INTEGRATIVE APPROACHES IN LIFE SCIENCE RESEARCH

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INTEGRATIVE APPROACHES IN LIFE SCIENCE RESEARCH

Edited by

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Dr. P. Dailiah Roopha

Dr. J. Stella Mary

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It is with great pleasure that I present this foreword to Integrative Approaches in Life Science Research, a scholarly volume edited by Dr. E. Joy Sharmila, Associate Professor and Head, Postgraduate & Research Department of Zoology, Dr. P. Dailiah Roopha, Assistant Professor, and Dr. J. Stella Mary, Assistant Professor, of The American College, Madurai.

The life sciences have entered an era in which meaningful discoveries increasingly emerge at the intersection of disciplines. The complex environmental, ecological, biomedical, and technological challenges confronting our world today demand research that transcends conventional boundaries and integrates diverse scientific perspectives. This volume is a commendable reflection of that vision. By bringing together contributions from researchers working across various domains of the life sciences, the editors have created a resource that is both timely and relevant.

This volume aptly reflects the remarkable breadth and interdisciplinary nature of contemporary life science research, bringing together studies on biodiversity, ecology, conservation, molecular biology, genetics, biotechnology, computational biology, environmental science, and public health. Through its diverse contributions, it demonstrates how integrative scientific inquiry enhances our understanding of living systems while addressing pressing global challenges affecting ecosystems and human well-being. The editors deserve commendation for their vision and dedicated efforts in curating this collection, and I congratulate all the contributing authors for their valuable scholarly contributions. This publication not only exemplifies The American College's enduring commitment to excellence in research and knowledge creation but also stands as a valuable resource for researchers, teachers, and students. I am confident that Integrative Approaches in Life Science Research will stimulate further scientific inquiry, encourage interdisciplinary collaboration, and make a meaningful contribution to the advancement of the biological sciences.

I extend my heartfelt congratulations to the editors and contributors for this commendable academic achievement and wish this publication every success among the scientific and scholarly community.

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Preface

Science has advanced by the accumulation of knowledge within defined disciplines. Life science research unravels the complexity of living systems. Life science is a challenging mystery to unfold the insights of various methodologies required to study the intricacies.

This book is a collection of scholarly contributions by integrating different domains of life sciences. It is an amalgamation of various approaches to understand the mysteries in life science. By convergence of various branches of life science, it reveals deeper insight into the complexity of living systems. The various approaches enable deeper insight into robust experimentation and innovative solutions. This book will serve as a resource for students, researchers and academicians. May this book encourage curiosity and contribute to a deeper understanding of life science.

Dr. E. Joy Sharmila

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CONTENTS

VEGETATION STRUCTURE SHAPES BUTTERFLY COMMUNITIES IN A TROPICAL THORN FOREST

B. Archana, E. Joy Sharmila, R. Prasanna, S. SanthoshKumar & M. Kayathri

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ABSTRACT: This study investigated butterfly assemblages in Kodimangalam Reserve Forest, a tropical thorn forest dominated by open and semi-open vegetation. Butterfly abundance was recorded using the Pollard Walk method across eight seasonal phases (pre monsoon, monsoon, post monsoon, and summer over two years). Vegetation cover was quantified using Landsat data, while surface temperature and rainfall were obtained from the NASA POWER grid database. Canonical Correspondence Analysis (CCA) in PAST 3.0 software showed that vegetation structure was the primary driver of butterfly assemblages, with surface temperature and rainfall moderately correlated with all families. Lycaenidae and Pieridae were strongly associated with herb- and shrub-dominated open habitats, Nymphalidae showed moderate representation, while Papilionidae and Hesperiidae, which favour dense vegetation, were less represented due to limited canopy cover. Sporadic rains and summer vegetation shifts supported higher butterfly richness in this habitat, highlighting the combined influence of vegetation structure and climatic variability on butterfly communities in thorn forest ecosystems.

KEYWORDS: Butterfly assemblages, Habitat structure, Vegetation dynamics, Tropical thorn forest, Species distribution.

INTRODUCTION

Kodimangalam Reserve Forest represents a distinctive tropical thorn ecosystem characterized by open scrub vegetation, scattered thorny trees, and resource-limited conditions that influence both plant and animal communities. Within this dry forest, variations in vegetation cover give rise to diverse microhabitats, each offering unique ecological settings. Such structural heterogeneity plays a central role in supporting insect diversity, particularly butterflies, which are widely recognized as sensitive indicators of habitat quality (Sadasivan & Sengupta, 2024; Bhakare & Ogale, 2018). Butterfly distribution and abundance are strongly shaped by vegetation composition, as it governs the availability

of resources, shelter, and favorable microclimatic conditions (Carlton and Ravichadran, 2018 and Nitin *et al.,* 2018). Open scrub patches, dense thickets, and tree-dominated sites each support different assemblages, reflecting the influence of vegetation structure on community organization. This study examines how vegetation types and their spatial patterns shape the distribution and diversity of butterflies in Kodimangalam Reserve Forest.

METHODOLOGY

The study was carried out in Kodimangalam Reserve Forest, which is characterized by open and semi-open thorn forest conditions with scattered shrubs, herbs, and tree patches. To capture seasonal variation, the study period was divided into eight phases pre monsoon, monsoon, post monsoon, and summer repeated across two consecutive years. Butterfly diversity was assessed using the standard Pollard Walk (Pollard & Yates, 1993) method along fixed transects that represented different habitat types, with observations made during peak activity hours under favorable weather conditions, and individuals identified to family and genus level using standard field guides. Vegetation cover was measured seasonally through field observations and Landsat satellite data, recording the dominance of herbs, shrubs, and trees to reflect habitat dynamics (Fig.1a and b). Climatic variables, including surface temperature and rainfall, were obtained from regional meteorological records, while terrain characteristics such as elevation and slope were also noted (Essaadia *et al.,* 2022; Rouse *et al.,* 1974). Butterfly diversity and vegetation cover were calculated for each seasonal phase, and their associations with environmental variables were analyzed using Canonical Correspondence Analysis (CCA), with ordination plots generated in PAST 3.0 statistical software (Thakur & Gosh, 2014; Uyanik & Güler, 2013).

RESULTS AND DISCUSSION

The present study highlights the association between vegetation structure and butterfly assemblages, along with climatic factors such as surface temperature and rainfall, as revealed by Canonical Correspondence Analysis (CCA) (Videvall *et al.,* 2016; Bianchi *et al.,* 2017). A total of 86 butterfly species belonging to 55 genera and five families were documented in the study area. Vegetation and butterfly diversity were assessed across eight seasonal phases (pre monsoon, monsoon, post monsoon, and summer), and the CCA plots were generated using PAST 3.0 statistical software. The analysis (Fig. 2a) indicates that

vegetation structure, rainfall, temperature, and terrain are key environmental gradients shaping Lycaenidae assemblages. Axis 1, the dominant and statistically significant gradient (65.2% variation, p = 0.013), contrasts sparse vegetation with high rainfall (supporting *Fereyeria sp.*, *Castalius sp.*, *Jamides spp.*) against dense vegetation habitats (*Lampides sp.*, *Discolampa sp.*, *Leptotes sp.*, *Zizina sp.*). Axis 2 explains 18.07% (p = 0.156) but lacks significance. Axis 3 (12.13%, p = 0.023) reflects a significant thermal–topographic gradient, where species like *Everes sp.*, *Cigaritis sp.*, and *Virachola sp.* associate with hotter, rocky environments. Axis 4 contributes 4.59% (p = 0.166), capturing minor variation, with generalist or microhabitat-sensitive taxa like *Chilades sp.*, *Hypolycaena* sp.*,* and *Zizeria* spp.. *Zizula* sp. lies at the margin of Axes 1 and 4, while *Euchrysops* sp. occupies the centroid, indicating ecological generalism. Together, the four axes explain 100% of the species–environment relationship, with Axis 1 and 3 emerging as the principal ecological gradients influencing butterfly distribution.

The CCA (Fig, 2b), results show that Axis 1 explains the largest portion of variation (78.6%, eigenvalue 0.04513, p = 0.001) in butterfly species distribution, with species like *Euthalia* and *Mycalesis* associated despite no clear environmental vectors. Axis 2 explains 18.47% of variation (eigenvalue 0.01061, p = 0.104) and aligns with sparse vegetation and rainfall, with species such as *Trimuala* and *Euploea* linked to this axis. Axis 3 accounts for 2.35% (eigenvalue 0.001351, p = 0.969), associated with rocky terrain and surface temperature, hosting species like *Melanitis*, *Arceae*, and *Dananus*. Species including *Ariadnae*, *Hypolimnas*, *Phalantha*, and *Naeptis* occur near the junction of Axes 1 and 2, while *Junonia* lies between Axes 2 and 3. Axis 4 explains only 0.57% of variation (eigenvalue 0.000329, p = 0.976) with species like *Bybilia* present but no environmental vectors. Axes beyond 4 have negligible eigenvalues and are not significant. Overall, Axis 1 is the main gradient influencing species distribution, with limited contribution from other measured environmental factors.

Canonical Correspondence Analysis (Fig. 2c) of Pieridae species shows that Axis 1 accounts for most variation (70.67%, eigenvalue = 0.01062, p = 0.083) but lacks strong alignment with specific environmental vectors, with species like *Pareronia*, *Leptosia*, and *Eurema* associated with this axis. Axis 2 (26%, eigenvalue = 0.00391, p = 0.053) correlates strongly with dense vegetation and includes *Appias*, *Hebomia*, and *Cepora*, indicating a

preference for vegetated habitats. Axis 3 (2.74%, eigenvalue = 0.00041, p = 0.824) relates to surface temperature, sparse vegetation, and rocky terrain, with species such as *Colotis* and *Catopsilia* linked to these mixed gradients. Axis 4 (0.58%, eigenvalue = 8.77E-05, p = 0.795) aligns near rainfall vectors, with *Belenois* associated. Overall, dense vegetation emerges as the primary factor influencing Pieridae composition, while temperature, terrain, and rainfall have lesser effects.

The CCA analysis of Papilionidae butterflies (Fig.4 d) reveals that Axis 1 explains 69.06% of the variation (eigenvalue = 0.02176, p = 0.016) and Axis 2 accounts for 30.94% (eigenvalue = 0.00975, p = 0.001), both statistically significant. Quadrant 1, defined by rocky terrain, surface temperature, and dense vegetation, includes *Troides* sp. and *Graphium* sp., indicating these species are associated with warm, rugged, and densely vegetated habitats. Quadrant 2 lacks environmental vectors but features *Papilio* sp. at the junction of Axes 1 and 3, suggesting a response to multiple or intermediate environmental gradients. Axis 3 is aligned with sparse vegetation and rainfall but contains no species, implying limited influence of these factors on Papilionidae distribution in this area. Quadrant 4, also lacking vectors, contains *Pachliopta* sp., which may be influenced by unmeasured habitat variables. Overall, Papilionidae species show strong associations with topography, temperature, and vegetation structure, particularly in relation to Axis 1 environmental drivers.

The Canonical Correspondence Analysis (Fig. 4 e) of Hesperiidae butterflies reveals that Axis 1 explains 82.76% of the variation (eigenvalue = 0.09623, p = 0.016), representing a significant environmental gradient. Axis 2 contributes 14.45% (eigenvalue = 0.0168, p = 0.248), while Axes 3 and 4 explain 1.81% (eigenvalue = 0.00211, p = 0.659) and 0.98% (eigenvalue = 0.00114, p = 0.084), respectively, and are not statistically significant. In Quadrant 1, surface temperature and rocky terrain are key vectors, associated with *Gomalia* sp. and *Parnara* sp. Quadrant 2 lacks environmental vectors but includes *Spialia* sp. and *Hasora* sp. Quadrant 3 is defined by rainfall and sparse vegetation, with *Sarangesa* sp. present. Quadrant 4 features dense vegetation and is associated with *Ancistroides* sp. Temperature, terrain, and vegetation structure emerged as key drivers of butterfly assemblages in Kodimangalam Reserve Forest. While surface temperature was not directly correlated with butterfly presence, higher post monsoon and summer temperatures, coupled with sporadic rains, enhanced nectar-rich ground flora and increased species richness. The

bar chart (Fig. 3a) shows the proportion of specialist and generalist genera across families based on CCA, while the pie charts (Fig. 3b) illustrate habitat-restricted specialists distributed across dense vegetation, sparse vegetation, and rocky terrain. Pieridae were particularly abundant due to their physiological adaptations, including HSP70 proteins (Schmalensee *et al.,* 2023; Subedi *et al.,* 2021; Karlsson & Wiklund, 2005), and the availability of small-flower nectar sources that also favored Lycaenidae and Pieridae. In contrast, Papilionidae and Hesperiidae, which depend on dense, humid forest habitats, were less represented. Overall, the open, seasonally dynamic vegetation, along with terrain and rainfall-driven habitat shifts, supported adaptive generalists such as Lycaenids, Pierids, and Nymphalids, while specialists remained constrained (Bruschini *et al.,* 2024). These results highlight the interplay of microclimate, terrain, and vegetation structure in shaping butterfly communities and emphasize the resilience of adaptable species in fluctuating thorn forest ecosystems.

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Fig. 1a. Seasonal vegetation cover of the study area (June 2022–May 2023).

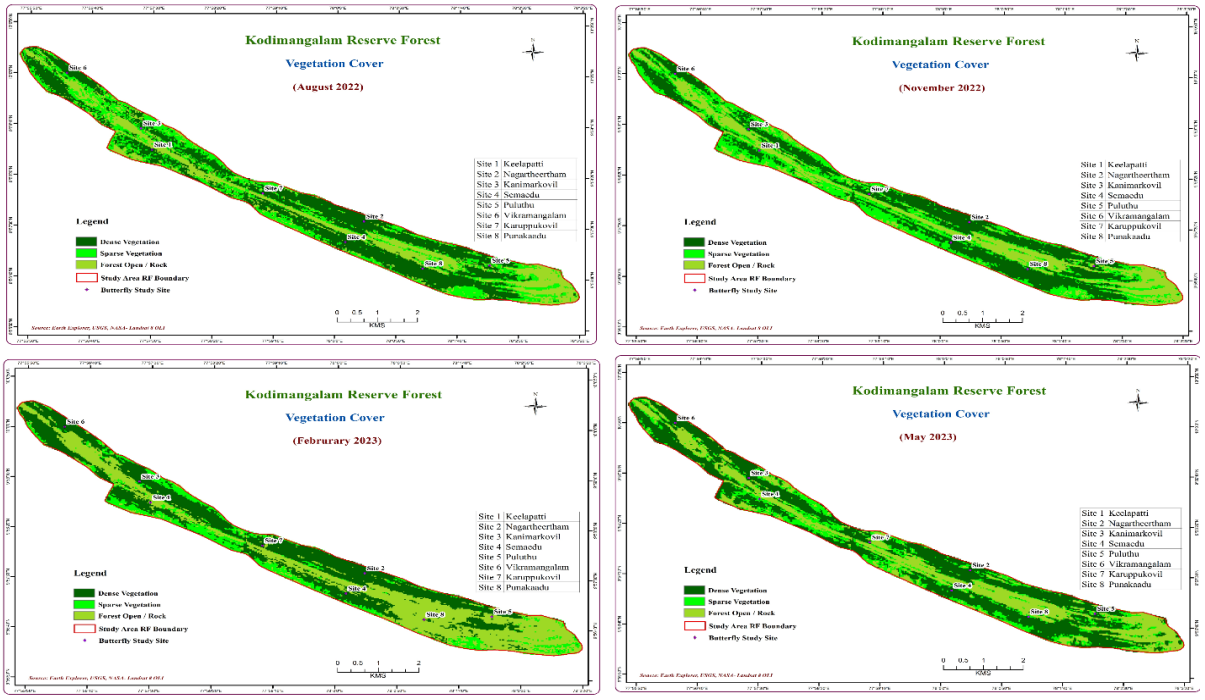


Fig.1b. Seasonal vegetation cover of the study area (June 2023–May 2024).

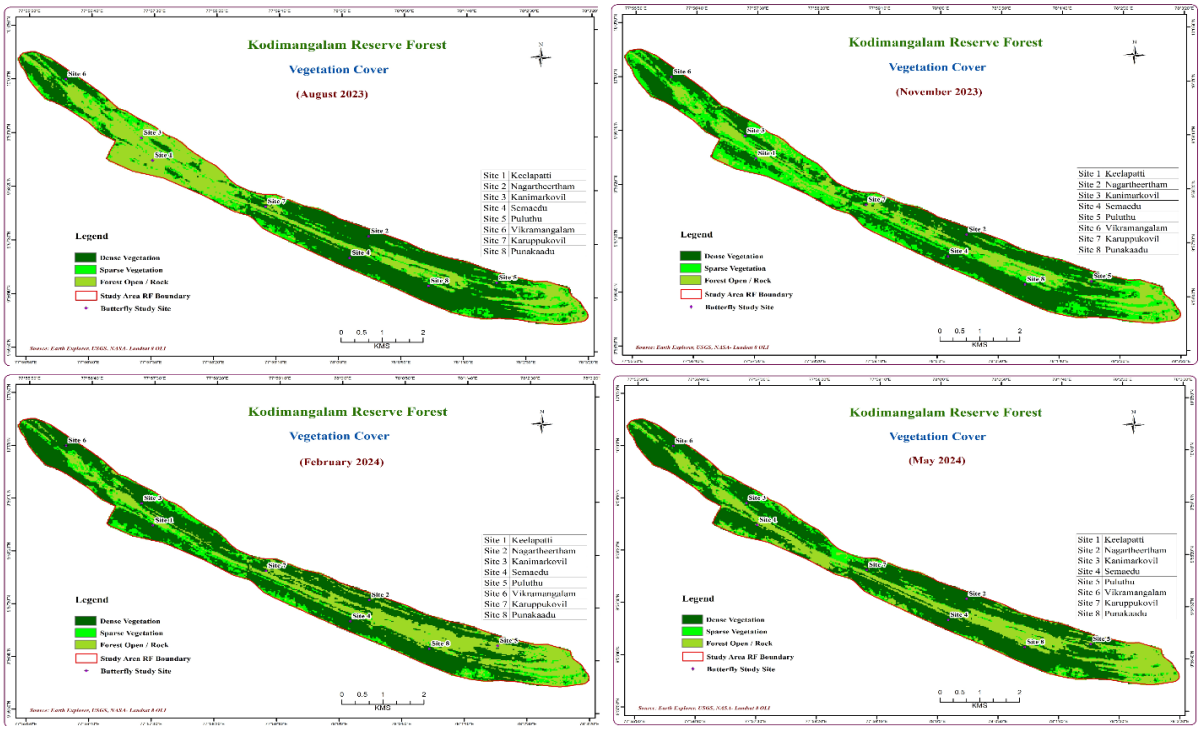


Fig.2. CCA plots showing vegetation and climate associations of butterfly families: (a) Lycaenidae, (b) Nymphalidae, (c) Pieridae, (d) Papilionidae, (e) Hesperiidae.

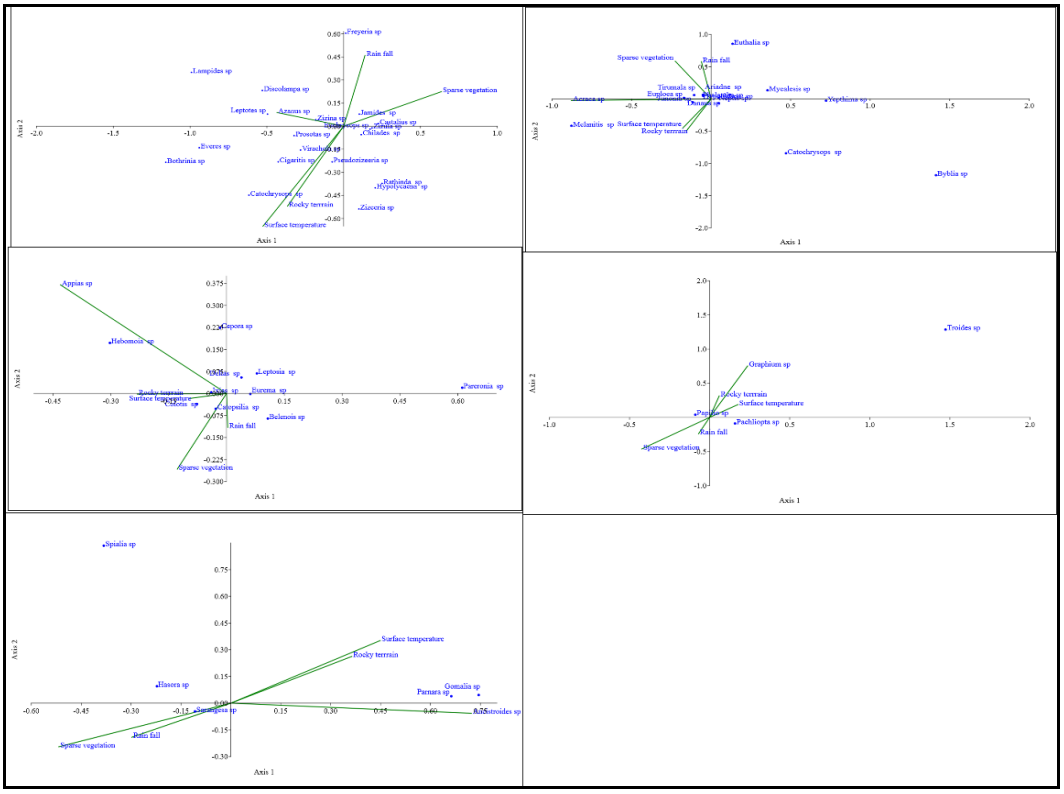
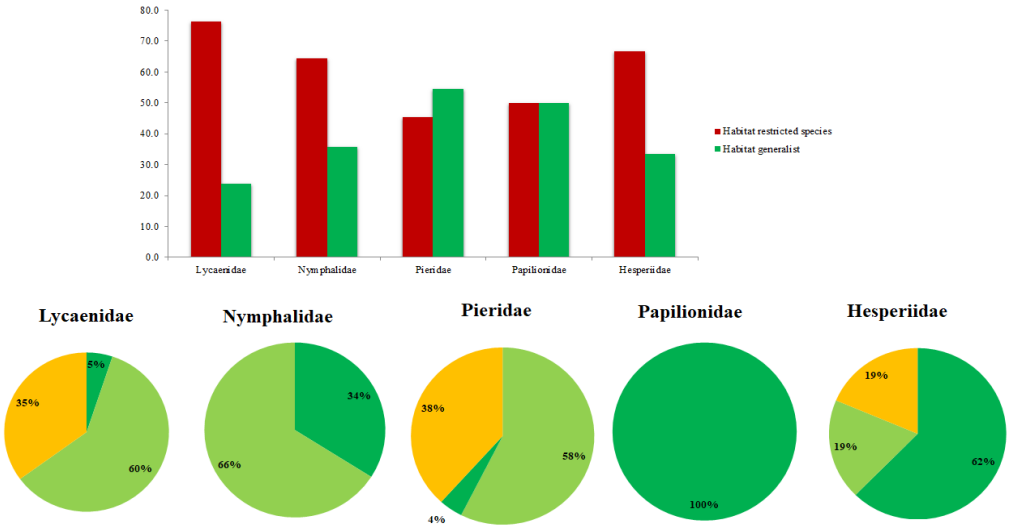


Fig.3. Butterfly Genera Specialization and Habitat Preferences in Kodimangalam Reserve Forest



DISTRIBUTION, DIVERSITY AND SEASONALITY OF SPIDERS IN THE AMERICAN COLLEGE (SATELLITE CAMPUS), CHATRAPATTI, MADURAI DISTRICT, TAMIL NADU

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ABSTRACT

Spiders play a crucial ecological role as natural biological control agents and indicators of environmental health. The present study investigates the distribution, diversity, and seasonal variation of spider fauna in The American College (Satellite Campus), Chatrapatti, Madurai District, Tamil Nadu, India. A systematic survey was conducted from October 2024 to March 2025, covering four habitat types: around pond, built-up areas, orchards, and amenity zones. A total of 39 species belonging to 15 families were recorded. The family Araneidae was the most dominant, followed by Lycosidae, Salticidae, and Thomisidae. Diversity indices such as Shannon–Wiener, Simpson, Margalef, and Evenness were calculated monthly. Seasonal variations showed peak diversity during February and March, while lower diversity was observed during January. Correlation analysis indicated a non-significant relationship between spider abundance and climatic factors such as temperature, humidity, and rainfall. The study highlights the ecological importance of campus habitats in conserving arachnid diversity and emphasizes the role of microhabitat heterogeneity in sustaining spider populations.

