismene</i>	–	<i>Melanitis leda</i>	
<i>Mycalesis mineus</i>	<i>Mycalesis mineus</i>	–	–	<i>Mycalesis mineus</i>	
<i>Mycalesis perseus</i>	–	<i>Mycalesis perseus</i>	–	<i>Mycalesis perseus</i>	
<i>Neptis hylas</i>	<i>Neptis varmona</i>	<i>Neptis varmona</i> , v. <i>eurymene</i>	<i>Neptis hylas</i>	<i>Neptis hylas</i>	

Accepted Name	Smith (1882)	Betham (1894)	Padhye et al. (2013)	Our list	Remarks
<i>Neptis jumbah</i>	–	<i>Neptis jumbah</i>	–	<i>Neptis jumbah</i>	
<i>Pachliopta aristolochiae</i>	–	–	<i>Pachliopta aristolochae</i>	<i>Pachliopta aristolochiae</i>	Erroneous generic and specific name in Padhye et al. (2013)
<i>Pachliopta hector</i>	<i>Papilio hector</i>	–	<i>Pachliopta hector</i>	<i>Pachliopta hector</i>	
<i>Papilio ambrax</i>	<i>Papilio epius</i>	–	–	–	
<i>Papilio clytia form dissimilis</i>	<i>Papilio form dissimilis</i>	–	–	<i>Papilio clytia form dissimilis</i>	
<i>Papilio clytia form clytia</i>	<i>Papilio form panope</i>	–	<i>Papilio clytia form clytia</i>	<i>Papilio clytia form clytia</i>	
<i>Papilio deiophobus</i>	<i>Papilio deiophobus</i>	–	–	–	This could be misidentification as the species is distributed in the Philippines, Moluccas and some parts of West Papua.
<i>Papilio demoleus</i>	–	–	<i>Papilio demoleus</i>	<i>Papilio demoleus</i>	
<i>Papilio iswara</i>	<i>Papilio iswara</i>	–	–	–	This could be misidentification as the species is distributed over the Sundaland.
<i>Papilio polymnestor</i>	<i>Papilio polymnestor</i>	<i>Papilio polymnestor</i>	<i>Papilio polymnestor</i>	<i>Papilio polymnestor</i>	
<i>Papilio polytes</i>	<i>Papilio pammon</i>	<i>Papilio Polytes</i>	<i>Papilio Polytes</i>	<i>Papilio polytes</i>	
<i>Parantica aglea</i>	<i>Danais aglea</i>	<i>Danais melanooides</i>	<i>Parantica aglea</i>	<i>Parantica aglea</i>	
<i>Pareronia valeria</i>	<i>Eronia valeria</i>	–	–	–	
<i>Pelopidas agna</i>	<i>Chapra agna</i>	–	–	–	
<i>Pelopidas mathias</i>	–	<i>Chapra mathias</i>	–	<i>Pelopidas mathias</i>	
<i>Phaedyma columella</i>	–	<i>Neptis ophiana</i>	–	<i>Phaedyma columella</i>	
<i>Phalanta phalantha</i>	<i>Atella phalanta</i>	<i>Atella phalantha</i>	–	<i>Phalanta phalantha</i>	Erroneous specific name by Smith (1882)
<i>Polyura bhārata</i>	<i>Charaxes athamas</i>	–	–	<i>Polyura bhārata</i>	
<i>Prosotas nora</i>	–	–	<i>Prosotas nora</i>	<i>Prosotas nora</i>	