Keywords: Spider diversity, Araneae, Seasonal variation, Biodiversity, Environmental indicators, Tamil Nadu

INTRODUCTION

Biodiversity plays a fundamental role in maintaining ecosystem stability, productivity, and resilience. Among all biological groups, arthropods represent the most diverse and ecologically significant component of terrestrial ecosystems (Wilson, 1987). Spiders (Order:

Araneae), as obligate predators, occupy a crucial position in food webs by regulating insect populations and maintaining ecological balance (Foelix, 2011). Their abundance, diversity, and sensitivity to environmental changes make them reliable indicators of habitat quality and ecosystem health (Marc *et al.,* 1999).

Spiders exhibit remarkable ecological adaptability, occupying a wide range of habitats including forests, grasslands, agricultural fields, wetlands, and urban environments. Their diverse foraging strategies—such as orb-web building, ambush predation, and active hunting— allow them to exploit various ecological niches (Uetz *et al.,* 1999). Because of their sensitivity to microclimatic conditions such as temperature, humidity, and vegetation structure, spider assemblages respond rapidly to environmental disturbances, making them valuable tools for biodiversity monitoring and conservation planning (Cardoso *et al.,* 2011).

Globally, more than 52,000 spider species have been described, with new taxa being discovered regularly (World Spider Catalog, 2024). India, recognized as one of the world's megadiverse countries, supports a rich spider fauna due to its wide range of climatic zones and habitat types. However, despite this diversity, comprehensive studies on spider assemblages remain limited, particularly in semi-natural and human-modified landscapes such as educational campuses and peri-urban regions (Sebastian & Peter, 2009).

The Eastern Ghats, a discontinuous range of hills extending along the eastern part of India, represent an ecologically significant yet underexplored biogeographic zone. The region is characterized by tropical climatic conditions, seasonal rainfall, and heterogeneous vegetation patterns, which collectively support diverse arthropod communities. The Madurai district, located within this region, experiences distinct seasonal variations that strongly influence arthropod population dynamics, especially spiders (Uetz *et al.,* 1999).

The American College (Satellite Campus), Chatrapatti, is characterized by a mosaic of habitats including orchards, open grasslands, built-up areas, and water bodies. Such habitat heterogeneity creates favorable conditions for diverse spider guilds, including orb-weavers, ground hunters, foliage dwellers, and ambush predators. Despite this ecological potential, systematic documentation of spider diversity within this campus has not been previously undertaken.

Understanding the distribution and seasonal dynamics of spider communities in such semi-natural ecosystems is essential for biodiversity conservation and environmental monitoring. Spiders serve as effective bioindicators due to their sensitivity to habitat disturbance, microclimatic variation, and prey availability (Pearce & Venier, 2006). Therefore, the present study aims to assess the distribution, diversity, and seasonal variation of spider fauna in the American College (Satellite Campus), Chatrapatti, Madurai District. The study further examines the influence of environmental factors such as temperature, humidity, and rainfall on spider assemblages, thereby contributing baseline ecological data for future conservation and management strategies.

MATERIALS AND METHODS

Study Area

The American College's satellite campus is situated in Chatrappati, 15 kilometers away along the Madurai-Natham Highway. The 60-acre campus was built in the slopes of Kiluvamali, a hillock on the Eastern Ghats that is a part of the Alagar Hills. The coordinates for latitude and longitude are 9.9252117N, 78.1197644° E. The campus comprises ponds, gardens, orchards, built-up areas, and open vegetation, providing diverse microhabitats for spiders. Four sites have been selected for the study in order to comprehensively evaluate the richness and distribution of spiders.

Site 1: Around Pond Site 2: Build Ups Site 3: Orchard

Site 4: Amenities

Sampling Period and Method

The study was conducted from October 2024 to March 2025 using the Visual encounter on Line transect - method as described by Burnham *et al.,* (1980) to estimate the abundance and population of spider diversity at The American College (Satellite Campus). Surveys were carried out four days each month, with observations recorded from 6:00 AM to 11:00 AM and from 7:00 PM to 11:00 PM to ensure comprehensive data collection. Spiders were collected using:

- Visual search method
- Hand collection
- Sweep netting
- Ground searching

Collected specimens were photographed, identified using standard taxonomic keys, and categorized up to species or genus level.

Data Analysis

Diversity indices such as Shannon–Wiener Index (H′), Simpson's Index (1–D), Margalef Richness Index, and Evenness (e^H/S) were calculated. Pearson correlation analysis was performed to assess relationships between spider abundance and climatic variables (temperature, humidity, and rainfall).

RESULTS

Species Composition and Overall Diversity

A total of 364 individual spiders belonging to 36 species, 29 genera, and 16 families were recorded during the study period (October 2024 – March 2025) from the four selected habitats of The American College (Satellite Campus), Chatrapatti (Table 1). The recorded taxa represented a wide range of ecological guilds, indicating a structurally diverse and ecologically stable habitat. Among the families recorded, Araneidae was the most dominant, contributing 25% of the total species, followed by Lycosidae (17%), Sparassidae (11%), and Oxyopidae, Salticidae, Theridiidae, and Uloboridae (each 6%). The remaining families such as Eresinidae, Ctenidae, Tetragnathidae, Ischnothelidae, and Theraphosidae were represented by one or two species each, indicating lower abundance but significant taxonomic diversity (Fig 1). The dominance of orb-weaving spiders reflects the availability of suitable vegetation structure and prey density within the study area, while the presence of ground-dwelling and wandering spiders indicates habitat heterogeneity and ecological stability.

Species Distribution Across Study Sites

The distribution of spiders varied considerably among the four sampling sites: Orchard area (S3) recorded the highest number of individuals (149), attributed to dense vegetation, shade, high prey availability, and minimal human disturbance. Built-up area (S2) supported 96 individuals, indicating that anthropogenic structures still provide suitable microhabitats for certain adaptable species. Pond area (S1) supported 90 individuals, particularly web builders benefiting from high insect availability. Amenity area (S4) recorded the lowest abundance (29 individuals), likely due to habitat modification and frequent human activity (Fig 2). This variation highlights the influence of vegetation complexity, moisture availability, and disturbance levels on spider assemblage.

TABLE1. Spider population from October 2024 to March 2025

S.NO	SPECIES	Order	Family	Oct	Nov	Dec	Jan	Feb	March	TOTAL
1.	*Argiope anusuja*	Araneae	Araneidae (Orb-Weaver Spiders)	0	0	0	0	1	0	**1**
2.	*Argiope aemula*			0	1	0	0	0	0	**1**
3.	*Bijoaraneus mitificus*			0	0	0	1	0	0	**1**
4.	*Cyrotophora cicatrasa*			2	5	3	0	0	1	**11**
5.	*Gasteracantha geminata*			0	0	0	0	0	1	**1**
6.	*Neoscona bilunaritis*			0	0	0	0	0	1	**1**
7.	*Neoscona mukerjei*			3	7	0	0	3	2	**15**
8.	*Cyclosa Hexatuberculata*			0	0	1	0	0	0	**1**
9.	*Crisoprizza* sp.			20	22	18	16	6	8	**90**
10.	*Stegodyphus* sp.		Eresinadae (Velvet Spiders)	12	14	0	0	0	0	**26**
11.	*Thomisus* sp.		Thomisidae (Crab Spiders)	0	0	0	0	0	1	**1**
12.	*Bowie* sp.		Ctenidae (Wandering Spiders)	0	2	0	0	0	0	**2**
13.	*Hersillia savignyi*		Hersiliidae (Tree Trunk Spiders)	1	2	0	0	1	1	**5**
14.	*Hippasa* sp.		Lycosidae (Wolf Spiders)	11	10	20	18	12	4	**75**
15.	*Arctosa* sp.			20	10	5	5	5	5	**50**
16.	*Allocosa* sp.			0	2	0	0	0	0	**2**
17.	*Pardosa milvina*			0	0	0	1	0	0	**1**
18.	*Leucage* sp.			1	0	3	2	0	2	**8**
19.	*Lycosa* sp.			6	15	3	2	2	2	**30**
20.	*Oxyopes* sp.		Oxyopidae (Lynx Spider)	3	2	2	1	0	0	**8**

21.	*Oxyopes shweta*			0	1	0	1	0	0	**2**
22.	*Hyptiotes* sp.		Uloboridae (Hackled Orb Weavers)	0	2	1	1	1	0	**5**
23.	*Miagrammopes* sp.			1	2	0	0	1	0	**4**
24.	*Plexippus paykulli*		Salticidae (Jumping Spiders)	0	2	0	0	0	0	**2**
25.	*Stenaelurillus* sp			0	0	1	0	1	0	**2**
26.	*Gnathpalystes* sp.		Sparassidae (Huntsman Spiders)	0	0	1	0	0	2	**3**
27.	*Olios* sp.			0	0	1	0	0	0	**1**
28.	*Heteropoda venatoria*			0	0	1	0	0	0	**1**
29.	*Heteropoda* sp.			0	0	1	0	2	0	**3**
30.	*Tetragnatha* sp.		Tetragnathidae (Long-Jawed Orb Weavers)	0	0	1	0	0	0	**1**
31.	*Perenethis* sp.		Pisuraidae (Nursery Web Spiders)	0	2	0	0	0	0	**2**
32.	*Enoplognatha* sp.		Theridiidae (Tangle Web Spider)	0	3	0	0	0	1	**4**
33.	*Indothele* sp.		Ischnothelidae (Mygalomorph Spiders)	0	0	0	0	1	0	**1**
34.	*Poecilotheria* sp.		Theraposidae (Tarantulas)	1	0	0	0	0	0	**1**
35.	*Heterophrictus* sp.			1	0	0	0	0	0	**1**
36.	*Solifuge* sp.	Solifugae	Eremobatidae (Sun Spiders)	0	0	0	0	0	1	**1**

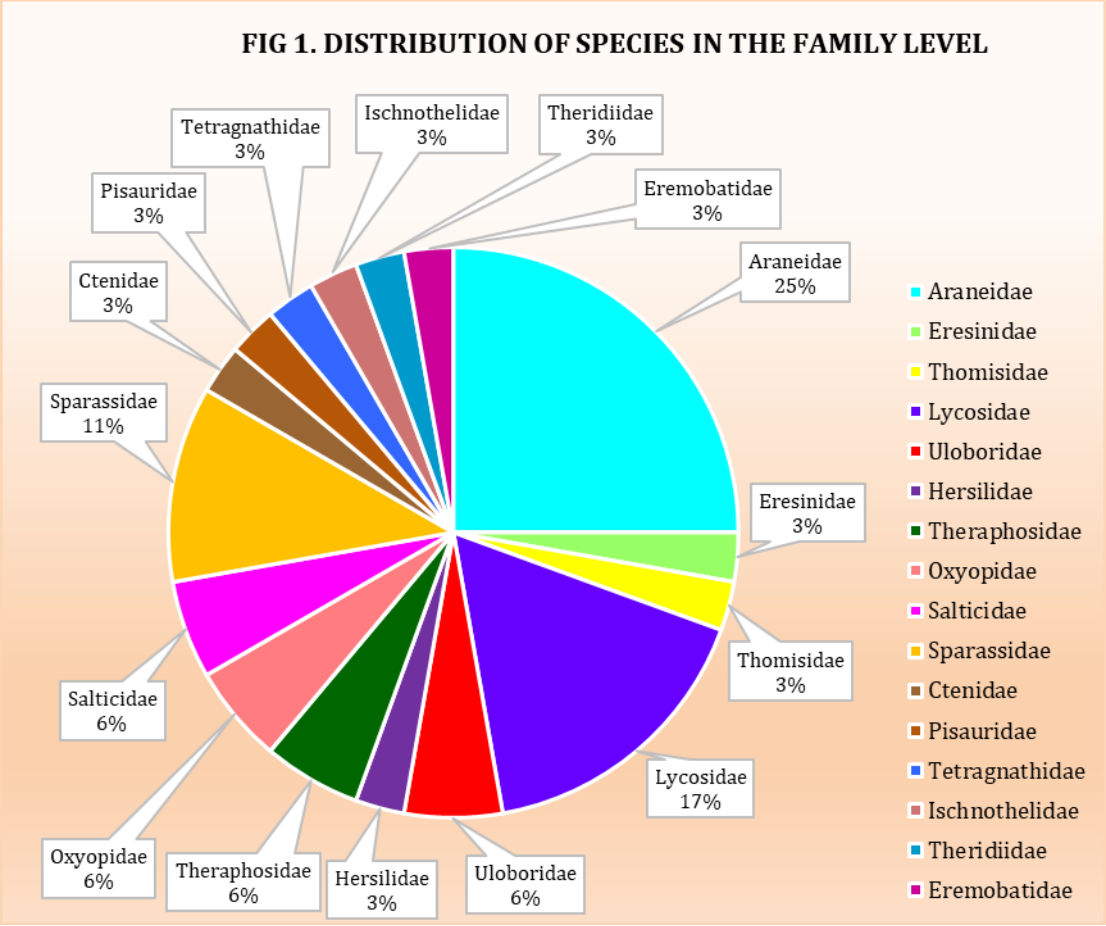


FIG 1. DISTRIBUTION OF SPECIES IN THE FAMILY LEVEL
Tetragnathidae 3%
Ischnothelidae 3%
Theridiidae 3%
Pisauridae 3%
Eremobatidae 3%
Araneidae 25%
Ctenidae 3%
Sparassidae 11%
Eresinidae 3%
Thomisidae 3%
Salticidae 6%
Lycosidae 17%
Oxyopidae 6%
Theraphosidae 6%
Hersilidae 3%
Uloboridae 6%
Araneidae
Eresinidae
Thomisidae
Lycosidae
Uloboridae
Hersilidae
Theraphosidae
Oxyopidae
Salticidae
Sparassidae
Ctenidae
Pisauridae
Tetragnathidae
Ischnothelidae
Theridiidae
Eremobatidae

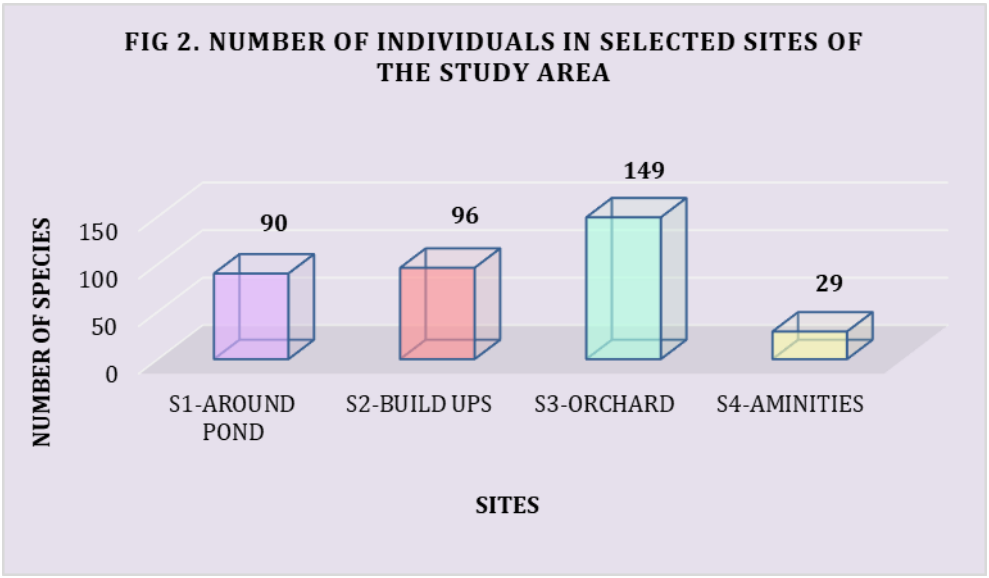


FIG 2. NUMBER OF INDIVIDUALS IN SELECTED SITES OF THE STUDY AREA
NUMBER OF SPECIES
150
100
50
0
90
96
149
29
S1-AROUND POND
S2-BUILD UPS
S3-ORCHARD
S4-AMINITIES
SITES

Guild Structure Analysis

The spider fauna was classified into functional guilds based on foraging strategies: Orb-web builders dominated the assemblage, reflecting the abundance of shrubs, trees, and artificial structures suitable for web construction. Ground hunters and ground weavers were well represented in leaf litter and open soil habitats, especially in orchard and pond regions.

Monthly Variation in Spider Abundance

Spider abundance varied significantly across months:

- Highest abundance: November (81 individuals) and December (76 individuals)
- Lowest abundance: March (32 individuals).

The decline in spider numbers from January to March may be attributed to increasing temperature, reduced humidity, and lower prey availability. Seasonal fluctuations strongly influenced activity patterns and detectability.

Diversity Indices Analysis

The Shannon–Wiener Index (H′) Values ranged from 1.52 (January) to 2.43 (March), indicating moderate to high species diversity. The increase during late winter suggests favorable microclimatic conditions and increased prey availability.The Simpson's Diversity Index (1–D) Values ranged between 0.68 and 0.88, indicating high diversity with moderate dominance of a few species. The Evenness Index (J′) Evenness values (0.41–0.76) suggested uneven distribution of individuals among species, with dominance by a few taxa such as *Crisoprizza* sp., *Hippasa* sp., and *Arctosa* sp (Table 2).

Table 2. Diversity Indices

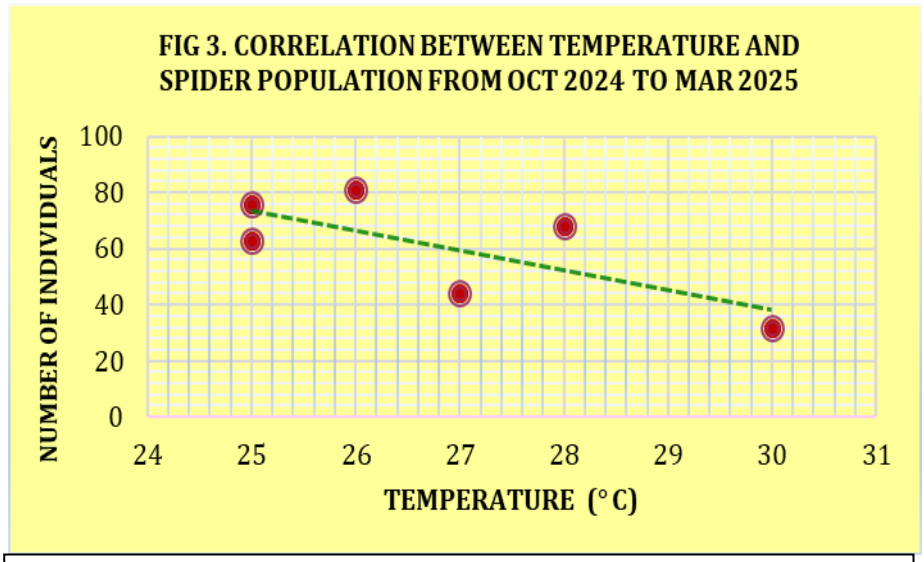
Simpson_1-D	0.7569	0.8444	0.7896	0.6814	0.8421	0.8824
Shannon_H	1.691	2.253	1.99	1.52	2.212	2.433
Evenness_e^H/S	0.5426	0.5946	0.4879	0.4155	0.6526	0.7598

Relationship Between Spider Population and Environmental Variables

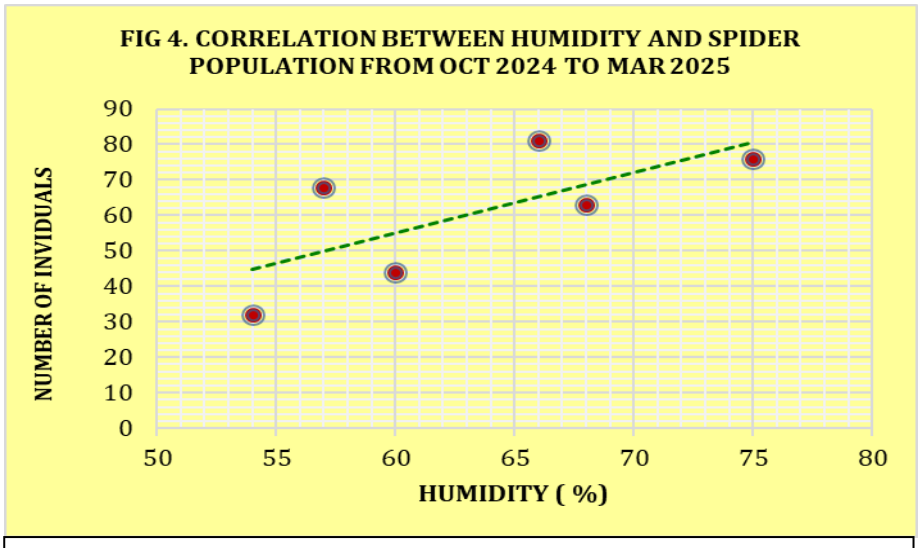
Temperature - A moderate negative correlation was observed between temperature and spider abundance ($r = –0.558$; $p = 0.249$). Although not statistically significant, this suggests that higher temperatures may negatively influence spider activity or survival.

Humidity - A positive correlation (r = 0.616; p = 0.193) was observed between humidity and spider abundance, indicating that moist conditions favour spider activity, web construction, and prey availability.

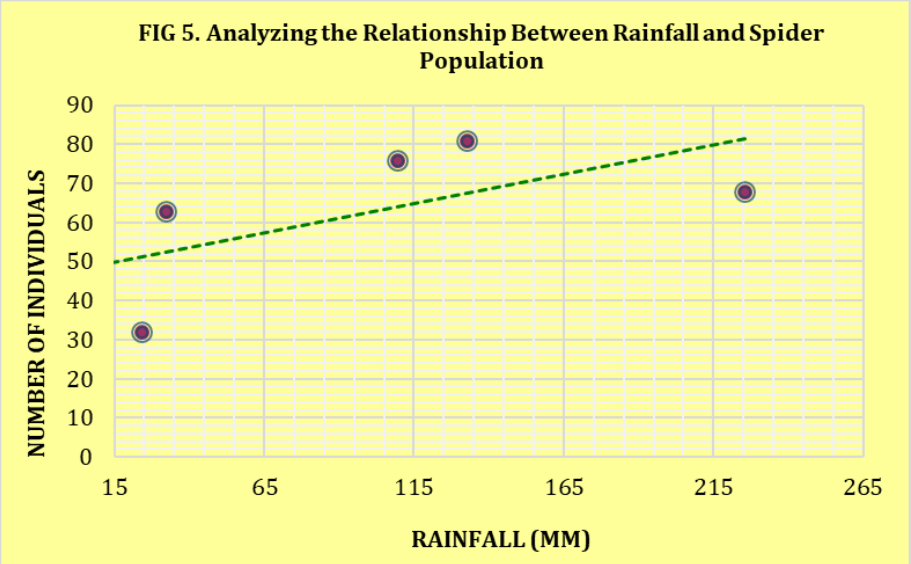
Rainfall - Rainfall showed a strong positive correlation with spider abundance (r = 0.734), though not statistically significant (p = 0.097). Increased rainfall likely enhances vegetation growth and prey density, indirectly supporting spider populations.



A non-significant large negative relationship between Temperature and Number of individuals, (r (4) = .558, p = .250). **r = -0.5583**



A non-significant large positive relationship between Humidity and Number of individuals, (*r* (4) = .616, *p* = .193).**r = 0.6156**



A non-significant large positive relationship between Rainfall and Number of individuals, (r (4) = .734, p = .097). **r = 0.7336**

DISCUSSION

The present study provides a detailed account of the diversity, distribution, and seasonal variation of spider assemblages in the American College (Satellite Campus), Chatrapatti, Madurai District. The findings reveal a rich and diverse spider fauna, reflecting the ecological heterogeneity and favourable microhabitat conditions of the study area. The dominance of multiple spider families and guilds highlights the ecological stability and functional complexity of the habitat. A total of 36 spider species belonging to 16 families were recorded during the study period, indicating a moderately high level of species richness when compared to similar studies conducted in semi-natural and institutional landscapes (Sebastian & Peter, 2009; Caleb & Sankaran, 2021). The dominance of families such as Araneidae, Lycosidae, Salticidae, and Thomisidae aligns with previous findings from tropical and subtropical ecosystems, where these groups are commonly reported due to their adaptability to diverse microhabitats (Uetz *et al.,* 1999; Cardoso *et al.,* 2011). Orb-weaving spiders (Araneidae) constituted the most dominant guild, suggesting the availability of suitable web-supporting vegetation and abundant flying insect prey. This pattern has been widely documented in agroecosystems and semi- natural habitats where vegetation complexity promotes higher prey density and web-building opportunities (Marc *et al.,* 1999; Foelix, 2011). The presence of ground-dwelling hunters such as Lycosidae and Gnaphosidae further indicates well-developed litter layers and undisturbed soil surfaces within the study area.

The variation in spider abundance among different habitat types highlights the strong influence of habitat structure on spider community composition. The orchard habitat supported the highest diversity and abundance, likely due to its dense vegetation, layered canopy structure, and relatively low anthropogenic disturbance. Similar patterns have been reported in orchard and agroforestry systems, where structural complexity enhances prey availability and niche diversity (Pinkus-Rendon *et al.,* 2006). In contrast, the amenity and built-up areas exhibited comparatively lower spider abundance, possibly due to frequent human interference, habitat simplification, and limited prey resources. Nevertheless, the presence of certain adaptable species in these areas indicates that urban and semi-urban environments can still support spider populations, albeit with reduced diversity. Such findings corroborate earlier studies emphasizing the importance of microhabitat quality rather than land-use category alone in determining spider assemblages (Shochat *et al.,* 2004).

Seasonal fluctuations significantly influenced spider abundance and diversity. Higher population densities observed during the post-monsoon and early winter months can be attributed to increased vegetation growth, prey availability, and favorable microclimatic conditions. Similar seasonal trends have been reported in tropical and subtropical regions, where spider abundance peaks during periods of moderate temperature and high humidity (Wise, 1993; Tuf *et al.,* 2015). The decline in spider abundance during the hotter months may be associated with increased desiccation stress, reduced prey availability, and changes in vegetation cover. Such seasonal dynamics emphasize the sensitivity of spider populations to climatic variables, particularly temperature and humidity, as supported by earlier ecological studies (Marc *et al.,* 1999; Uetz *et al.,* 1999). The correlation analysis indicated a positive relationship between spider abundance and environmental factors such as humidity and rainfall, although these relationships were not statistically significant. This pattern suggests that while climatic factors influence spider activity and distribution, their effects are often mediated by habitat structure and vegetation complexity. Temperature exhibited a negative correlation with spider abundance, consistent with observations that extreme heat can limit spider activity and survival (Foelix, 2011). These findings support previous studies suggesting that spiders respond more strongly to habitat heterogeneity than to individual climatic parameters alone (Pearce & Venier, 2006). Consequently, maintaining structurally complex habitats is critical for sustaining diverse spider communities.

Spiders play a vital role in regulating insect populations and maintaining ecological balance. Their sensitivity to environmental disturbances makes them valuable bioindicators

for assessing habitat quality and ecological integrity. The presence of diverse spider guilds within the study area underscores the ecological value of institutional campuses as reservoirs of biodiversity. The results of this study emphasize the importance of conserving green spaces within educational and semi-urban landscapes. Maintaining native vegetation, minimizing chemical usage, and preserving microhabitats such as leaf litter and shrubs can significantly enhance arthropod diversity. Such measures not only support biodiversity conservation but also promote ecosystem services such as natural pest regulation. The observed spider assemblage reflects a stable and heterogeneous ecosystem capable of supporting multiple functional guilds. The dominance of web-building species highlights the importance of vegetation complexity, while the presence of ground-dwelling and hunting spiders indicates intact soil and microhabitat conditions. Seasonal variation in abundance is largely driven by abiotic factors, particularly temperature and moisture availability.

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IN SILICO ANALYSIS OF TRICIN FOR TOPOISOMERASE INHIBITION IN ANTICANCER DRUG DISCOVERY

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ABSTRACT

DNA topoisomerases are essential enzymes that regulate DNA topology during replication and transcription. Because of their critical function in rapidly proliferating cells, they serve as key molecular targets in cancer therapy. Although several topoisomerase inhibitors are clinically available, challenges such as drug resistance, toxicity, and limited selectivity highlight the need for novel, and non-toxic compounds. Tricin, a plant-derived flavone with reported anticancer and anti-inflammatory properties, offers potential as a natural therapeutic agent; however, it's interaction with DNA topoisomerases has not been comprehensively investigated. The objectives of this *in silico* study are to evaluate the molecular and druglikeness properties of tricin and also to analyse whether tricin exhibit anticancer property by acting as an inhibitor of topoisomerases by visualizing the computational simulation. Molinspiration software was used to calculate physicochemical properties, Lipinski's rule of five for tricin and topotecan. The three-dimensional structures of DNA topoisomerase I (PDB ID: 1K4T) and DNA topoisomerase II (PDB ID: 3QX3) were retrieved from protein data bank, and tricin was retrieved from PubChem database. Using CASTpFold, the active sites of DNA topoisomerases were predicted and molecular docking was performed using AutoDock 4.2 to analyse the binding affinity and key interactions between tricin, and topotecan (control) with DNA topoisomerases. Tricin satisfies major drug-likeness properties and Lipinski's rule of five making it a potent drug molecule. Docking studies demonstrated that tricin interacts strongly with the active sites of DNA topoisomerase I and II, yielding binding energies of –7.70 and –6.21 kcal/mol. These values indicate higher affinity when compared with topotecan, which showed weaker binding energies of –6.60 and –4.52 kcal/mol, respectively. Tricin demonstrated higher binding affinity towards DNA topoisomerase than the control drug topotecan. Combined with its

positive drug-likeness features, tricin emerges as a promising natural lead molecule for further anticancer investigation.

INTRODUCTION

Cancer is a significant global health threat, owing to difficulties in early identification, low survival rates for those found at advanced stages, and the disease's increasing global incidence. Current treatments seldom result in complete remission or significantly improved survival, highlighting the critical need for innovative preventive techniques and therapeutic agents (Ding *et al.,* 2008: Tiwari *et al.,* 2017).

Among various molecular targets, DNA topoisomerases play a vital role in key cellular activities, including DNA replication, transcription, genome duplication, chromatin organization and chromosome segregation. They exhibit both nuclease and ligase activity and also have the ability to regulate the topological state of DNA (Liu *et al.,* 2020). Topoisomerases are categorized into two primary classes based on their catalytic mechanisms: topoisomerase I (TOPO I) and topoisomerase II (TOPO II). Topoisomerase I recognizes and binds particular DNA sequences, frequently single-stranded areas, *via* non-covalent interactions (Dasgupta *et al.,* 2020). Its main functions include relaxing DNA supercoils and reducing torsional stress. TOPO I then forms a covalent link between its catalytic tyrosine residue and the 3'-phosphoryl end of the DNA to produce a reversible single-strand break (Marini *et al.,* 2023).

Topoisomerase II selectively recognizes double-stranded DNA sequences during its catalytic cycle (Hu *et al.,* 2018). It executes coordinated cleavage of both DNA strands, generating transient double-strand breaks. Distinct from type IB topoisomerases, TOPO II functions as a homodimeric ATPase with α/β catalytic domains. Like all topoisomerases, they employ catalytic tyrosine residues for DNA cleavage and ligation. TOPO II initiates strand scission *via* nucleophilic attack by the tyrosine's catalytic site on the 3'-phosphate of the DNA backbone (Deweese and Osheroff, 2009: Bailly, 2012).

Flavonoids, which are abundantly found natural compounds, serve as a valuable reservoir for drug development owing to their diverse chemical structures and wide-ranging biological activities. Notably, substantial evidence has demonstrated that flavonoids possess significant chemopreventive and chemotherapeutic properties against cancer. Consequently, their potential as anticancer agents have attracted considerable research interest over several decades. Structurally, these are C6–C3–C6 compounds consisting of two benzene rings, joined by the C3 aliphatic chain with a heterocyclic pyran ring (Kumar and Pandey 2013).

Tricin (TC), chemically known as 5,7,4'-trihydroxy-3',5'-dimethoxy flavone, has the molecular formula $C_{17}H_{14}O_7$ and a molecular weight of 330.3 g/mol (Figure 1). The aglycone structure features hydroxyl groups at the C5 and C7 positions of ring A, and a hydroxyl group at the C4' position on ring B, flanked by two methoxy groups located at C3' and C5'. The C ring of TC is oxygenated at position 1, contains a double bond between C2 and C3, and has a carbonyl (C=O) group at C4. The presence of the hydroxyl group at C4' on ring B sandwiched by methoxy groups enhances TC's bioavailability, metabolic stability, and intestinal absorption. The multiple hydroxyl groups typically afford flavonoids potent antioxidant properties, while methoxy substitutions increase lipophilicity and facilitate membrane permeability. Furthermore, the double bond and carbonyl functionalities contribute to the increased molecular stability, augmenting the biological activity. Collectively, methylation of flavonoids such as TC improves metabolic stability and promotes enhanced cellular uptake during intestinal absorption (Han *et al.,* 2016).

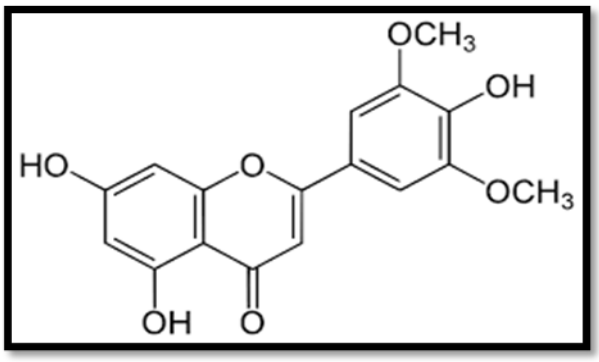


Figure 1: Chemical structure of tricin

Therefore, exploring the binding interactions of tricin with DNA topoisomerases *via in silico* docking is a promising approach to identify its potential as a natural anticancer agent that can inhibit critical enzymes involved in DNA replication and transcription processes, ultimately contributing to the development of safer and more effective cancer therapeutics. Hence, the objectives of this *in silico* study are to evaluate the molecular and druglikeness properties of tricin and also to analyse whether tricin exhibit anticancer property by acting as an inhibitor of topoisomerases by visualizing the computational simulation.

METHODOLOGY

Molecular descriptor calculation:

Molinispiration online database was used to calculate the molecular descriptors of tricin and topotecan. The canonical SMILES were retrieved from PubChem (Table 1) and uploaded in the search bar of molinspiration database to calculate the molecular properties.

Using the molecular properties obtained from molinspiration, the Lipinski's rule of five was analysed.

Table 1: Canonical SMILES of ligand

Ligand	Canonical SMILES
Tricin	COC1=CC(=CC(=C1O)OC)C2=CC(=O)C3=C(C=C(C=C3O2)O)O
Topotecan	CC[C@@]1(C2=C(COC1=O)C(=O)N3CC4=CC5=C(C=CC(=C5C N(C)C)O)N=C4C3=C2)O

Molecular interactions of Tricin with DNA topoisomerases I and II

Preparation of Target proteins:

The 3D crystal structures of DNA topoisomerase I (PDB ID: 1K4T) and DNA topoisomerase II (PDB ID: 3QX3) were obtained from the protein data bank (https://www.rcsb.org/) in PDB format (Figure 2). It was made sure that the protein is in 3-dimensional conformation without any breaks. AutoDock tool 4.2 was used to prepare the target proteins by removing the water molecules, and addition of polar hydrogen and kollman charges. The prepared proteins were saved in PDBQT format.

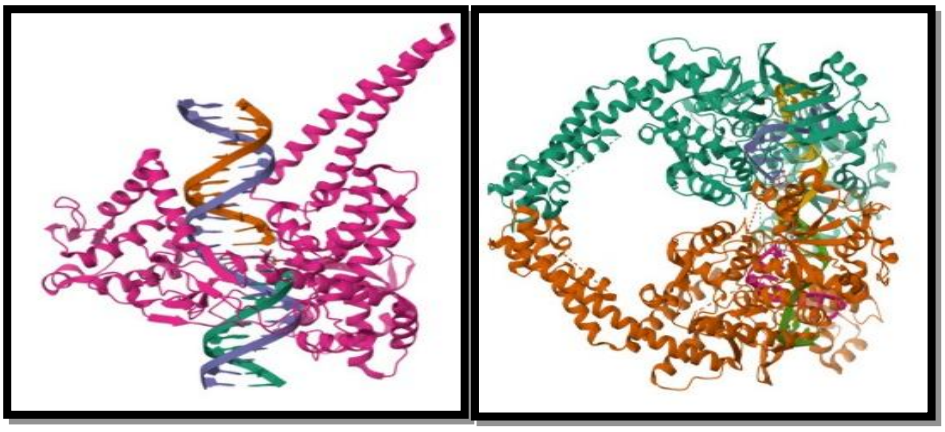


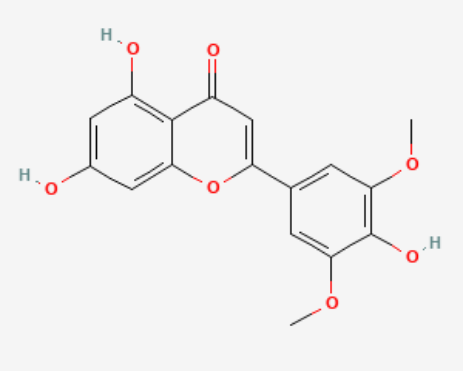
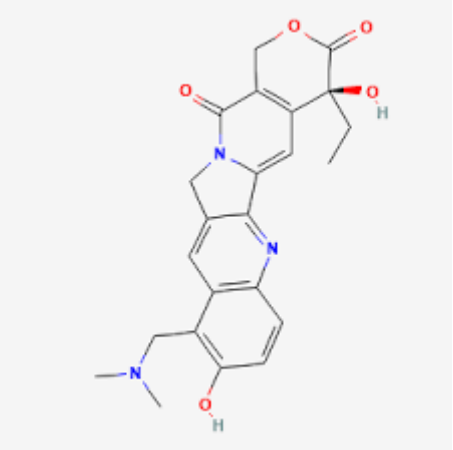
Figure 2: Structure of target molecules: a) 1K4T and b) 3QX3 retrieved from Protein databank

Determination and Preparation of Ligands:

The 3-dimensional structure of tricin and topotecan (control drug) were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format (Table 2). Using

OpenBabel tool the SDF format is converted to PDB format. Further, AutoDock tool was used to add gasteriger charge and the ligand is saved in PDBQT format.

Table 2: Ligand parameters retrieved from PubChem database

Ligands	PubChem Id	Molecular formula	Chemical structure
Tricin	5281702	$C_{17}H_{14}O_7$	
Topotecan	60700	$C_{23}H_{23}N_3O_5$	

Prediction of active site:

The Computed Atlas of Surface Topography of Protein Folds (CASTpFold) server (https://cfold.bme.uic.edu/castpfold/) was utilized to identify the active sites in DNA topoisomerases I and II. The PDB file for each protein was uploaded to the server, which predicted ligand binding sites. The binding site with the greatest surface area and volume was selected, and the amino acid residues involved in ligand binding were recorded (Ye *et al.,* 2024).

Molecular Docking:

Molecular docking of tricin and topotecan with DNA topoisomerases were conducted using the AutoDock 4.2 (Morris *et al.,* 2009), to analyze drug–biomolecule interactions and

explore possible binding modes and energies. The pdbqt format of protein and ligand were further used for docking. Site-specific docking was carried out for DNA topoisomerases. A grid box covering the predicted active site area was constructed and the number of points in X, Y, Z coordinates of the target proteins were saved (Table 3). The output was saved as grid parameter file (.gpf file). Docking was performed using Lamarckism genetic algorithm and the output was obtained in docking log file (.dlg file) format. The molecules with lowest binding energy obtained was selected to visualize the ligand-protein interaction. The molecular interactions werevisualized using Biovia Discovery Studio Visualizer.

Table 3: Grid box coordinates of DNA topoisomerases with tricin and topotecan

Target proteins	X, Y, Z coordinates	
	Tricin	Topotecan
DNA topoisomerase I (PDB ID: 1K4T)	72 × 48 × 50	42 × 64 × 40
DNA topoisomerase II (PDB ID: 3QX3)	116 × 94 × 94	122 × 114 × 114

RESULTS

Molecular descriptor calculation:

Table 4 depicts the molecular descriptor values and drug-likeness properties, as predicted by Lipinski's Rule of Five, for tricin and topotecan.

Table 4: Lipinski rule of five for tricin and topotecan

Compound	Tricin	Topotecan	Acceptable values
No. of hydrogen bond donor	3	2	<5
No. of hydrogen bond acceptor	7	7	<10
Molecular weight (g/mol)	330.29	421.4	<500
LogP	2.30	0.5	<5
No. of rotatable bond	3	3	<10

Prediction of active site:

The active sites of DNA topoisomerases I and II were predicted using the CASTpFold server (Figure 3). Spheres indicate the cavities surrounding the active sites of DNA topoisomerase I and II. The active site areas for DNA topoisomerase I and II were measured at 4846.6 Å² and 15292.8 Å², respectively, while their corresponding volumes were calculated to be 14077.9 Å³ and 39118.5 Å³.