Accepted Name	Smith (1882)	Betham (1894)	Padhye et al. (2013)	Our list	Remarks
<i>Sarangesa purendra</i>	<i>Sarangesa purendra</i>	<i>Sarangesa purendra</i>	–	<i>Sarangesa purendra</i>	
<i>Spialia galba</i>	<i>Hesperia galba</i>	–	–	<i>Spialia galba</i>	
<i>Spindasis lohita</i>	<i>Aphneus lohita</i>	–	–	<i>Spindasis lohita</i>	
<i>Tarucus theophrastus</i>	<i>Tarucus theophrastus</i>	–	–	–	
<i>Tirumala limniace</i>	<i>Danais limniace</i>	<i>Danais limniace</i>	<i>Tirumala limniace</i>	<i>Tirumala limniace</i>	
<i>Udaspes folus</i>	<i>Udaspes folus</i>	<i>Udaspes folus</i>	–	<i>Udaspes folus</i>	
<i>Vanessa indica</i>	<i>Pyrameis indica</i>	–	–	–	
<i>Ypthima philomela</i>	<i>Ypthima philomela</i>	<i>Ypthima philomela</i>	–	–	
<i>Ypthima singala</i>	<i>Ypthima singala</i>	–	–	–	
<i>Zeltus amasa</i>	<i>Zeltus etolus</i>	–	–	–	
Not found	<i>Danais careta</i>	–	–	–	Doubtful record by Smith (1882). Put ? by Betham (1894)
Not found	<i>Poritia</i>	–	–	–	
Not found	<i>Lampides elianus</i>	–	–	–	
Not found	–	<i>Terias esiope</i>	–	–	
Not found	–	<i>Isoteinon nilgheriensis</i>	–	–	Monotypic genus contains <i>Isoteinon lamprospilus</i>

## Seasonal Turnover

The butterfly diversity and distribution is known to be affected by seasons (Brower 1995, Kunte 2000, Tiple et al. 2009). This is especially true in the case of tropical butterflies which may experience extreme wet and dry seasons (Bonebrake et al. 2010). Further, it has also been observed in the case of southern Indian danaine butterflies that they avoid extreme wet and torrential monsoon conditions through longitudinal migration to drier areas (Kunte 2004). The highest number of butterflies in the winter (N = 125), observed during this survey, could be a result of the fact that winters have lower temperature, lower dampness and moderate water availability with no torrential precipitation in and around the study area. We also observe a dry season ‘pocket effect’ (similar to ‘ithomiine pocket’ observed by Vasconcellos-Neto (1991)) in butterflies of the genus *Mycalesis*, *Lethe*, *Ypthima* (Family Nymphalidae) and *Celaenorhinus*, *Taractrocera* and *Spialia* (Family Hesperidae). These butterflies could be observed in open areas on hill-tops and hill-slopes during monsoon and winter months, but their number becomes less in these areas during

the months of summer when they could be observed in dark, shady habitats. We were, however, unable to determine the cause of the high number of hesperiid observations during the monsoon and this needs a detailed behavioural study.

### **Spatial Turnover**

The patterns for the diversity of butterflies of Matheran are very similar to those of the California Channel Island Birds and Vanuatu Birds, mentioned by Podani and Schmera (2011). High overall similarity for the entire butterfly diversity (Suppl. material 2) and family-wise similarity between the sites (Fig. 8a-e) indicate the possibility of very stable diversity in the area with very low emigration to, or immigration from, surrounding areas. However, a detailed study from surrounding areas would be required to confirm this fact. The high overall similarity between the pairs of study sites (N = 28) also suggests a higher percentage of habitat generalist species surveyed in and around Matheran.

### **Colour coding**

This novel approach is expected to improve the representation of the data for seasons and activities of the Indian butterflies. We encourage adding more activities and unique colour codes to make this system more universal, uniform and reader friendly. We also recommend its use while uploading records on open databases, such as Butterflies of India (Kunte et al. 2020) and iNaturalist (<https://www.inaturalist.org/>) for conveying information regarding the seasons and activities of butterflies.

### **Conclusions**

A total of 140 species of butterflies belonging to six families were recorded from Matheran, India. This list includes 77 new records for Matheran. We observed a strong seasonal variation in butterfly diversity. The maximum diversity (N = 125) of butterflies was recorded during winter, while the least (N = 80) during monsoon. A high similarity of butterfly species composition was observed between the pairs of sites studied, tending towards perfect nestedness. This also emphasises the fact that the butterfly diversity in the region is quite stable and chances of emigration to, or immigration from, surrounding regions are very low. A strong seasonal gradient for activity patterns was not observed; however, we did observe a 'pocket effect' of dry season on butterflies. Butterflies during the dry season tend to aggregate near damp and shady places. Further, we introduce a novel barcode system for denoting seasons and activities of Indian butterflies and hope that this will help butterfly biologists to concisely and effectively present the data.