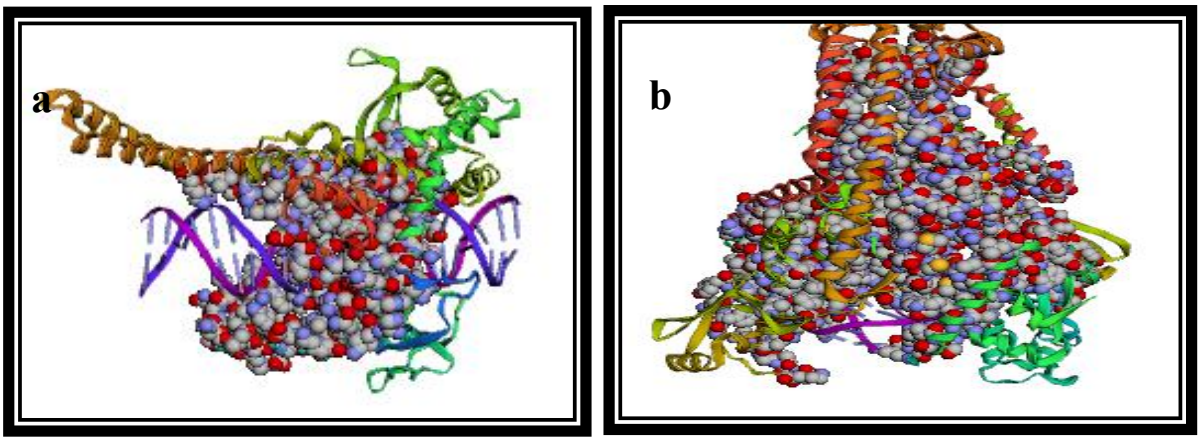


Figure 3: Active sites of DNA topoisomerase I and II predicted using CASTpFold

Molecular interactions of Tricin with DNA topoisomerases I and II

In this study, tricin and topotecan (control drug) were docked with DNA topoisomerases I and II using AutoDock 4.2 software. The binding energies, interacting amino acids and interacting bonds of tricin and topotecan docked with DNA topoisomerases I and II were depicted in table 5 and figure 4,5,6,7.

Table 5: Binding affinities and interaction profile of tricin and topotecan with DNA Topoisomerases

Ligand	Target proteins	PDB ID	Binding affinities (kcal/mol)	Interacting amino acids	Interacting bonds
	DNA topoisomerase I	1K4T	-7.70	*Gln312, Tyr308, Thr258, Glu255, Pro229, Pro233, Glu232, Pro235,*	Conventional Hydrogen bond, carbon hydrogen bond, alkyl, π-

Tricin				*Lys262, Tyr231, Phe309*	alkyl, π-cation, π- π T shaped
	DNA topoisomerase II	3QX3	-6.21	*Gln516, Leu568, Ile506, Arg503, Glu522, Asn520, Leu507*	Conventional Hydrogen bond, carbon hydrogen bond, π- sigma, alkyl
Topotecan	DNA topoisomerase I	1K4T	-6.60	*His514, His575, Val509, Ile517Lys536, Asn576, Pro578*	Conventional Hydrogen bond, carbon hydrogen bond, π- cation, alkyl
	DNA topoisomerase II	3QX3	-4.52	*Lys374, Met263, Gly359, Glu232, Lys262*	Conventional Hydrogen bond, carbon hydrogen bond, π- π T-shaped, alkyl, π-alkyl

Figure 4: Molecular interactions of tricin with DNA topoisomerase I

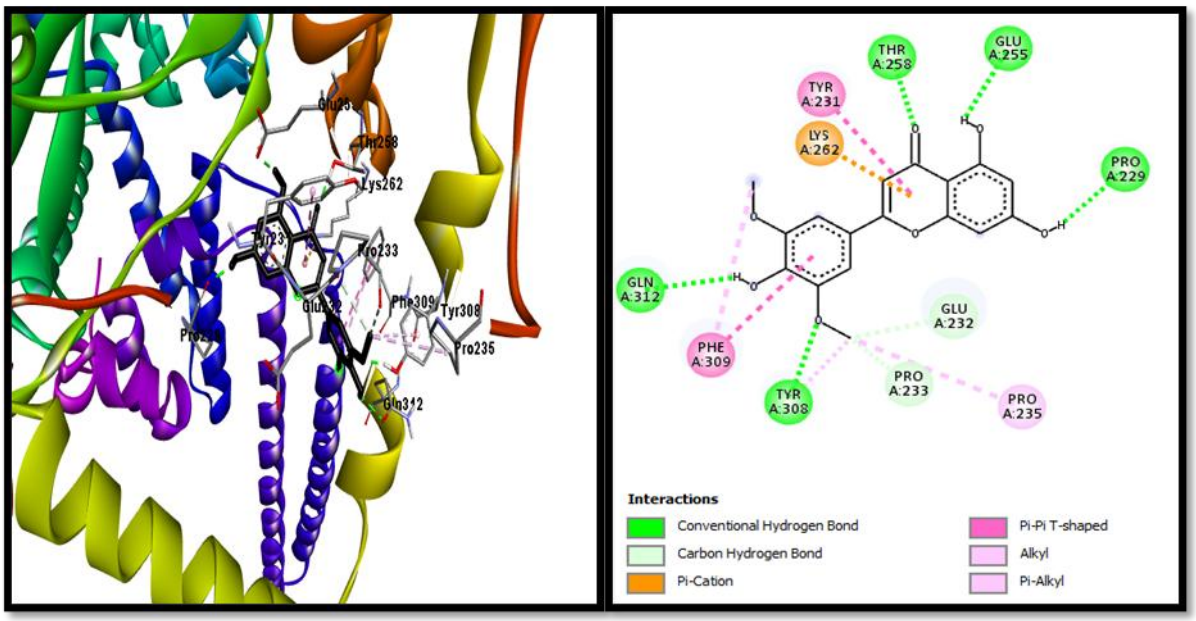


Figure 5: Molecular interactions of topotecan with DNA topoisomerase I

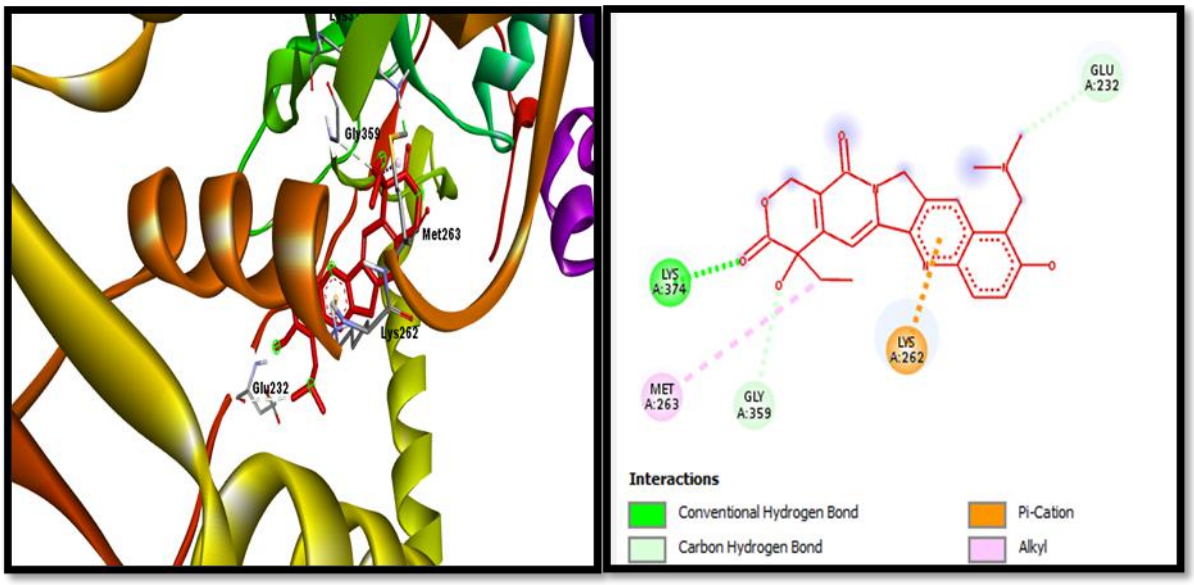


Figure 6: Molecular interactions of tricin with DNA topoisomerase II

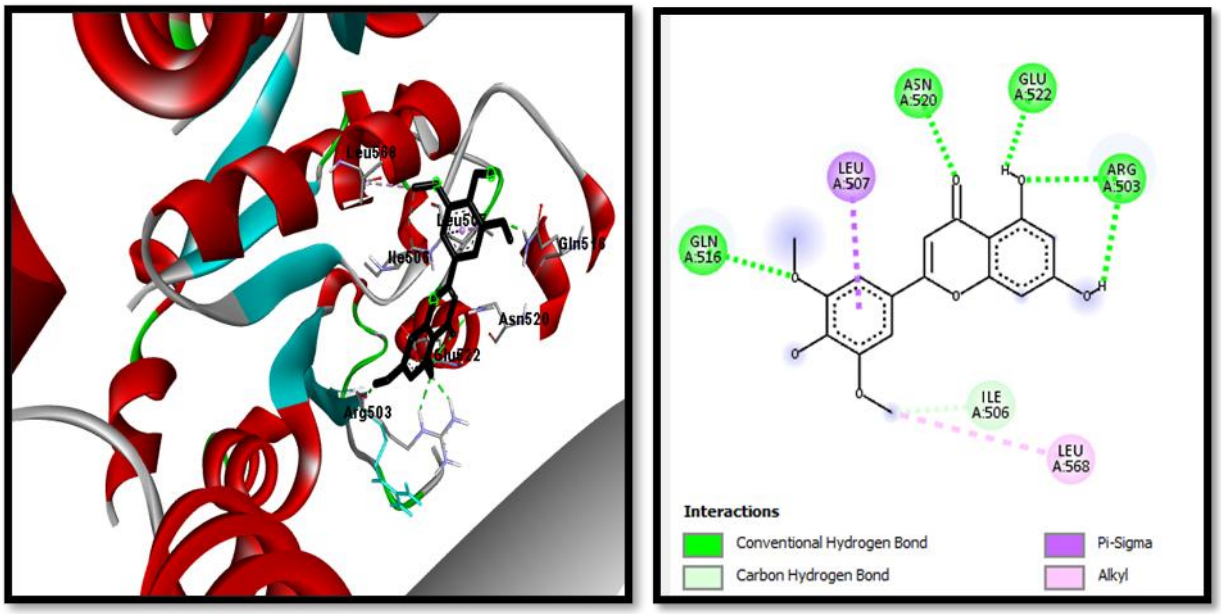
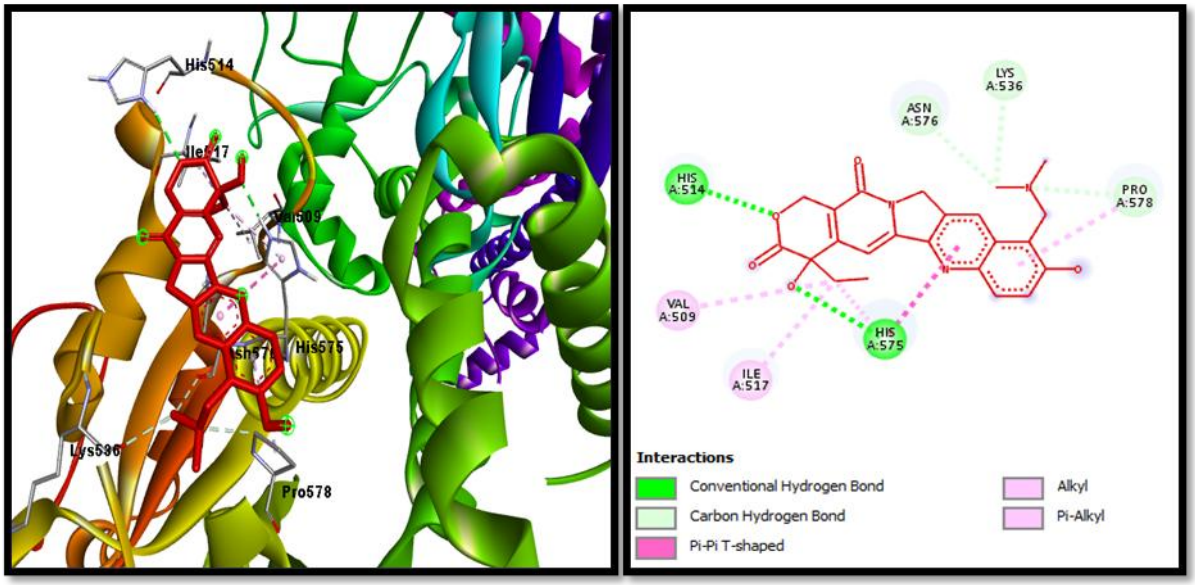


Figure 7: Molecular interactions of topotecan with DNA topoisomerase II



DISCUSSION

When the drug-likeness of tricin and the reference compound topotecan was assessed using the Molinspiration cheminformatics tool based on Lipinski's criteria it was identified that tricin had a molecular weight of 330.29 g/mol, which is clearly below the 500 Da threshold defined by Lipinski's rule, suggesting a favorable profile for absorption, distribution, and systemic transport relative to bulkier compounds. Its LogP of approximately 2.30 indicates moderate lipophilicity, supporting sufficient membrane permeability and oral bioavailability without undue hydrophobicity. Tricin contains three hydrogen bond donors and seven hydrogen bond acceptors, values that remain within the limits specified by the Rule of Five and are consistent with adequate solubility and the capacity for productive hydrogen bonding with biological targets (Table 4).

Topotecan, a synthetic anticancer agent that acts through inhibition of DNA topoisomerase I, also conforms to Lipinski's criteria, with a molecular weight of about 421.4 g/mol and a LogP near 0.5, both within the recommended range for orally active drugs. The compound possesses two hydrogen bond donors, approximately seven hydrogen bond acceptors, and three rotatable bonds, and shows no violations of the Rule of Five, supporting its established oral bioavailability and systemic exposure as a chemotherapeutic agent (Table 4).

Although both tricin and topotecan comply fully with Lipinski's rule, their lipophilicity profiles differ significantly, with tricin exhibiting a higher LogP and therefore greater lipophilic character than topotecan. This difference suggests that tricin may display

enhanced membrane partitioning and potentially greater tissue accumulation in lipid-rich compartments, whereas topotecan's lower LogP is typical of many anticancer agents designed to balance aqueous solubility and permeability for effective systemic distribution. The observed divergence in molecular weight and lipophilicity reflects their distinct chemical classes, with tricin representing a lower-molecular-weight flavonoid and topotecan a more structurally complex synthetic small molecule.

Both compounds exhibit similar numbers of hydrogen bond donors and acceptors, contributing to their aqueous solubility and enabling productive hydrogen bonding with biological macromolecules, which is critical for drug–target recognition and affinity. Consequently, tricin and topotecan both comply with Lipinski's drug-likeness criteria, supporting their suitability as orally active agents. Their distinct lipophilicity and structural features, however, are consistent with their different pharmacological roles, with tricin representing a naturally occurring flavonoid with balanced physicochemical properties and topotecan a synthetic chemotherapeutic, optimized for specific DNA interaction and systemic antitumor efficacy.

Induction of DNA damage through direct interference with DNA topoisomerases is a well-established strategy in the design of antiproliferative agents. In oncology, selective targeting of DNA topoisomerase exploits tumor vulnerabilities and offers a means to circumvent certain forms of drug resistance (Halazonetis *et al.,* 2008). In this context, the preferred binding modes of tricin to topoisomerase I and II were explored using AutoDock, revealing that its two methoxy and three hydroxy substituents confer limited conformational flexibility while providing multiple hydrogen bond–forming sites.

The pharmacological effectiveness of a small molecule such as tricin is largely governed by its binding affinity, which depends on the spectrum of non-covalent interactions established with the target receptor. These include van der Waals forces, conventional and carbon–hydrogen bonds, and π-mediated contacts such as π–π stacking and π–alkyl interactions. Collectively, these forces are essential for stabilizing ligand–receptor complexes, including those formed between tricin and topoisomerase, thereby supporting its potential anticancer activity.

Topoisomerase inhibitors, often described as "topoisomerase poisons," act by converting the native enzyme into a cytotoxic species that induces DNA damage, and hold particular relevance in oncology for tumors that are resistant to other targeted modalities. The present molecular docking analysis indicates that tricin exhibits stronger binding affinities toward topoisomerase I and II than the reference inhibitor topotecan, with topotecan showing

binding energies of −6.60 kcal/mol and −4.52 kcal/mol for topoisomerase I and II, respectively, whereas tricin achieves −7.40 kcal/mol and −6.21 kcal/mol for the same targets (Table 5). The higher affinity of tricin, especially toward topoisomerase I, suggests a promising potential as an inhibitor of these enzymes and as a lead candidate for anticancer drug development.

Both tricin and topotecan engage the active site of DNA topoisomerase I through networks of non-covalent interactions that stabilize ligand positioning and support inhibitory function, yet distinct interaction profiles emerge from their docking analyses. Tricin forms conventional hydrogen bonds with key residues such as *Gln312*, *Tyr308*, *Thr258*, and *Glu255*, leveraging its methoxy and hydroxy substituents for strong, directional contacts that anchor the ligand precisely within the catalytic pocket. These bonds are complemented by weaker carbon–hydrogen interactions with *Pro233* and *Glu232*, which fine-tune orientation, alongside π–cation contacts with Lys262 and π–π T-shaped stacking involving *Tyr231* and *Phe309* for electrostatic and aromatic stabilization. Hydrophobic contributions from alkyl contacts (*Pro235*) and π–alkyl interactions (*Phe309*) further promote compact packing and desolvation (Figure 4).

In comparison, topotecan (−6.60 kcal/mol docking score) relies on a conventional hydrogen bond with *Lys374* for primary specificity, augmented by carbon–hydrogen bonds with *Glu232* and *Gly359* that engage its polarized C–H groups with protein electronegative centers. Electrostatic reinforcement occurs *via* a π–cation interaction with *Met263*, while an alkyl interaction with *Lys262* enhances hydrophobic consolidation and van der Waals forces (Figure 5). Although both ligands exploit shared residues like *Glu232*, tricin's broader repertoire of hydrogen bonding sites and π-mediated contacts suggests greater versatility in stabilizing the enzyme–DNA–ligand ternary complex, potentially contributing to its reported superior binding energy (−7.40 kcal/mol).

The interaction between DNA topoisomerase II and tricin, characterized by a docking score of −6.21 kcal/mol, is governed by a dense network of non-covalent forces that collectively confer a highly stable binding mode. Conventional hydrogen bonds with *Gln516*, *Glu522*, *Asn520*, and *Arg503* generate directional and energetically favorable contacts between tricin’s polar substituents and complementary heteroatoms in the protein, effectively anchoring the ligand within the catalytic site. A carbon–hydrogen bond involving *Ile506* adds further, though weaker, stabilization by subtly adjusting tricin’s orientation relative to electron-rich regions, while a π–sigma interaction with *Leu507* and an alkyl contact with

Leu568 provide additional aromatic and hydrophobic reinforcement, promoting tight packing and desolvation of the binding pocket (Figure 6).

In contrast, the interaction of DNA topoisomerase II with topotecan, with a less favorable docking score of −4.52 kcal/mol, is maintained by a somewhat different but still coherent set of non-covalent contacts. Conventional hydrogen bonds with *His514* and *His575* ensure strong and selective recognition between topotecan's polar groups and the histidine side chains, whereas carbon–hydrogen bonds with *Asn576*, *Lys536*, and *Pro578* cumulatively enhance the overall affinity by engaging polarized C–H groups with electron-rich protein sites. A π–π T-shaped interaction with *His575*, together with hydrophobic alkyl contacts involving *Pro578* and π–alkyl interactions with *Val509* and *Ile517*, further stabilizes the complex through aromatic and hydrophobic packing (Figure 7).

Comparatively, tricin displays a more negative docking energy and engages a broader array of interaction types at the topoisomerase II active site, suggesting a tighter and more finely optimized fit than topotecan. The predominance of multiple strong hydrogen bonds, combined with π–sigma and hydrophobic contacts, indicates that tricin can achieve a robust and specific binding orientation likely conducive to effective enzyme inhibition. Taken together, these observations support the notion that tricin may act as a potent topoisomerase II–directed anticancer agent and, on an *in-silico* basis, may exhibit stronger inhibitory potential than the reference drug topotecan, while still requiring comprehensive experimental validation to confirm these predicted advantages.

Anthocyanins and several flavonoid subclasses have been reported as functional topoisomerase modulators with anticancer relevance. Tang *et al*. (2019) showed that blackberry anthocyanins reduced irinotecan-induced topoisomerase I cleavable complex formation and DNA strand breaks in rat colon, supporting the concept that dietary polyphenols can attenuate topoisomerase-mediated DNA damage and act as functional inhibitors of this enzyme system. Planar flavonoids such as quercetin, myricetin, and fisetin can intercalate into DNA by inserting between base pairs, perturbing helix geometry and thus interfering with the assembly and catalytic activity of topoisomerases required for replication and transcription, which contributes to their established anticancer properties (López *et al.,* 2010).

The integrated assessment of tricin and topotecan using Lipinski's Rule of Five indicates that both compounds possess molecular weight, lipophilicity, hydrogen-bonding

capacity, and rotatable bond numbers compatible with good oral bioavailability and drug-likeness. Tricin, however, exhibits a lower molecular weight and a relatively higher LogP than topotecan, features that may favor membrane permeability and potentially more favorable pharmacokinetic behavior compared with the synthetic reference drug.

Molecular docking results further demonstrate that tricin forms multiple stabilizing interactions within the active sites of topoisomerase I and II, including conventional and carbon–hydrogen bonds, π–π stacking, and π–cation contacts, yielding binding energies more favorable than those observed for topotecan in the same systems. These interaction patterns suggest a high degree of structural complementarity between tricin and critical residues within the catalytic domains of both topoisomerase isoforms, consistent with effective enzyme inhibition.

Collectively, these findings indicate that tricin not only complies with classical drug-likeness criteria but also displays substantial inhibitory potential against DNA topoisomerases through a network of non-covalent interactions that stabilize drug–enzyme–DNA complexes and impede DNA religation, a mechanism central to many clinically used topoisomerase poisons. The observed binding affinities and interaction profiles support the hypothesis that tricin could induce apoptosis in neoplastic cells *via* topoisomerase inhibition, positioning it as a compelling lead for further preclinical anticancer evaluation, including detailed pharmacodynamic characterization, toxicity assessment, and *in vivo* efficacy studies.

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STUDY ON PERSISTENCE AND ANTIBACTERIAL EFFICACY OF ACETAMINOPHEN AND STREPTOMYCIN AGAINST *E. COLI* IN *IN VITRO*

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ABSTRACT: This study investigates the potential antibacterial properties of Acetaminophen (Paracetamol), a common non-antibiotic pharmaceutical, and its interaction with the conventional antibiotic Streptomycin against clinical isolates of *E. coli*. While *E. coli* is a standard model organism, the global rise of multidrug-resistant strains has created an urgent need for novel antimicrobial strategies, including drug repurposing. Using an *in vitro* agar well diffusion assay, the research evaluated the efficacy of varying concentrations (50–500 mg/mL) of both compounds over a 72-hour period. The results demonstrate that acetaminophen possesses a measurable, dose- and time-dependent inhibitory effect. While lower concentrations showed negligible activity, higher doses (400–500 mg/mL) consistently produced visible zones of inhibition, peaking at 0.8 mm at 72 hours. In comparison, streptomycin exhibited significantly stronger and more immediate bactericidal activity across all tested levels, reaching a maximum inhibition zone of 2.0 mm. The findings suggest that acetaminophen may serve as a potential antimicrobial adjunct, possibly by inducing oxidative stress or compromising bacterial membrane integrity, thereby facilitating better antibiotic uptake. Notably, the bacterial isolates did not develop rapid resistance to acetaminophen during the study, supporting its potential as a stable supplemental agent. However, the high concentrations required for effective inhibition highlight the need for further research into localized delivery methods to mitigate risks like hepatotoxicity. Ultimately, this study underscores the importance of understanding how common pharmaceutical contaminants influence bacterial persistence and offers a new perspective on combating multidrug-resistant infections.

Keywords: Paracetamol, Acetaminophen, *E. coli, antibacterial, Synergistic*

INTRODUCTION

Escherichia coli is a Gram-negative, rod-shaped bacterium belonging to the family *Enterobacteriaceae* and is widely regarded as a fundamental model organism in

microbiological research due to its rapid growth, metabolic flexibility, and genetic tractability. While non-pathogenic strains are normal inhabitants of the gastrointestinal tract of humans and other warm-blooded animals, pathogenic *E. coli* strains possess distinct virulence determinants and resistance traits that enable them to cause a broad spectrum of intestinal and extra-intestinal diseases. Whole-genome sequencing studies have revealed that *E. coli* exhibits remarkable genomic plasticity, characterized by a dynamic pan-genome comprising a conserved core genome and an accessory genome encoded on plasmids, transposons, and pathogenicity islands, which facilitate adaptation, virulence, and associated with multidrug resistance and invasive infections, underscoring their clinical and epidemiological significance (Pitout & DeVinney, 2019; Manges *et al.,* 2023). Recent surveillance data indicate that *E. coli* continues to be a leading cause of both community- and hospital-acquired infections worldwide, with a worrying rise in multidrug-resistant strains (Manges *et al.,* 2023). Antimicrobial resistance in *E. coli* has emerged as a critical public health concern, with studies since 2018 documenting increasing resistance to commonly used antimicrobials such as β-lactams, fluoroquinolones, and aminoglycosides, largely driven by extended-spectrum β-lactamases, aminoglycoside-modifying enzymes, ribosomal mutations, and the dissemination of mobile genetic elements. Environmental studies have further demonstrated that resistant *E. coli* populations persist and spread through wastewater treatment plants, hospital effluents, and surface waters, reinforcing the importance of a One Health framework that integrates human, animal, and environmental health perspectives (Berendonk *et al.,* 2019; Hendriksen *et al.,* 2019). Streptomycin, a classical aminoglycoside antibiotic, has historically been effective against Gram-negative bacteria including *E. coli*, however, increasing resistance to streptomycin has been reported in recent years, though it remains a standard reference compound in antimicrobial susceptibility testing and combination studies exploring synergistic or adjuvant effects (Tripathi *et al.,* 2022; Ghosh *et al.,* 2023). In parallel, non-antibiotic pharmaceuticals such as Acetaminophen (Paracetamol), one of the most widely used analgesic and antipyretic drugs globally, have gained attention due to their frequent detection in aquatic environments resulting from incomplete removal during wastewater treatment. Emerging evidence suggests that paracetamol can influence bacterial physiology, stress responses, metabolic activity, and antimicrobial susceptibility in *E. coli*, potentially contributing to selective pressures within microbial ecosystems (Grenni *et al.,* 2018; Patel *et al.,* 2021). Consequently, investigating interactions between conventional antibiotics like streptomycin and widely used non-antibiotic pharmaceuticals such as paracetamol is increasingly relevant for understanding their combined effects on *E. coli*

growth, survival, and resistance development, particularly in the context of escalating antimicrobial resistance and pharmaceutical contamination of the environment. The primary goal of the present study is to evaluate how varying concentrations of acetaminophen and Streptomycin influence the growth patterns of *E. coli* within a controlled *in vitro* condition.

MATERIALS AND METHODOLOGY

Study Site

This study was conducted at the Postgraduate and Research Department of Zoology, The American College, Madurai. All procedures were performed in a sterile environment using a Laminar Air Flow cabinet and rigorous sterilization protocols.

Bacterial Strain Acquisition and Identification

The *E. coli* strains were obtained from a clinical laboratory and sub cultured to ensure viability. Standard procedures were followed to prevent cross-contamination during collection. Isolates were grown on MacConkey agar at 37ºC for 24 hours. Identification was confirmed through morphological characteristics and biochemical assays, including indole production, Methyl red, and Voges-Proskauer tests. Confirmed isolates were preserved in glycerol stocks under refrigeration. To obtain pure colonies and determine bacterial density, a ten-fold serial dilution was performed. A single colony was inoculated into 5 mL of sterile nutrient broth and incubated at 37ºC for 18-24 hours. The culture was diluted in phosphate-buffered saline (PBS) from 10^{-1} up to 10^{-6}. 100 µL from each dilution was plated onto MacConkey agar using the spread plate technique.

Media Preparation and Inoculation

Culture media, specifically MacConkey and Nutrient agar, were prepared using HiMedia™ dehydrated powders. Media were dissolved in distilled water, heated until clear, and sterilized by autoclaving at 121ºC and 15 for 15 minutes. Once cooled to room temperature, media were poured into sterile Petri dishes within a laminar flow cabinet. For the experimental use, *E. coli* was inoculated using the streak plate method to ensure discrete colony isolation.

Preparation of Acetaminophen and Streptomycin Concentrations

To evaluate dose-dependent effects, acetaminophen solutions were prepared from a 500 mg tablet source. A stock solution was initially prepared and filtered through a syringe filter for sterility. Using the dilution formula $C_1V_1 = C_2V_2$, a series of working solutions were curated.

Final test concentrations ranged from 50mg/mL to 500mg/mL. Streptomycin concentrations prepared from the commercially available injection form.

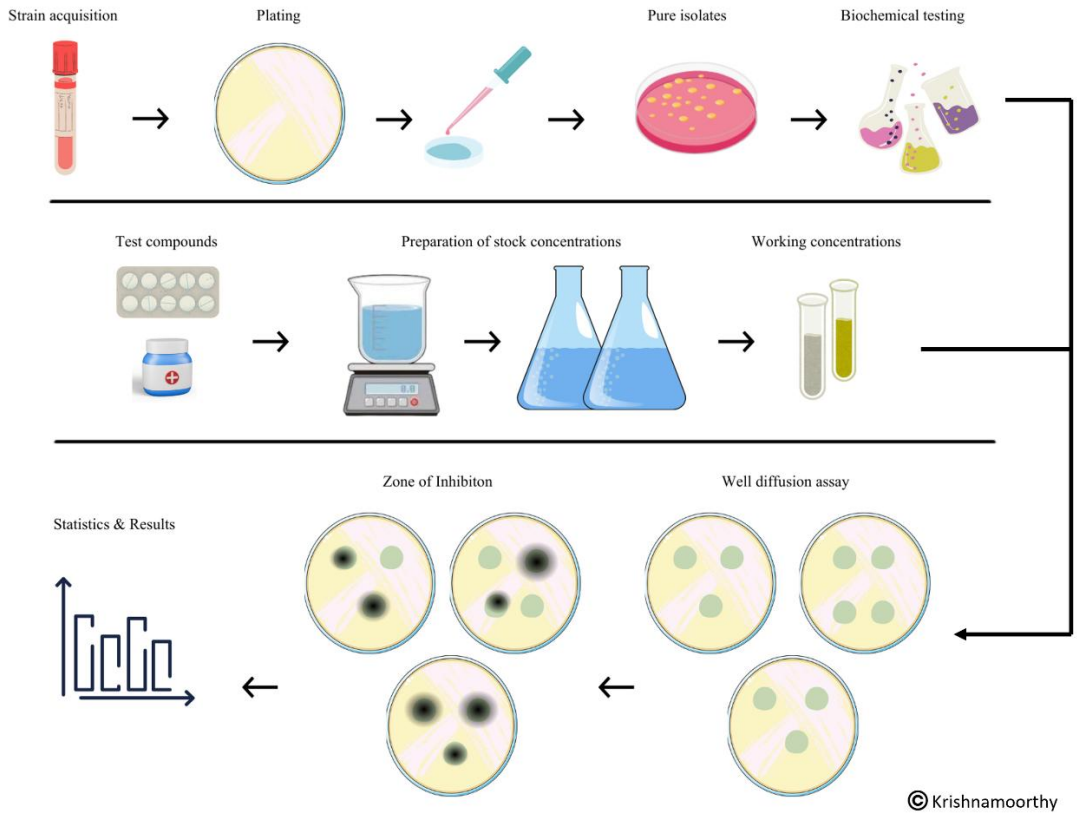

Figure 1. Illustration showing the methodology from the sample acquisition to data interpretation

Antimicrobial Susceptibility Testing

The Agar Well Diffusion method was employed to test the efficacy of the acetaminophen and Streptomycin concentrations. A sterile swab was used to create a uniform "carpet culture" of *E. coli* on the agar surface. After the inoculum was absorbed, wells with a diameter of approximately 6–8 mm were punched aseptically into the agar using a sterile cork borer. Sample Loading: 100 µL of each acetaminophen and streptomycin concentration was carefully pipetted into the designated wells. Plates were incubated in an inverted position at 37ºC for 24 hours to allow for bacterial growth and drug diffusion. Post-incubation, the plates were examined for visible Zones of Inhibition and the results were recorded for analysis.

RESULTS

The antimicrobial potential of acetaminophen against *Escherichia coli* was analyzed using the Agar well diffusion assay, with streptomycin as reference antibiotic. Acetaminophen used, ranged from 50-500 mg/mL, and zones of inhibition (ZOIs) were measured at 12, 24, 48, and 2 hours (h) in experimental period under standardized incubation conditions. Control plates containing solvent alone showed no inhibition throughout the study. Acetaminophen

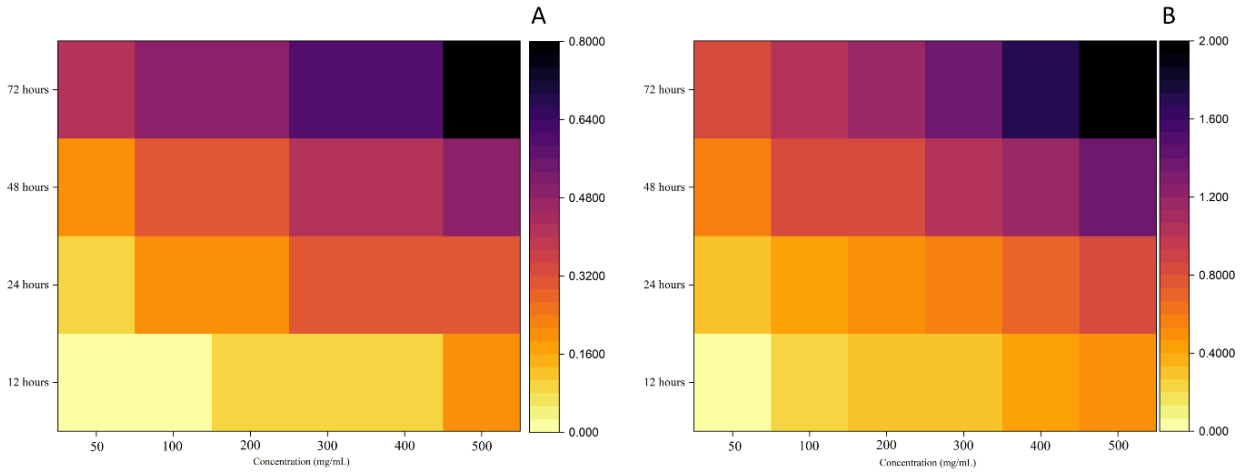

Figure 1. Heatmaps A and B showing the antibacterial effect against the *E. coli* isolates. Heat map A represents the acetaminophen and heatmap B represents streptomycin respectively, wherein Concentrations were plotted in X axis and the treatment time in Y axis

exhibited a clear concentration and time-dependent inhibitory effect against *E. coli*. No inhibition was recorded at 12 h, at the lowest concentration of 50 mg/mL with minimal activity emerging only after prolonged exposure, reaching a ZOI of 0.4 mm at 72 hours. Larger zones of inhibition resulted from incremental increases in acetaminophen concentration. At 200-300 mg/mL, ZOIs became detectable within 24 h and increased modestly with time, whereas higher concentrations (400 and 500 mg/mL) increased the inhibition magnitude. The maximum ZOI for acetaminophen was 0.8 mm at 500 mg/mL at 72 h. This trend was consisted throughout experiment showing the substantial response, while lower concentrations continued to exhibit weak inhibition. These findings indicate that acetaminophen exerts measurable but limited antibacterial activity against *E. coli*, which becomes evident primarily at higher concentrations and extended exposure times. In contrast, streptomycin exhibited higher antimicrobial activity across all concentrations and time points. Detectable inhibition was observed even at 50 mg/mL, with ZOIs increasing consistently with both dose and incubation time. At 500 mg/mL, streptomycin produced a ZOI of 2.0 mm at 72 h and comparable inhibition levels were sustained in subsequent weeks. Across all concentrations, streptomycin consistently showed greater activity that acetaminophen,

highlighting the moderate autonomous antimicrobial effect. Heatmaps 1 and 2 emphasized that acetaminophen produce relatively limited but reproducible inhibition, streptomycin exhibited strong and consistent antibacterial activity and the combined treatment showed a steep and sustained enhancement of inhibition across concentrations and incubation periods. Collectively, these results show that acetaminophen possesses mild antibacterial activity against *E. coli* in a dose- and time-dependent manner, underscoring its potential as an adjunct in antimicrobial therapy.

DISCUSSION

The present study investigated the antimicrobial activity of acetaminophen and streptomycin against clinically isolated *E. coli* strain. The findings are particularly significant in the context of increasing antimicrobial resistance, as they highlight how the non-antibiotic pharmaceuticals possess the potential to modulate bacterial susceptibility and enhance the efficacy of conventional antibiotics. The well diffusion assays demonstrated that acetaminophen exhibits a concentration-dependent inhibitory effect against *E. coli*. At low concentrations (50–100 mg/mL) the inhibitory effect was negligible, while higher concentrations (400–500 mg/mL) consistently yielded measurable ZOI across all four weeks. The gradual increase in inhibition magnitude over time, culminating in a maximum ZOI of 1.6 mm at 500 mg/mL, suggests that acetaminophen exerts a mild but persistent antibacterial effect that becomes evident primarily under prolonged exposure. These observations align with earlier reports indicating that certain non-antibiotic drugs can interfere with bacterial growth through indirect mechanisms such as oxidative stress induction or disruption of membrane integrity (Smirnova et al. 2005; Hall et al. 2024). Streptomycin demonstrated greater antimicrobial activity, inhibiting growth at lowest concentration and maintaining strong bactericidal activity across all time points. This outcome is consistent with the established aminoglycosides potency against Gram-negative bacteria and puts the relatively mild dose response effect of acetaminophen in proper perspective. This suggests that acetaminophen may enhance antibiotic activity by adjunct with the streptomycin treatments. One explanation is that acetaminophen induces oxidative or metabolic stress compromising the bacterial membrane integrity or redox homeostasis and in turn facilitating the antibiotic uptake. This interpretation is consistent with previous observations reporting acetaminophen-induced stress responses in bacteria, including the modulation of antioxidant defence systems and redox-sensitive pathways (Smirnova et al. 2005; Shaikh et al. 2024; Abdullah et al. 2024). Synergistic interactions between non-antibiotic pharmaceuticals and antibiotics have

been documented with other drug classes, lending further support to the concept that such interactions may represent a broader and underappreciated phenomenon (Drees et al. 2023). An important observation from this study is the antimicrobial effects were consistent over the experimental period, with no apparent attenuation of inhibitory capacity. This suggests that *E. coli* doesn't develop resistance rapidly to acetaminophen under repeated exposure, a finding consistent with reports shown that non-antibiotic drugs do not readily drive cross-resistance to antibiotics (Hall et al. 2024). Such characteristic makes the acetaminophen more appealing as a supplement rather than a replacement for antibiotics. Despite this, it should be stated that the translational relevance of this data has several limitations. Firstly, the concentrations required to display significant antimicrobial activity is higher than that used in typical systemic therapeutic levels of acetaminophen, raising concerns regarding toxicity and feasibility. While localized or topical applications may overcome some of these limitations, issues related to hepatotoxicity and environmental persistence warrant careful consideration (Agarwal 2022; Gorrochategui et al. 2023). Overall, this study suggesting that non-antibiotic drugs can possess latent antibacterial properties more importantly, can potentiate antibiotic activity that they may adequately enhance the efficacy of antibiotics.

CONCLUSION

The present study demonstrates that acetaminophen (paracetamol), a common non-antibiotic pharmaceutical, possesses measurable dose- and time-dependent antibacterial activity against clinical isolates of *E. coli* in the *in vitro* conditions. Compared to streptomycin, acetaminophen exhibits a consistent and persistent ability, particularly at higher concentrations and extended exposure periods. The experimental results suggest a potential synergistic or adjunct role for acetaminophen in antimicrobial therapy. this study suggest that may acetaminophen enhance the antibiotic efficiency of some antibiotics by changing the membrane integrity and the lack of rapid resistance development to acetaminophen during the study period highlights its potential as a stable supplemental agent. Despite these promising *in vitro* findings, the high concentrations required for significant antimicrobial action, necessitate further investigation into localized delivery methods and potential toxicity. Ultimately, this research underscores the importance in understanding how pharmaceutical contaminants in the environment influence bacterial persistence and highlights a novel pathway for drug-repurposing strategies to combat the global challenge of multidrug-resistant *E. coli* strains.

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DISTRIBUTION OF MAYFLIES FROM SELECTED FRESHWATER HABITATS IN AROUND MADURAI

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ABSTRACT: Distributions of mayflies were investigated in selected freshwater habitats in and around Madurai, Tamil Nadu. A total of 453 specimens belonging to 8 genera and 4 families were collected from 5 streams and 10 ponds in study sites. Among Stream ecosystems, the Shannon index was highest in AVM bridge (1.938) and lowest in Kamatchipuram (0.9653). In pond habitats, the Shannon index was highest in Thanakankulam (0.6693) while the lowest was recorded at Melamathur (0.2573). Simpson's index was highest in AVM bridge (0.8336) and lowest in Kamatchipuram (0.5877). In pond highest Simpson's index was observed at Thanakankulam (0.4764), whereas the lowest value was recorded at Melamathur (0.1327). In stream, highest abundance of mayflies was collected in the site of the Saibaba temple and lowest abundance of was observed at Vilachery. In pond ecosystems, the greater number of mayflies were collected in the site of Thanakankulam and least number of mayflies were collected in the site of Harvey patti. The Canonical Correlation Analysis (CCA) results revealed that pH, Alkalinity, Hardness, water temperature and Magnesium are the major stressors influencing the distribution of mayfly taxa in freshwater habitats.

KEYWORDS: AVM bridge, Thanakankulam, Mayflies, Shannon index, Simpson's index.

INTRODUCTION

Mayflies (Ephemeroptera) are a globally distributed group of insects, with the highest diversity in tropical areas and over 3000 worldwide known species (Barber - James *et al.,* 2008). They have been utilized as ideal organisms for biological monitoring studies (Barbour *et al.,* 1996). Mayfly larvae feed on detritus and are thus highly sensitive to changes in the substrate and to any toxic material entering the water (Landa and Soldán, 1995). The nymphs undergo a series of moults as they grow, the precise number being variable within a species,

depending on external factors, such as temperature, food availability and current velocity. The various freshwater ecosystem consists of ponds, lakes, streams, reservoirs, rivers, temporary puddles, swamps, thermal springs and pool. There are two types of inland water habits are lotic (flowing) and lentic (stagnant). The lotic system encompasses rivers and streams. Lentic systems comprise lakes and ponds. Mayflies are considered as ‘‘keystone’’ species and their presence is believed to be an important environmental indicator of oligotrophic to mesotrophic (i.e. low to moderately productive) conditions in running waters. A high sensitivity of mayfly taxa to oxygen depletion, acidification, and various contaminants, including metals, ammonia and other chemicals, was demonstrated in both observational and experimental studies (Hubbard & Peters, 1978; Resh Tand Jackson, 1993; Hickey & Clements, 1998). Several regional studies have documented the diversity and community structure of Ephemeroptera in Indian freshwater ecosystems, including streams of, Temporal variation of mayflies in response to ecological attributes in Gadana (Sivaruban *et al.,*2020), Community structure of mayflies (Insecta: Ephemeroptera) in tropical streams of Western Ghats of Southern India (Barathy *et al.,* 2020),Distribution of mayflies in thirty streams of Western Ghats, Southern India (Barathy *et al.,* 2021a),Chinnasuruli Falls in the Megamalai Hills (Barathy *et al.,* 2021b),Diversity of the EPT complex (Ephemeroptera, Plecoptera and Trichoptera)in the Western and Eastern Ghats (South India) caused by the variations of landscape elements and mesohabitats (Sivaruban et al 2022) and the “sky islands” of the Palni Hills in the Western Ghats (Selvakumar *et al.,* 2025).