### **Acknowledgements**

MS and NM thank Dr. Deepak Apte, the Director, Bombay Natural History Society, Mumbai; for his support and encouragement during this project. MS and SS are grateful to the people of Matheran for providing local support during the survey. We thank the Biodiversity

Heritage Library for making rare old manuscripts readily available online. We are grateful to Dr. Thomas Vattakven and India Biodiversity Portal for helping us upload raw data of the project and providing the URL for citation. NM thanks Manas Modak for helping prepare the raw dataset in Darwin Core Format through his excellent skills of programming in java. NM thanks Shruti Paripatyadar for introducing him to SDR simplex and its uses. We thank Rohan Bhagat for helping us prepare the map of the study site. MS and SS also thank Abhinav Nair, Gargi Geedh and Tejas Mehendale for helping them variously. We thank reviewers and subject editor for their invaluable comments which helped improve the manuscript. We are grateful to the editorial board and the journal for providing a generous waiver on article processing charges upon our request. Finally, we thank our families for keeping up the working environment at home amidst these chaotic COVID-19 situations.

## Author contributions

MS and SS conducted the field survey. NM did data analysis. MS, SS and NM conceptualised and developed the colour code. MS, SS and NM wrote the manuscript.

## Conflicts of interest

Authors declare no conflict of interest.

## References

- Betham JA (1894) Note on some of the butterflies of Matheran. *Journal of the Bombay Natural History Society* 8 (3): 421-423.
- Bhakare M, Ogale H (2018) A guide to butterflies of Western Ghats (India) includes butterflies of Kerala, Tamilnadu, Karnataka, Goa, Maharashtra and Gujarat State. Milind Bhakare (Privately Published), x+496 pp.
- Birdwood HM (1886) A catalogue of the flora of Matheran. *Journal of Bombay Natural History Society* 1 (4): 203-214.
- Bonebrake TC, Ponisio LC, Boggs CL, Ehrlich PR (2010) More than just indicators: A review of tropical butterfly ecology and conservation. *Biological Conservation* 143 (2010): 1831-1841. <https://doi.org/10.1016/j.biocon.2010.04.044>
- Brower LP (1995) Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America (1857-1995). *Journal of the Lepidopterists' Society* 49: 304-385.
- Evans WH (1932) The identification of Indian butterflies. Second Edition Revised (1985 reprint). Bombay Natural History Society, Mumbai, 454 pp.
- Kehimkar I (2008) The book of Indian butterflies. Bombay Natural History Society, Mumbai, 497 pp.
- Kehimkar I (2016) Butterflies of India. Bombay Natural History Society, Mumbai, xii+528 pp.