MATERIALS AND METHODS

Study area:

Ponds - Kumaran colony pond, Vadivelkarai pond, Harveypatti, Sambakulam pond, SN college, Near Kamatchipuram govt school, Thanakankulam, Melamathur, Thuvariman, Thiruparankundram.

Streams – Vilacheri, Keelamathur, Near AVM bridge, Saibaba temple, Kamatchipuram stream.

Water Sample Analysis

Measuring the physico-chemical properties of stream water, including dissolved oxygen, pH, temperature, calcium, magnesium, hardness, and alkalinity, using the APHA (2005) guidelines.

Macroinvertebrate collection and identification

Mayflies are collected by the hand-picking method. Larval specimens were preserved in 80% ethanol. Examined under a stereomicroscope and light microscopy. Identified with the help of a field guide by Sivaramakrishnan *et al.* (1998) & Barathy *et al.* (2021).

Data Analysis

The data analysis was done with the help of PAST software (version 4.2) to measure various diversity indices (Hammer *et al.,* 2001).

RESULTS

Samples of Ephemeroptera larvae were collected from 10 different sites of the pond and 5 different sites of the stream in 2024 – 2025. Sampling results in a total number of 453 specimens belonging to 8 genera and 4 families. Shannon-Wiener and Simpson diversity indices were used to evaluate the total diversity of mayfly taxa of the study sites. In the pond ecosystem, the Shannon index was highest at Thanakankulam (0.6693), indicating the presence of the highest diversity of mayfly taxa in the ecosystem and lowest at Melamathur (0.2573). Simpson's index was highest in Thanakankulam (0.4764) and lowest in Melamathur(0.1327). The evenness value was almost high at Thanakankulam (0.9765) and lowest at Vilachery (0.8774). In stream habitats, the Shannon index was highest at AVM bridge (1.938), which indicates the presence of the highest diversity of mayflies in the ecosystem and lowest at Kamatchipuram (0.9653). Simpson's index was highest at the AVM bridge (0.8336) and lowest at Kamatchipuram (0.5877). The evenness value was almost high in Kamatchipuram (0.8752) and lowest at Vilachery (0.761) (Table 3). Water quality parameters such as DO, Water Flow, Alkalinity, Hardness and pH were analysed.

In Stream ecosystems, the greater number of mayflies were collected in the site of the Saibaba temple and least number of mayflies were collected in the site of Vilachery. In Ponds habitats, a greater number of mayflies were collected in the site of Thanakankulam, whereas least number of mayflies were collected in the site of Harvey Patti. Among the observations, family Baetidae was almost abundant across all study sites (both stream and pond ecosystems) *In* stream*, Acentrella vera* was the dominant species and contributed the highest number of specimens across sites. In Ponds**,** *Cloeon bicolor* was the most abundant species and occurred at all sampling locations.

The Family Caenidae is the abundant mayfly taxa exist in the river. In stream, *Caenis kaegies* is collected only a few study sites, whereas in the pond, *caenis kaegies* was rich in all study sites. *Trichorythus meenakshi* is collected only from a few study sites. In the Leptophlebiidae family, *Choroterpes latus* were collected in more numbers. Canonical Correspondence Analysis (CCA) revealed that pH, water temperature, alkalinity, and calcium were the major environmental factors influencing mayfly distribution in freshwater habitats in and around Madurai **(**Fig. 1**).**

DISCUSSION

Environmental characteristics such as water temperature, dissolved oxygen, and habitat heterogeneity remain key drivers of mayfly assemblages. Studies of macroinvertebrate communities in subtropical African river systems indicate that spatiotemporal environmental variation strongly structures aquatic insect communities, including mayflies, reinforcing the importance of physical and chemical habitat variables in shaping diversity and community composition (Munyai *et al.,* 2025). This study, Ephemeropteran of fresh water habitats in and around studied in 15 selected sites. The high scores on diversity indices, such as the Shannon index and Simpson index, indicate that unpolluted drivers support a greater diversity of animals (Lenatand Penrose, 1996). In this study, Shannon and Simpson index values in the stream were very less in the site of Kamatchipuram (0.9653), and Kamatchipuram (0.5877) (Table 3). In the pond, the study Shannon and Simpson index values in the stream were very less in the site of Melamathur (0.2573) and Melamathur (0.1327) (Table 3). It results in a total number of 453 specimens belonging to 8 genera and 4 families (Tables 4 and 5). So, this shows that a comparison between the stream water and pond water, the pond water is more polluted than the stream water, and the diversity of insects is less in pond water than stream.

The Canonical Correspondence Analysis shows the species like *Labiobaetis pulchellus, Tenuibaetis kaltenbachi, Nigrobaetis klugei,* and *Tricorythus meenakshi* were positively correlated with a strong concentration of physicochemical parameters, Hardness, calcium, pH, and negatively correlated with DO and Magnesium. Rainfall, water flow, turbidity and air temperature are vital in governing the diversity and distribution of mayfly larvae (Barathy *et al.,* 2021a). Water temperature directly affects both the nature of aquatic fauna and species diversity. Temperature can also affect organisms indirectly as a consequence of oxygen saturation levels. As water temperature rises, the metabolism of aquatic insects increases, which leads to more oxygen requirements.

At higher water temperatures, the oxygen-carrying capacity of water decreases because of a diminished affinity of water for oxygen. In the present investigation, the water temperature was recorded between 28℃ to 33℃. Higher water temperature was seen in the sites of pond water, such as near SN college, 33°C and Melamathur, 28°C. Normal DO level in the lentic and lotic waters is 2.0 – 7.2 mg/L. In the present investigation, the DO was found to be low in the site of pond water near SN college, 3.0 mg/L. The lowest DO, which might be caused by contamination from community wastewater, anthropogenic impacts and lack of rainfall and due to an increase in temperature. The main source of dissolved oxygen for running water is from the atmosphere through the mixing process. It is different for standing water due to the photosynthesis of microorganisms. Previous studies show significant mortality occurred during the experiments as the proportion of dead individuals increased exponentially with decreasing DO. In the present study, the DO level shows that decrease in the freshwater habitats, which leads to less Ephemeropteran richness.

The family Baetidae of order Ephemeroptera from this study can be proposed as an indicator of water quality and ecosystem health primarily because of its presence in both the polluted and unpolluted water. However, it appears to be sensitive to pollution as its number significantly reduced in September and January. The genera Baetis and Caenis from earlier studies have been reported to be tolerant to organic pollution (Menetrey *et al.,*2007). Human activities and continue to have a significant effect on mayfly diversity throughout the world (LandaandSoldán,1995). Several mayfly species have become extinct, and many are endangered and figure in the national Red Lists. Overall, the findings emphasize that anthropogenic pressures are a major threat to freshwater ponds and streams, leading to habitat degradation and reduced biological diversity. Therefore, immediate conservation measures and sustainable management practices are necessary to protect these ecosystems and preserve their ecological functions.

TABLE 1 PHYSICO- CHEMICAL PARAMETERS OF SELECTED FRESH WATER HABITATS (PONDS) IN AND AROUND MADURAI, TAMIL NADU

S.NO	SITE	DO(mg/L)	ALKALINITY (mg/L)	HARDNESS (mg/L)	CALCIUM (mg/L)	MAGNESIUM (mg/L)	TEMPERATURE	PH (mg/L)
1	Kumaran colony	4.096	32	70	8.016	12.182	32	7.8
2	VadivelKarai	4.096	40	50	16.032	2.436	32	7
3	Harveypatti	5.19	36	60	16.833	4.384	31	7.5
4	Sambakulam	4.096	36	60	15.230	5.360	32	7
5	SN College	3.072	28	50	16.833	4.38	33	7
6	Kamatchipuram	6.144	48	40	8.016	4.872	29	7.2
7	Thanakankulam	4.096	36	70	16.032	7.304	32	7.8
8	Melamathur	3.072	24	90	14.428	13.15	33	7
9	Thuvariman	7.242	40	104	9.214	20.46	28	7.8
10	Thiruparankundram	7.242	40	90	10.42	15.634	28	6.8

TABLE 2 PHYSICO CHEMICAL PARAMETERS OF SELECTED FRESH WATER HABITATS (STREAM) IN AND AROUND MADURAI, TAMIL NADU

Sl. NO	SITE	DO (mg/L)	ALKALINITY (mg/L)	HARDNESS (mg/L)	CALCIUM (mg/L)	MAGNESIUM (mg/L)	TEMPERATURE	PH (mg/L)
1	Near avm bridge	4.096	200	14	8.016	9.25	30	7
2	Saibaba temple	7.168	80	6	15.23	8.76	29	7.6
3	Keelamathur	6.144	240	10	6.412	4.86	29	7.2
4	Velachery	3.072	200	8	16.032	9.74	31	7
5	Kamatchipuram	2.048	120	6	15.230	7.79	31	7.2

TABLE 3 DIVERSITY INDICES

	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 6	SITE 7	SITE 8	SITE 9	SITE 10	SITE 11	SITE 12	SITE 13	SITE 14	SITE 15
Simpson_1-D	0.7377	0.8188	0.8336	0.8271	0.5877	0	0	0	0.4444	0.4592	0	0.4764	0.1327	0	0.375
Shannon_H	1.519	1.789	1.938	1.88	0.9653	0	0	0	0.6365	0.6518	0	0.6693	0.2573	0	0.5623

site 1- Vilacheri, site 2- Keelamathur, site 3- near Avm Bridge, site 4- saibaba temple, site 5-kamatchipuram, site 6-kumaran colony, site 7- Vadivel Karai, site 8- Harvey Patti, site 9- Sambakulam, site 10- SN College, site 11- near Kamatchipuram Govt School, site 12- Thanakankulam, site 13- Melamathur, site 14- Thuvariman, site 15- Thiruparankundram

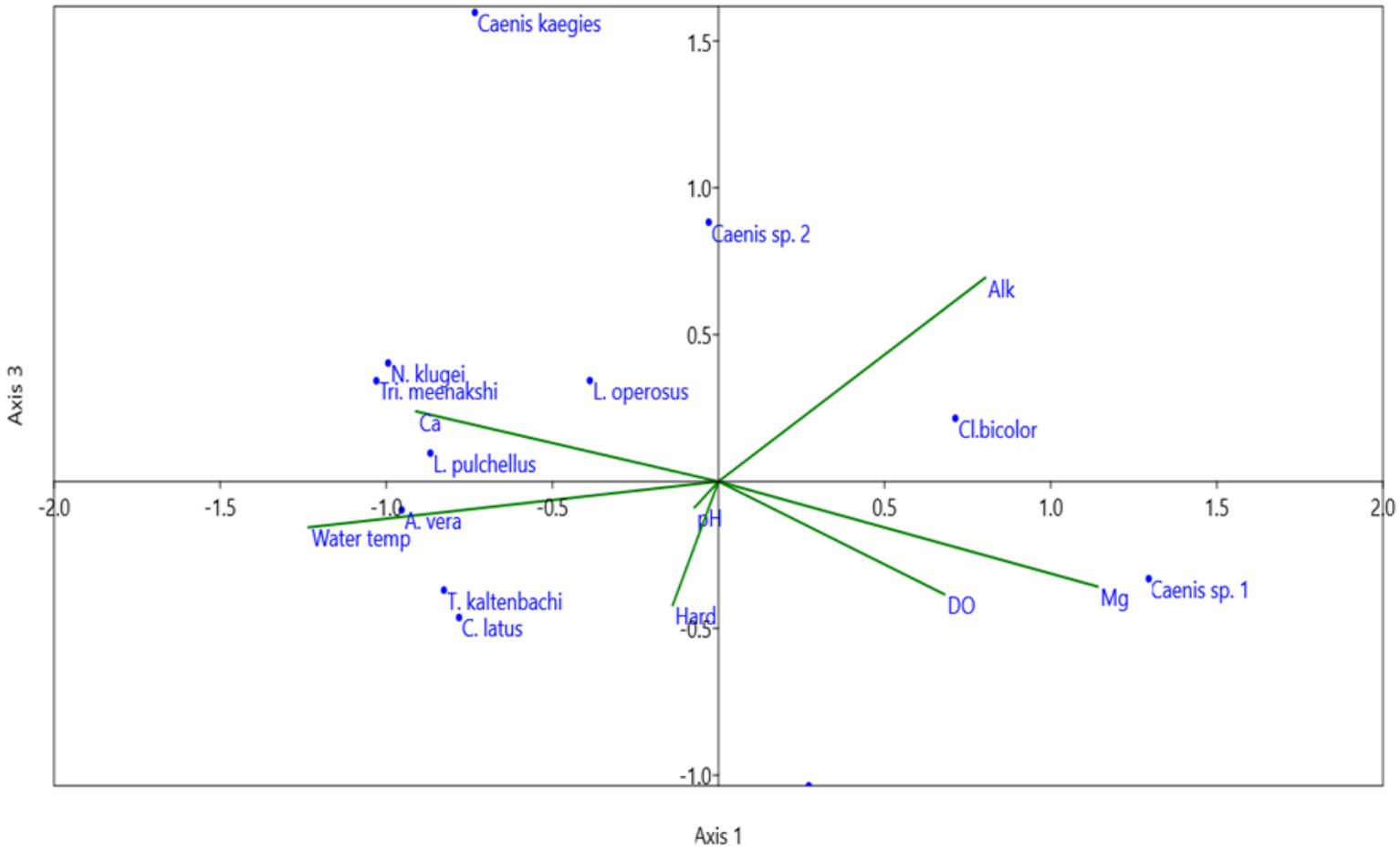
TABLE 4 TAXONOMIC INVENTORY OF EPHEMEROPTERA FROM THE SELECTED FRESH WATER HABITATS (PONDS) IN AROUND MADURAI, TAMIL NADU

FAMILY	**GENUS AND SPECIES**	**SITE 1**	**SITE 2**	**SITE 3**	**SITE 4**	**SITE 5**	**SITE 6**	**SITE 7**	**SITE 8**	**SITE 9**	**SITE 10**
Caenidae	*Caenis venkataramani*	0	0	0	0	0	0	18	0	0	0
	Caenis kaegies	0	2	0	3	11	0	0	8	0	0
	Caenis sp.1	0	0	0	6	18	0	0	2	0	0
	Caenis sp.2	0	0	0	0	0	0	0	0	0	6
Baetidae	*Cloeonbicolor*	28	11	13	12	10	17	28	26	17	18

TABLE 5 TAXONOMIC INVENTORY OF EPHEMEROPTERA FROM THE SELECTED FRESH WATER HABITATS (STREAM) IN AROUND MADURAI, TAMIL NADU

FAMILY	GENUS AND SPECIES	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5
Leptophlebiidae	*Choroterpes latus*	13	11	4	6	12
Baetidae	*Nigrobaetis klugei*	0	12	9	12	0
	Labiobaetis pulchellus	3	8	5	9	0
	Labiobaetis operosus	2	0	3	0	0
	Acentrella vera	5	8	10	14	17
	Tenuibaetis kaltenbachi	3	5	0	7	0
Caenidae	*Caenis kaegies*	0	0	10	0	0
	Caenis venkatarmani	15	1	1	2	4
	Caenis sp.	0	0	1	2	0
Tricorythidae	*Tricorythus Meenakshi*	0	4	2	3	0

FIG. 1 CCA ANALYSIS OF MAYFLY TAXA



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HIGH-RESOLUTION INSIGHTS INTO BAT MOVEMENT: THE ROLE OF GPS TRACKING IN CONSERVATION ECOLOGY

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ABSTRACT: Global Positioning System (GPS) technology represents a revolutionary step in wildlife ecology, offering unprecedented precision in tracking animal movements. In bats, which are critical providers of ecosystem services such as pollination, seed dispersal, and insect pest control, GPS tracking enables researchers to obtain fine-scale insights into flight paths, roost fidelity, commuting ranges, and foraging landscapes. Traditional methods such as roost surveys, mist-netting, mark–recapture studies, and acoustic monitoring have yielded valuable ecological knowledge but remain limited in their ability to capture continuous spatiotemporal data. The miniaturization of GPS loggers, now deployable on medium- to large-bodied bat species, has transformed movement ecology by facilitating automated and high-resolution data collection. This paper develops a conceptual framework for GPS tracking in bats, focusing on species selection, device deployment, data acquisition, analysis, and conservation applications. By standardizing protocols, GPS-based research can provide fine-scale ecological insights, inform habitat and corridor design, and strengthen biodiversity conservation strategies.

KEYWORDS: GPS tracking, bats, movement ecology, biologging, conservation, ecosystem services

INTRODUCTION

Bats (Order Chiroptera) are the second most diverse order of mammals, with more than 1,400 species described worldwide (Simmons & Cirranello, 2021). They are keystone species in many ecosystems, providing vital ecological services ranging from pollination and seed dispersal to insect population regulation (Kunz *et al.,* 2011; Maas *et al.,* 2016). These services extend beyond natural ecosystems into agricultural landscapes, where bats significantly reduce pest abundance and thereby enhance crop productivity (Boyles *et al.,* 2011; Wanger *et al.,* 2014). Despite their ecological and economic importance, bats are threatened globally by habitat destruction, roost disturbance, climate change, and emerging

infectious diseases (Kingston, 2016; Brook & Dobson, 2015). Effective conservation of bats requires not only the protection of roost sites but also a detailed understanding of their spatial ecology, movement dynamics, and interactions with human-modified landscapes.

For decades, the study of bat movement relied on techniques such as visual surveys, banding, radiotelemetry, and acoustic monitoring (White & Garrott, 2012; Weller *et al.,* 2016). Radiotelemetry in particular advanced understanding of nightly foraging ranges and roost switching but suffered from limitations in signal range, manpower requirements, and data resolution (Singaravelan & Marimuthu, 2004; Wikelski *et al.,* 2007). The introduction of GPS tracking has overcome many of these constraints, allowing researchers to record precise location data over extended periods with minimal disturbance to the animal. GPS has enabled detailed reconstructions of bat flight paths, migratory corridors, and foraging patterns, offering a fine-scale perspective that is essential for conservation planning and ecosystem management (Kays *et al.,* 2015; O'Mara *et al.,* 2019).

This paper proposes a conceptual framework for the integration of GPS tracking in bat research. It emphasizes species selection, device deployment, data collection, analysis, and conservation applications, while also addressing the challenges and future directions of this rapidly evolving field.

CONCEPTUAL FRAMEWORK FOR GPS TRACKING IN BATS SPECIES SELECTION

The first step in designing a GPS-based study is careful species selection. Device size and weight remain important constraints, and ethical guidelines recommend that attached equipment not exceed 5% of an animal's body weight (Aldridge & Brigham, 1988). This makes large-bodied bats, such as fruit bats (Pteropus, Rousettus) and carnivorous bats (Megaderma lyra), suitable candidates for GPS tracking (O'Mara *et al.,* 2019). These species are particularly important for ecological and conservation studies because of their long-distance dispersal, migratory behaviour, and significant roles in ecosystem service provision (Fleming *et al.,* 2009). By focusing on such species, researchers can maximize the ecological relevance of GPS datasets while minimizing animal welfare risks.

DEVICE SPECIFICATIONS AND DEPLOYMENT

Modern GPS loggers are compact and lightweight, with some models weighing as little as1.5–2 g and offering location accuracy of <10 m (Ripperger *et al.,* 2020). They can

bedeployed in two primary ways: glue-on attachments for short-term studies and backpack harnesses for long-term tracking (Weller *et al.,* 2016). The choice of attachment depends on study objectives. Short-term deployments are useful for investigating nightly foraging behavior, whereas long-term studies are essential for mapping migratory routes and seasonal habitat use.

Device specifications, including fix frequency and battery life, must be optimized to balance data resolution with deployment duration. For example, frequent fixes provide high-resolution flight paths but reduce battery life, whereas less frequent fixes extend monitoring but capture less detail (Kays *et al.,* 2015). The integration of additional sensors, such as accelerometers or acoustic microphones, offers the possibility of linking movement data with behavior and foraging activity (Toledo *et al.,* 2020).

DATA COLLECTION

Once deployed, GPS devices provide continuous, automated spatiotemporal data on bat movements. Parameters recorded include geographic coordinates, altitude, speed, and distance traveled. Data can be stored onboard or transmitted remotely, depending on the device. Remote data transmission, while more expensive, reduces the need for device recovery and minimizes data loss (Kranstauber *et al.,* 2011). Increasingly, researchers are integrating GPS data into global platforms such as Movebank, which facilitate standardized storage, sharing, and analysis of movement data across species and regions (Kays *et al.,* 2020).

DATA ANALYSIS

GPS-derived datasets are analyzed using a variety of spatial and statistical tools. Kernel density estimators and minimum convex polygons are commonly employed to estimate home ranges and core foraging areas (Calenge, 2006). Habitat selection analyses link bat movements to landscape features, revealing preferences for specific vegetation types, crop systems, or roosting habitats (O'Mara *et al.,* 2019). GIS mapping further supports the visualization of movement patterns and their overlap with anthropogenic features such as farmland or urban areas. Recent advances in machine learning offer opportunities for automated classification of bat behaviors from movement trajectories, enabling predictive modeling of responses to environmental change (Kays *et al.,* 2020).