- Kocher SD, Williams EH (2000) The diversity and abundance of North American butterflies vary with habitat disturbance and geography. *Journal of Biogeography* 27 (4): 785-794. <https://doi.org/10.1046/j.1365-2699.2000.00454.x>
- Kothari MJ, Moorthy S (1993) Flora of Raigad District, Maharashtra State. Botanical Survey of India, Calcutta, 581 pp.
- Kremen C (1992) Assessing the indicator properties of species assemblages for natural areas monitoring. *Ecological Application* 2 (2): 203-217. <https://doi.org/10.2307/1941776>
- Kunte K (2000) India, a lifescape: Butterflies of Peninsular India. Universities Press (India) Limited, Hyderabad, 272 pp.
- Kunte K (2004) Species composition, sex-ratios and movement patterns in danaine butterfly migrations in southern India. *Journal of the Bombay Natural History Society* 102: 280-286.
- Kunte K, Sondhi S, Roy P (Eds) (2020) Butterflies of India. v.2.90. Indian Foundation for Butterflies URL: <https://www.ifoundbutterflies.org/>
- Larsen TB (1988) The butterflies of the Nilgiri mountains of the Southern India (Lepidoptera: Rhopalocera). *Journal of the Bombay Natural History Society* 84: 26-43.
- Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MH, Szoecs E, Wagner H (2019) vegan: Community Ecology Package. R package version 2.5-6. URL: <https://CRAN.R-project.org/package=vegan>
- Padhye A, Patwardhan A, Jadhav A, Shelke S, Modak N, Mujumdar N, Chhaya K, Mhaske P, Patil K, Koparde P, Patil R, Deulkar R, Sahasrabudhe A, Bangal P, Narvekar A, Chikne S, Dhamale R, Gaikwad S, Pande S, Patil R, Khatavkar S, Viswasrao V, Pendharkar A, Malkar R, Ogale H, Naik H, Mirza Z, Sanap R, Jagdale S, Patwardhan A (2013) Butterflies of northern Western Ghats: A compilation of checklists. *Ela Journal* 2 (1): 3-22.
- Pascal JP (1988) Wet evergreen forests of the Western Ghats of India: ecology, structure, floristic composition, and succession. French Institute, Pondicherry, India, 345 pp.
- Podani J (2001) SYN-TAX 2000. Computer programs for data analysis in ecology and systematics. User's Manual. Scientia, Budapest.
- Podani J, Schmera D (2011) A new conceptual and methodological framework for exploring and explaining pattern in presence-absence data. *Oikos* 120 (11): 1625-1638. <https://doi.org/10.1111/j.1600-0706.2011.19451.x>
- Ramesh BR, Pascal JP, Nouguié C (1997) Atlas of endemics of the Western Ghats (India): distribution of tree species in the evergreen and semi-evergreen forests. 38. Institut Français Pondichéry, Pondicherry, India, 403 pp.
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>
- Saji K, Ogale H (2020) *Cheritra freja* (Fabricius, 1793) – Common Imperial. In: Kunte K, Sondhi S, Roy P (Eds) Butterflies of India. v. 2.88. Indian Foundation for Butterflies URL: <http://www.ifoundbutterflies.org/sp/689/Cheritra-freja>
- Sawchik J, Dufrêne M, Lebrun P (2005) Distribution patterns and indicator species of butterfly assemblages of wet meadows in southern Belgium. *Belgian Journal of Zoology* 135 (1): 43-52.
- Smith JY (1882) Matheran Hill: Its People, Plants and Animals. Thacker, 184 pp.

- Thomas CD, Malorie HC (1985) Rarity, species richness and conservation: Butterflies of the Atlas Mountains in Morocco. *Biological Conservation* 33 (2): 95-117. [https://doi.org/10.1016/0006-3207\(85\)90098-9](https://doi.org/10.1016/0006-3207(85)90098-9)
- Tiple A, Agashe D, Khurad AM, Kunte K (2009) Population dynamics and seasonal polyphenism of *Chilades pandava* butterfly (Lycaenidae) in central India. *Current Science* 97: 1774-1779.
- Van Gasse P (2013) Butterflies of India-annotated checklist. Butterflies of India, Kruibekke, Belgium, 161 pp.
- Varshney RK, Smetacek P (2015) A synoptic catalogue of the Butterflies of India. Butterfly Research Centre, Bhimtal & Indinov Publishing, 8 plates+261 pp.
- Vasconcellos-Neto J (1991) Interactions between Ithomiine butterflies and Solanaceae feeding and reproductive strategies. In: Price PW, Lewinsohn TM, Fernandes GW, Benson WM (Eds) *Plant animal interactions: evolutionary ecology in tropical and temperate regions*. Wiley-Interscience, New York.
- Vattakaven T, George R, Balasubramanian D, Réjou-Méchain M, Muthusankar G, Ramesh BR, Prabhakar R (2016) India Biodiversity Portal: An integrated, interactive and participatory biodiversity informatics platform. *Biodiversity Data Journal* 4: e10279. <https://doi.org/10.3897/BDJ.4.e10279>
- Wynter-Blyth MA (1957) *Butterflies of the Indian Region* (1982 Reprint). Today and Tomorrows Printers and Publishers, New Delhi, 523 pp.

## Supplementary materials

### Suppl. material 1: Percentage matrix fill and percentage contributions from the SDR-simplex analyses of family-wise and overall species richness. [doi](#)

Authors: Sawant, M., Sarang, S., Modak, N.

Data type: Table

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### Suppl. material 2: Similarity-Richness difference-Species replacement simplex plot for overall butterfly diversity of Matheran showing high similarity. Points denote pair of sites (N = 28) [doi](#)

Authors: Sawant, M., Sarang, S., Modak, N.

Data type: Image

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