CONSERVATION APPLICATION

The conservation applications of GPS tracking are diverse and profound. Long-term GPS studies have revealed the transboundary migrations of flying foxes, highlighting the need for regional cooperation in bat conservation (Tidemann & Nelson, 2004). Similarly, GPS- based monitoring of vampire bats has improved understanding of their commuting patterns and interactions with livestock, informing strategies to mitigate rabies transmission and human–wildlife conflict (Streicker *et al.,* 2016). In agricultural landscapes, GPS data have identified foraging hotspots where bats provide significant pest suppression, underscoring their role as natural biocontrol agents (Wanger *et al.,* 2014). Beyond ecosystem services, GPS studies inform the design of ecological corridors, guide roost protection efforts, and contribute to assessments of zoonotic disease spillover risks (Brook & Dobson, 2015).

The integration of GPS technology into bat ecology can best be understood as a structured, cyclical process. Each stage—species selection, device deployment, data collection, data analysis, and conservation applications—forms a vital link in this workflow. Together, they ensure that technological advancements are ethically implemented, scientifically rigorous, and practically relevant for conservation. The conceptual workflow is summarized in Figure 1, which illustrates how these interconnected components create a continuous cycle of knowledge generation. Beginning with the careful choice of species suitable for GPS tagging, the process extends through the technical aspects of device use and the systematic collection of spatiotemporal data. These data are then analyzed with spatial and ecological tools to derive meaningful insights, which in turn feed directly into conservation strategies such as roost protection, corridor design, and pest management. By presenting the framework in a visual cycle, Figure 1 highlights the iterative nature of research where conservation outcomes inform future studies and species selection, thereby reinforcing an adaptive and dynamic approach to bat movement ecology.

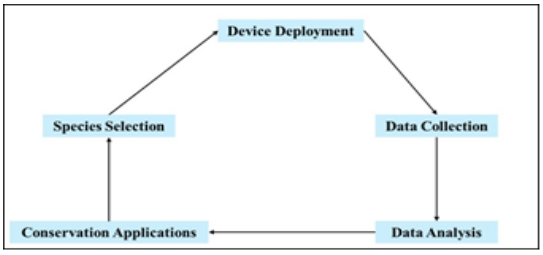

Figure – 1: Conceptual framework of GPS tracking in bat ecology, highlighting species selection, device deployment, data collection, data analysis, and conservation applications.

Challenges and Future Directions

Despite its transformative potential, GPS tracking in bats faces several challenges. Devices remain expensive, limiting accessibility for researchers in resource-constrained regions. Battery life is another constraint, particularly when high-resolution data are required. GPS signals may be obstructed in caves, dense forests, or urban environments, leading to data gaps (Ripperger *et al.,* 2020). For smaller bat species, the miniaturization of devices remains a technological barrier.

Future directions include the development of ultra-light GPS loggers (<1 g), solar-assisted charging systems, and integrated biologgers combining GPS with accelerometers, gyroscopes, heart rate monitors, and acoustic sensors (Toledo *et al.,* 2020). Advances in artificial intelligence and big data analytics will enable automated classification of movement behaviors and predictive modeling of bat responses to climate change, habitat loss, and emerging diseases (Kays *et al.,* 2020). Furthermore, the expansion of collaborative platforms such as Movebank will facilitate cross-continental comparisons and the establishment of a global bat movement ecology network.

CONCLUSION

The integration of GPS technology into bat research represents a paradigm shift in the study of movement ecology. By enabling fine-scale, continuous, and automated monitoring, GPS devices provide researchers with the tools to answer fundamental questions about bat behavior, ecology, and ecosystem services. Establishing standardized frameworks for species selection, device deployment, data collection, analysis, and conservation application will ensure that GPS-based studies maximize both scientific output and conservation impact. As technological advancements reduce costs and improve device efficiency, GPS tracking will play an increasingly central role in bat research, offering critical insights into biodiversity conservation, ecosystem service management, and human–wildlife coexistence.

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FOURIER TRANSFORM INFRA-RED (FTIR) SPECTROCHEMICAL ANALYSIS OF SELECTED BUTTERFLY WINGS OF LYCAENIDAE FAMILY

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ABSTRACT: The Lycaenidae family, renowned for its vibrant and intricate wing patterns, offers unique insights into the structural and chemical composition of butterfly wings. This study utilizes Fourier Transform Infrared (FTIR) spectroscopy to analyze the wings of butterfly species within the Lycaenidae family, including *Azanus jesous*, *Discolampa ethion*, *Prosotas nora*, *Castalius rosimon*, *Jamides bochus*, *Euchrysops cnejus*, *Zizina otis*, *Chilades lajus*, *Lampides boeticus*, *Zizula hylax*, and *Leptotes plinius*. The analysis focuses on the variations in their cuticular hydrocarbons (CHCs), revealing the predominant presence of alkanes, alkenes, carboxylic acids, and nitro compounds. Significant differences were observed in the presence of alkynes, aromatic compounds, isothiocyanates, aliphatic and aromatic amines, and sulfoxide groups among the species' cuticular hydrocarbons. The spectrochemical profiles obtained in this study have potential applications in biomimetic materials science, offering bio-templates for use in environmental, industrial, and medical fields, while also contributing to the broader field of lepidopterology.

KEY WORDS: Lycaenidae, FTIR, Cuticular Hydrocarbons, functional groups, bio template.

INTRODUCTION

Insects have unique hydrocarbon profiles over their body surfaces, with each individual having a unique CHC profile (Menzel *et al.,* 2017). These CHCs are crucial for insect survival, acting as a barrier against microbes, lubricating, and involved in regeneration, lubrication, mutualistic interactions, host parasite interactions, and mate recognition (Cooper *et al.,* 2009). Butterfly colour patterns are influenced by sexual selection, conspecific recognition, camouflage, and mimicry (Pask *et al.,* 2017). They generate structural and pigmentary colours through microstructures and pigments (Sharmila *et al.,* 2022. Giraldo,

2008). Lycaenids have different microstructures for light diffusion and melanin pigment for contrast enhancement. Chemical heterogeneity on micron scales provides hydrophobic and antimicrobial properties (Chung and Carroll, 2015).

Butterfly wings are composed of chitin and proteins such as resilin and sclerotin, which contribute to their mechanical strength and optical properties (Archana *et al.,* 2022; Guan *et al.,* 2018). While FTIR spectroscopy has revealed these components, comprehensive analyses on the Lycaenidae family remain limited. Spectrochemical studies offer insights into the chemical complexity of butterfly wing scales (Barboux *et al.,* 2021; Blomquist *et al.,* 2020 & 2010). This study utilizes FTIR spectroscopy to analyze insect cuticles and wings, focusing on the functional groups of hydrocarbons present in the blue regions of butterflies, particularly within the Lycaenidae family. The aim is to identify key functional groups and molecular interactions that contribute to their unique properties, while also exploring variations in chemical composition among different species within this family.

MATERIALS AND METHODS

Sample preparation

Lycaenidae (blues) are well known for its structural colours. To study the chemical complexity behind the structural colours, Lycaenids with different shades of blues were selected for FTIR analysis, It includes metallic blue colors from the basal region of *Castalius rosimon*, iridescent blue suffusions from the post-discal region of *Discolampa ethion*, dark purple colors from the discal region of *Prosotas nora*, *Jamides bochus*, *Euchrysops cnejus*, *Zizina otis*, *Chilades lajus*, *Lampides boeticus* and *Zizula hylax and* pale blue colors from the apical region of *Azanus jesous and sub discal region of Leptotes plinius*. Collected butterfly wings were detached from the thorax using fine forceps under a stereomicroscope to avoid any damage to the wing structures. The selected region of wings was then cut into a small piece (1 cm x 1 cm) and taken into an eppendorf tube.

Fourier -Transform Infra-Red spectroscopy (FTIR)

FTIR spectra were recorded using a JASC0- FT/IR-4600 (model) FTIR spectrometer equipped with a DLATGS detector. In this study, FTIR was used to acquire data about chemical composition of the blue regions of butterfly wings. The FTIR spectrum was obtained by crushing the wing portion with Potassium Bromide crystal, the wings of the

butterflies were directly placed and spectrum was recorded. The spectral range was set from 4000 $^{cm-1}$ to 400 $^{cm-1}$ with a resolution of 4 $^{cm-1}$. Since each bond absorbs different wave length of light, spectrogram of absorbed light provides a unique finger print for specific molecule, thus allowing for an effective chemical identification (Archana *et al.,* 2022; Sackey *et al.,* 2018). The spectra were plotted in the graph by using Origin Pro software (version 9.85).

RESULTS AND DISCUSSION

FTIR analysis of blue wing regions in Lycaenidae butterflies revealed diverse compounds, including hydrocarbons, proteins, lipids, aromatics, nitro compounds, alcohols, aldehydes, ketones, esters, isothiocyanates, and sulfoxides (Fig. 1, 2). Hydrocarbons such as alkanes, alkenes, and alkynes were central to waterproofing, signaling, and mate attraction (Tobin *et al.,* 2015). Long-chain alkanes like n-nonacosane (C29) provided cuticular waterproofing and reduced water loss (Omura *et al.,* 2012; Arsene *et al.,* 2002), while alkenes (monoenes, dienes) mediated sexual communication and species differentiation, as shown in *Papilio polytes* (Omura *et al.,* 2021; Omura *et al.,* 2020; Omura & Honda, 2005).

Alkynes, with their reactive triple bonds, likely contribute to intraspecific communication and chemical defense, consistent with findings in Pieridae butterflies (Archana *et al.,* 2022). FTIR spectra also revealed protein-related groups: carboxylic acids essential for peptide bond formation and protein stability (Liu *et al.,* 2003), aliphatic amines supporting hydrophobic interactions in protein folding, and aromatic amines (tyrosine, phenylalanine, tryptophan) critical for wing cuticle sclerotization, enhancing durability and stress resistance (Machovic *et al.,* 2016). Lipids such as esters, ethers, and ketones play key physiological roles: esters form protective wax layers against desiccation, ethers support membrane stability, and certain ketones act in pheromonal signaling (Zohry *et al.,* 2019). Volatile alcohols and aldehydes contribute to olfactory communication, while esters can function as aphrodisiac pheromones (e.g., *Colias philodice*) or antiaphrodisiac agents (e.g., *Pieris rapae*) depending on species context (Ehlers & Schulz, 2023; Arsene *et al.,* 2003).

The identification of isothiocyanates in species such as *Zizula hylax* and *Pieris rapae* suggests a defensive role for these compounds, which are commonly sequestered from glucosinolate-containing plants like those in the Brassicaceae family (Badenes-Pérez, 2023). Interestingly, *Zizula hylax*, which feeds on Fabaceae species typically lacking glucosinolates, appears to have evolved a novel strategy for acquiring or synthesizing these defensive

metabolites, demonstrating ecological plasticity and adaptive biochemical innovation. The detection of sulfoxides, characterized by S=O stretching vibrations, also points to the presence of sulfur-based defensive compounds. These are likely sequestered from host plants and stored in wing tissues as part of a broader chemical defense strategy, enhancing unpalatability or toxicity to predators (Opitz *et al.,* 2009).

Spectrochemical profiles varied notably among species. *Zizina otis* showed the highest chemical diversity, while *Chilades lajus* and *Lampides boeticus* exhibited similar profiles with imines, alkynes, alkanes, and nitro groups. *Jamides bochus* displayed an intermediate mix of alcohols, alkanes, aldehydes, and esters. *Zizula hylax* stood out with unique isothiocyanates, and *Freyeria trochylus* had a minimal profile dominated by alkanes and nitro compounds. *Euchrysops cnejus* and *Castalius rosimon* shared aromatic amines, alcohols, and alkanes, while the same amines in *Catochrysops strabo* and *Curetis thetis* suggested roles in cuticular hardening. Nitro compounds were widespread, likely linked to pigmentation or deterrence. Overall, these functional group patterns reflect both phylogenetic divergence and ecological specializations.

Hierarchical clustering and heat map analyses revealed distinct spectrochemical patterns among Lycaenidae species, with dendrograms showing species groupings and a Venn diagram highlighting shared and unique compounds between Lycaenidae and Pieridae (Fig. 3). Lycaenidae were characterized by aliphatic ethers, ketones, amines, isothiocyanates, and sulfoxides, while Pieridae showed alkyl halides, brominated compounds, carbonyls, and chlorinated derivatives. Both families shared core compounds such as alcohols, aldehydes, alkanes, alkenes, alkynes, aromatic amines, and carboxylic acids (Archana *et al.,* 2022), reflecting conserved traits linked to physiology and ecology. Variation in chemical composition was shaped by lineage, ecology, and biosynthetic pathways, with selective production influenced by predation, mating strategies, and host plant interactions, underscoring the role of evolutionary and ecological dynamics in shaping chemical phenotypes.

This study highlights the utility of FTIR spectroscopy for identifying species-specific cuticular hydrocarbons (CHCs) and surface compounds, with applications in forensic entomology and integrated pest management through improved species identification and ecological monitoring (Butterworth *et al.,* 2020; Ando *et al.,* 2004). Its capacity to

discriminate cryptic species and reveal interspecific chemical diversity underscores its relevance for taxonomy and systematics (Snellings *et al.,* 2018; Chung & Carroll, 2015;

Seppa *et al.,* 2011; Kather & Martin, 2012; Braga *et al.,* 2013). Moreover, the conservation and diversification of CHCs provide insights into speciation, phylogeny, and adaptive strategies, emphasizing the ecological and evolutionary significance of butterfly surface chemistry.

Table 1: FTIR profiling of chemical components in selected Lycaenids

]	Functional groups	Sp 1	Sp 2	Sp 3	Sp 4	Sp 5	Sp 6	Sp 7	Sp 8	Sp 9	Sp 10	Sp 11	Chemical bonding
	Alcohol	+		+	+	+			+				O-H stretching
	Aldehyde								+				C-H stretching
	Aliphatic amine		+										N-H stretching
	Aliphatic ether					+						+	C=O stretching
	Alkane	+	+	+	+	+	+	+	+	+	+	+	C-H stretching
	Alkene	+	+		+		+						C=C stretching
	Alkyne						+	+		+		+	C=C stretching
	Alpha-beta unsaturated ketone					+							C=C stretching
	Amine	+						+		+	+		C-N stretching
	Anhydride group							+					C=O stretching
	Aromatic amines								+				C-N stretching
	Aromatic compounds		+										C-H bending
	Aryl alkyl ester		+										C-O stretching
	Aryl alkyl ether			+	+	+	+					+	C-O stretching
	Carboxylic acid	+					+				+	+	O-H bending
	Ester	+					+	+					C-O stretching
	Imine			+					+	+	+	+	C=N stretching
	Isothiocynate										+		N=C=S stretching
	Nitro compounds	+	+	+	+	+	+	+	+	+	+	+	N-O stretching
	Sulfoxide				+						+	+	S=O stretching

Fig. 1. a) ***Azanus jesous,*** **b)** ***Discolampa ethion,*** **c)** ***Prosotas nora,*** **d)** ***Castalius rosimon***

e) ***Jamides bochus*** **and f)** ***Euchrysops cnejus***

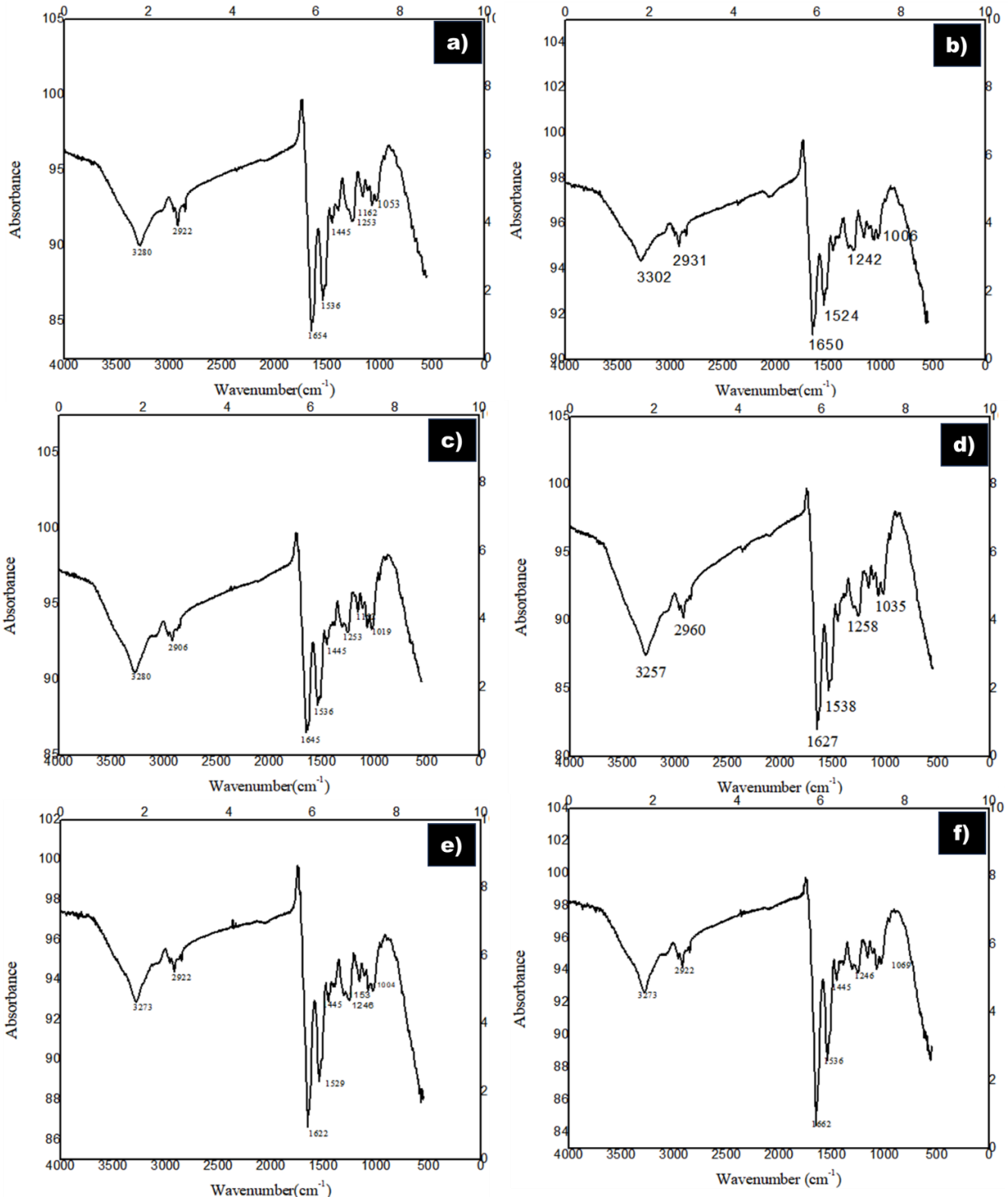


Fig. 2. a) ***Zizina otis,*** **b)** ***Chilades lajus,*** **c)** ***Lampides boeticus,*** **d)** ***Zizula hylax*** **and e)** ***Leptotes plinius***

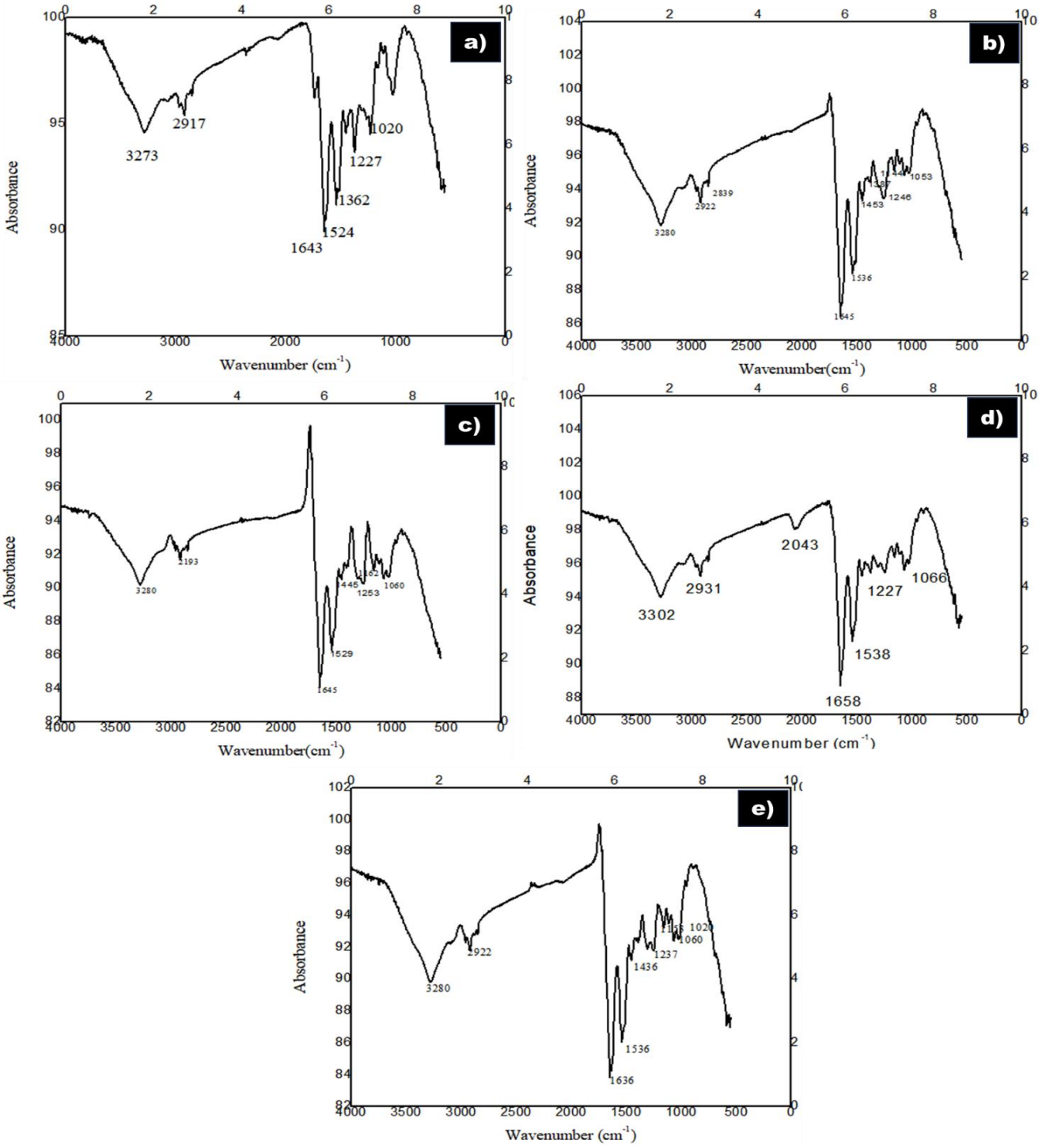
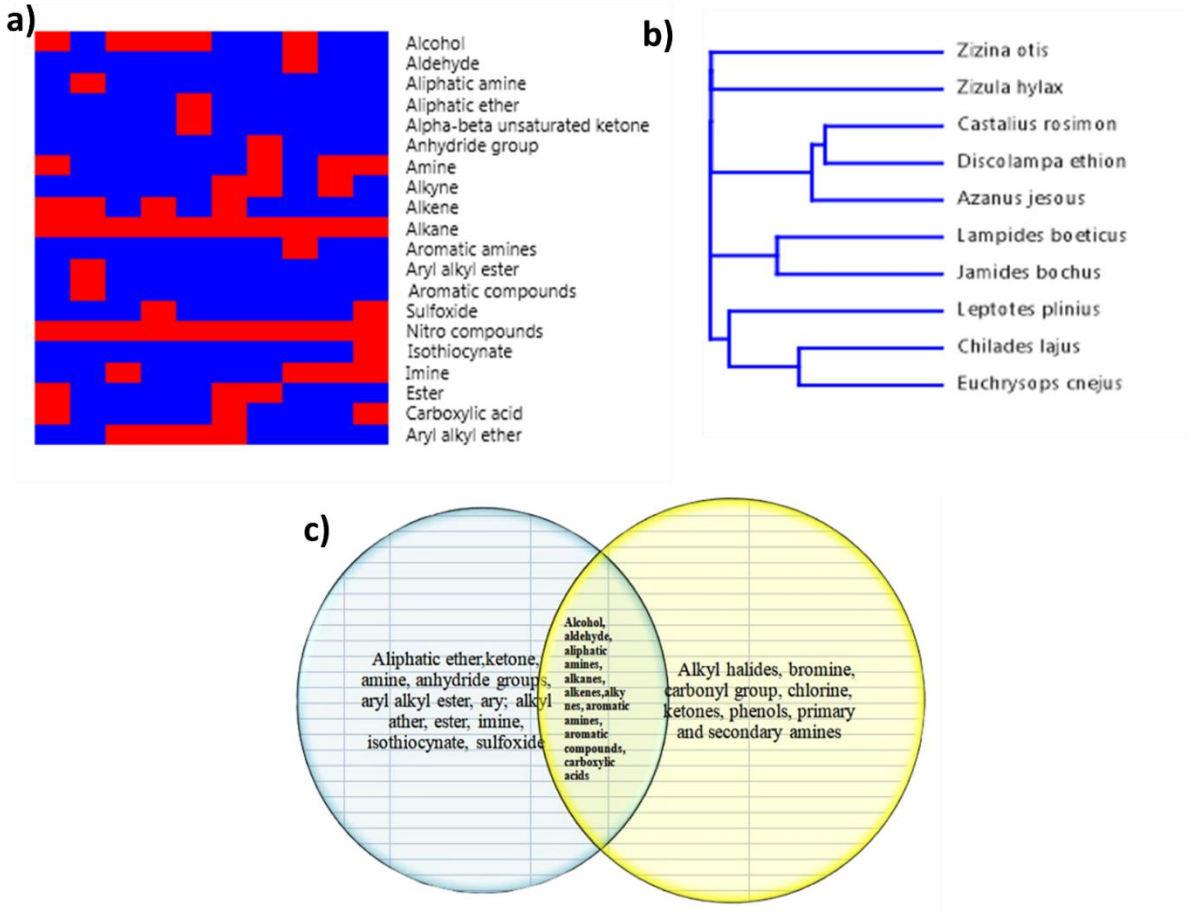


Fig. 3. a) Heat map showing chemical profile of selected lycaenid wings, b) Dendrogram showing species relationships within the family Lycaenidae and c) Venn Diagram: Wing chemical composition in Lycaenidae (blue) and Pieridae (yellow) butterflies.



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PHYLOGEOGRAPHY OF *DANAUS CHRYSIPPUS* USING MITOCHONDRIAL COI

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ABSTRACT

DNA barcoding of *Danaus chrysippus* from Madurai, Tamil Nadu, was conducted to confirm species identity and assess genetic variation. The mitochondrial COI gene was amplified, sequenced, and analysed using a Neighbour-Joining phylogenetic tree with bootstrap support. The results revealed clear geographic clustering, with the Tamil Nadu population forming a distinct clade, while North Indian sequences clustered closer to populations from other countries. Partial overlap with African clades reflected migratory connections, highlighting the species' geographic variation. This study demonstrates that COI barcoding reliably distinguishes populations and provides valuable insights into the genetic structure and biogeography of *D. chrysippus*, supporting future conservation and biodiversity studies.

KEYWORDS: *Danaus chrysippus,* DNA barcoding, COI gene, Phylogenetic analysis and Geographic variation.

INTRODUCTION

DNA barcoding has become an essential molecular tool for improving species identification, especially in taxonomically challenging groups (Wilson *et al.,* 2017). This approach relies on sequencing short, standardized DNA regions that exhibit sufficient interspecific variation to distinguish species reliably. Since its formal proposal by Hebert in 2003, the mitochondrial cytochrome c oxidase subunit I (COI) gene has been widely adopted as the standard animal barcode because of its rapid evolutionary rate, conserved primer-binding sites, and efficient amplification across diverse taxa (Jinbo and Kato,2011). The development of global initiatives such as the Barcode of Life project and the International Barcode of Life project (iBOL) has further enhanced the utility of DNA barcoding by establishing extensive reference libraries for species comparisons (Mampang *et al.,* 2023).

Butterflies are ideal subjects for DNA barcoding because of their high diversity, ecological importance, and the frequent morphological similarities that complicate traditional taxonomy. DNA barcoding is particularly valuable in this group, as cryptic species, mimicry, sexual dimorphism, and colour variation often make morphology unreliable. The COI

barcode region provides a consistent genetic tool for confirming species identity, resolving taxonomic ambiguity, and detecting hidden diversity, thereby strengthening butterfly systematics and conservation (Singh *et al.,* 2021). *Danaus chrysippus*, a widely distributed and toxic butterfly classified as Least Concern by the IUCN and common in Tamil Nadu, was chosen for this study due to its ecological relevance and involvement in mimicry (Bhakare and Ogale, 2018). Using COI-based DNA barcoding, this study investigates its genetic variation and species identity, offering a molecular framework to clarify evolutionary relationships and support biodiversity assessment and conservation efforts.

MATERIALS AND METHODS

Sample Collection and Genomic DNA Isolation

Tissue samples of *Danaus chrysippus* were used for genomic DNA extraction. DNA was isolated using the NucleoSpin® Tissue Kit (Macherey-Nagel) following the manufacturer's instructions. Briefly, approximately [specify tissue type, e.g., leg or thoracic muscle] was placed in a 1.5 ml microcentrifuge tube, and 180 µl of T1 buffer and 25 µl of proteinase K were added. Samples were incubated at 56°C in a water bath until complete lysis. Following lysis, 5 µl of RNase A (100 mg/ml) was added, and the mixture was incubated at room temperature for 5 minutes. Subsequently, 200 µl of B3 buffer was added and incubated at 70°C for 10 minutes. After incubation, 210 µl of 100% ethanol was added, mixed thoroughly, and the lysate was transferred into a NucleoSpin® Tissue column placed in a 2 ml collection tube. The column was centrifuged at 11,000 × g for 1 minute, washed sequentially with 500 µl of BW buffer and 600 µl of B5 buffer, and DNA was eluted using 50 µl of BE buffer into a clean 1.5 ml microcentrifuge tube.

Assessment of DNA Quality

The quality of isolated DNA was evaluated by agarose gel electrophoresis. Five microliters of DNA were mixed with 1 µl of 6X gel loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer, pH 8.0) and loaded onto a 0.8% agarose gel prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was conducted at 75 V until the dye front reached the bottom of the gel. DNA bands were visualized using a UV transilluminator (Genei), and images were captured with a Gel Documentation System (Bio-Rad).

PCR Amplification of COI Gene

The mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using universal primers LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions were performed in a total volume of 10 µl containing 5 µl of 2X Phire Master Mix, 0.25 µl of each primer, 1 µl of genomic DNA, and 4 µl of nuclease-free water. Amplification was carried out in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) under the following conditions: initial denaturation at 98°C for 30 seconds; 10 cycles of denaturation at 98°C for 5 seconds, annealing at 45°C for 10 seconds, and extension at 72°C for 15 seconds; followed by 30 cycles of denaturation at 98°C for 5 seconds, annealing at 50°C for 10 seconds, and extension at 72°C for 15 seconds; and a final extension at 72°C for 60 seconds. PCR products were verified on 1.2% agarose gels prepared in 0.5X TBE buffer with 0.5 µg/ml ethidium bromide, using a 2-log DNA ladder (NEB) as a molecular size marker. Electrophoresis was performed at 75 V for 1–2 hours, and gels were visualized under UV illumination.

PCR Product Clean-up and Sequencing

PCR products were treated with ExoSAP-IT (GE Healthcare) to remove residual primers and nucleotides. Five microliters of PCR product were mixed with 0.5 µl of ExoSAP-IT and incubated at 37°C for 15 minutes, followed by enzyme inactivation at 85°C for 5 minutes. Sequencing reactions were conducted using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) in a 10 µl reaction mixture consisting of 6.6 µl nuclease-free water, 1.9 µl of 5X sequencing buffer, 0.3 µl of each primer, 0.2 µl of sequencing mix, and 1 µl of ExoSAP-treated PCR product. Thermal cycling conditions included an initial denaturation at 96°C for 2 minutes, followed by 30 cycles of denaturation at 96°C for 30 seconds, annealing at 50°C for 40 seconds, and extension at 60°C for 4 minutes, with a final hold at 4°C.

Post-Sequencing Clean-up and Data Analysis

Sequencing reactions were purified using ethanol precipitation. Fifty microliters of a mixture containing nuclease-free water, 0.1 µl EDTA (125 mM), 1 µl of 3 M sodium acetate (pH 4.6), and 44 µl of 100% ethanol were added to each sequencing reaction, vortexed, and incubated at room temperature for 30 minutes. Samples were centrifuged at 3700 rpm for 30 minutes, washed twice with 70% ethanol, air-dried, and resuspended in 10 µl of highly deionized formamide (Hi-Di). Sequencing was performed on an ABI 3500 DNA Analyzer (Applied Biosystems). Raw sequences were checked for quality using Sequence Scanner Software v1

(Applied Biosystems) and edited and aligned using Geneious Pro v5.1 (Drummond *et al.,* 2010) for downstream analyses including species identification and phylogenetic inference.

BLAST Similarity Assessment and Phylogenetic Evaluation

A COI gene sequence of *Danaus chrysippus* collected from Madurai, Tamil Nadu, was obtained and used for molecular identification. The sequence was subjected to BLASTn analysis in NCBI, and the top ten closest matches from different geographic regions were selected based on percentage identity. These included sequences from Uttar Pradesh, Jharkhand, Mizoram, Tamil Nadu, New York, Republic of Korea, Canada, and the United Kingdom, with similarity values ranging from 97.18% to 99.67%. (Table.1) All selected sequences, along with the Madurai COI sequence, were aligned using ClustalW in MEGA 12. A phylogenetic tree was constructed in MEGA 12 using the best-fitting evolutionary model, and node reliability was assessed using 1,000 bootstrap replicates. The resulting phylogeny was used to compare the genetic relationship of the Madurai specimen with global populations of *Danaus chrysippus*.

Table.1. Selected COI Sequences of *Danaus chrysippus* from Different Geographic Locations

Gene Accession Number	Geographic Location
MH675605.1	Uttar Pradesh
LC877963.1	Jharkhand
KP007596.1	New York
JX185863.1	Republic of Korea
KP007635.1	New York
KP007572.1	New York
KC306719.1	Mizoram, India
KP007574.1	Mizoram, India
JX226068.1	Tamil Nadu
GU67598.1	Canada
LR743639.1	United Kingdom (UK)

RESULTS AND DISCUSSION

In the present study, the mitochondrial COI gene of *Danaus chrysippus* was analysed to examine its phylogenetic relationships. A Neighbour-Joining tree was constructed, and bootstrap analysis assessed branch support (Fig.1). Most branches showed high bootstrap values, indicating reliable groupings. Some branches had moderate or lower support,

reflecting the species' wide distribution, mobility, and regional mixing. These lower-support nodes likely represent natural variation and do not affect the overall reliability of the phylogenetic tree. Geographically, the tree revealed clear clustering of sequences from India, Korea, North America, Canada, and the United Kingdom, indicating that populations generally group according to their location. South Indian samples formed a distinct cluster, separate from North Indian, African, and other global populations. A few Indian sequences showed partial overlap with African clades, consistent with the known migratory behaviour of *Danaus* butterflies and suggesting the sharing of some mitochondrial haplotypes across continents.

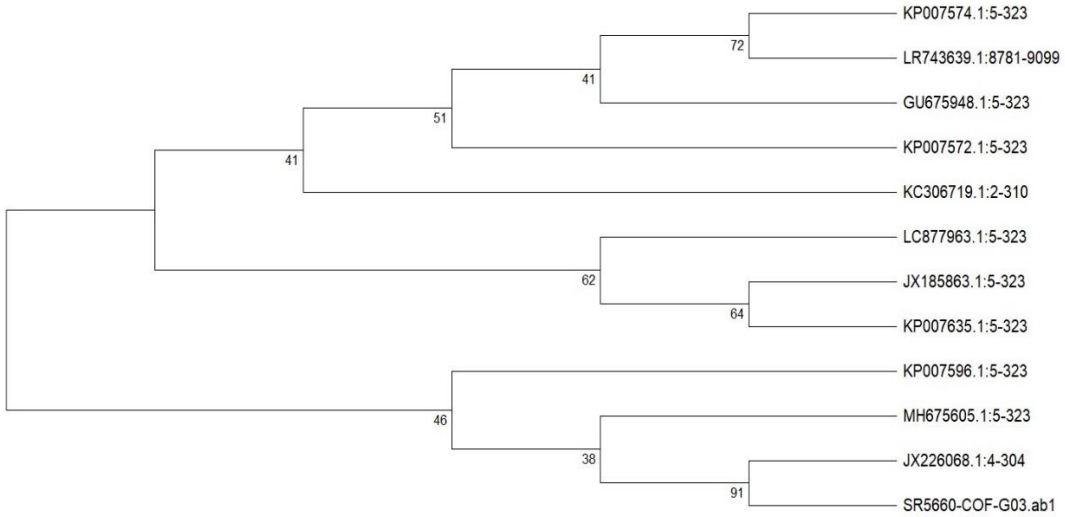

Figure 1: Phylogenetic Tree of *Danaus chrysippus* from Different Regions

These patterns can be attributed to several biological factors. The high dispersal ability of *D. chrysippus*, ongoing gene flow among populations, and relatively recent divergence events may reduce bootstrap support at mixed nodes and produce overlapping genetic signals (Singh *et al.,* 2021). Conversely, long-term geographic isolation, local climatic variation, and environmental pressures contribute to region-specific clusters with strong bootstrap support. Overall, the phylogenetic tree suggests that *D. chrysippus* maintains a largely stable mitochondrial lineage, while also exhibiting early signs of geographic divergence. This indicates that the species is genetically cohesive at a global scale, yet displays subtle genetic differentiation influenced by geographic factors.

The significance of this study is substantial. COI-based phylogenetic analysis facilitates the confirmation of species identity, detection of hidden genetic structure, and

identification of potential cryptic diversity (Antil *et al.,* 2023). It also provides a scientific framework for understanding population connectivity, migratory pathways, and historical colonization patterns (Livraghi *et al.,* 2018; Wang *et al.,* 2020). Such information is valuable for conservation planning, ecological monitoring, and broader evolutionary investigations (Singh *et al.,* 2021). Despite minor variations in bootstrap support, the overall phylogeny robustly indicates that geographic separation plays a key role in shaping the genetic structure of *Danaus chrysippus*. Consequently, this study represents a meaningful contribution to the understanding of butterfly genetics and biogeography.

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MICROPLASTICS IN THE ENVIRONMENT AND HUMANS: PATHWAYS OF EXPOSURE AND POTENTIAL HEALTH HAZARDS

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ABSTRACT: Microplastics are pervasive environmental contaminants, and their potential impacts on human health are a growing scientific and public concern. The presence and accumulation of microplastics in the environment threaten the ecological balance, the water environment, food sustainability and safety, and ultimately human health. This review synthesizes current knowledge on the pathways of human exposure—including ingestion, inhalation, and dermal contact—and evaluates the resulting potential health hazards. On the other hand, it can quickly enter the soil and plants, causing drastic changes in the physicochemical characteristics of soil (pH, water retention, nutrient content), microbial community, and fertility, thereby impeding the plant's whole performance and response. This review will be helpful to the understanding of the exposure risk and potential health hazards of microplastics. Evidence from experimental studies involving cells, organoids, and animal models indicates that microplastics can trigger oxidative stress, DNA damage, immune disturbances, metabolic dysfunction, neurotoxicity, and reproductive as well as developmental toxicity. Finally, we identify critical knowledge gaps and suggest priorities for future research to better understand the risks and mechanisms of microplastic toxicity for human health.

KEYWORDS: Microplastics, Human Exposure, Environmental Pollution, Health Issuse.

INTRODUCTION

Microplastics, defined as plastic particles smaller than 5 millimeters, have become ubiquitous contaminants in the global environment, marking what some scientists call "The Plasticene" epoch (Campanale *et al.,* 2020). These nano- and microplastic particles (NMPs) originate from multiple sources including the degradation of larger plastic waste, textile and tire wear, personal care products containing intentionally added plastics, and plastic packaging materials (Brachner *et al.,* 2020). Secondary microplastics form when larger plastic debris breaks down through physical, chemical, and biological processes in the

environment, while primary microplastics come directly from consumer products like liquid soaps, exfoliants, and industrial applications (Kochanek *et al.,* 2025).

The widespread distribution of microplastics has been documented across all environmental compartments. These particles contaminate marine organisms, drinking water, table salt, fruits and vegetables, and other human food sources worldwide. Microplastics have been detected in tap water with contamination reaching up to 628 particles per liter and in bottled water with levels as high as 4,889 particles per liter (Danopoulos *et al.,* 2020). Air pollution represents another major exposure route, with microplastics contributing to airborne particulate matter that can be directly inhaled or deposited on surfaces and soil (Zhang *et al.,* 2020).

Human exposure to microplastics is now inevitable and occurs through three main pathways: ingestion through contaminated food and water, inhalation of airborne particles, and potentially through soil contact (Kochanek *et al.,* 2025). Exposure estimates suggest humans may consume between 0-73,000 particles per year through table salt, 0-4,700 particles through drinking water, and inhale up to 30 million particles annually (Zhang *et al.,* 2020). Particularly concerning is infant exposure, with calculations showing that babies fed from polypropylene bottles can invest up to 3 million microparticles daily (Brachner *et al.,* 2020).

This perpetual and annually increasing human exposure has gained notable scientific and public attention, with major health organizations including the European Commission, European Chemicals Agency, and World Health Organization recognizing microplastics as potential health hazards (Brachner *et al.,* 2020) (Campanale *et al.,* 2020) (Toussaint *et al.,* 2019) (Wang *et al.,* 2020) (Rochman *et al.,* 2020). However, despite this recognition, precise data on human health impacts remain limited due to the lack of standardized detection methods and definitions (Toussaint *et al.,* 2019).

SOURCES MICROPLASTICS IN THE ENVIRONMENT

Microplastics enter the environment through two distinct pathways that create fundamentally different types of contamination. Primary microplastics are intentionally manufactured small particles found in consumer products such as liquid soaps, exfoliants, cleaning agents, cosmetics, paints, abrasives, and plastic pellets or powders used in industrial applications (Brachner *et al.,* 2020) (Kochanek *et al.,* 2025). These particles are deliberately

added to products for specific functional purposes and are directly released into the environment during use.

Secondary microplastics represent the larger category and form through the environmental breakdown of larger plastic debris. These particles result from degradation and disintegration processes that occur in nature due to physical, chemical, and biological factors acting on macroplastic waste. The fragmentation process transforms plastic items like bottles, bags, fishing nets, agricultural plastic films, industrial resin pellets, waste incineration, landfill leachate, and other plastic waste into progressively smaller particles, eventually reaching microplastic and nanoplastic sizes. Several specific sources contribute significantly to microplastic pollution. Plastic packaging materials continuously release particles, as demonstrated by research showing that infants fed from polypropylene baby bottles can be exposed to up to 3 million microparticles daily (Brachner *et al.,* 2020).The scale of potential contamination from secondary sources is substantial, given that global plastic production has reached 359 million tonnes, with land-based sources responsible for 80-90% of microplastic pollution compared to only 10-20% from ocean-based sources (Osman *et al.,* 2023).

CLASSIFICATION OF MICROPLASTICS

The most common polymer types found in environmental microplastic contamination include polypropylene, polyethylene, polyvinyl chloride (PVC), polystyrene, polyurethane, and polyethylene terephthalate (PET), listed in decreasing order of abundance (Zhang *et al.,* 2018a; Yan *et al.,* 2019). In drinking water specifically, polyethylene terephthalate (PET) and polypropylene (PP) emerge as the most frequently identified polymers (Danopoulos *et al.,* 2020).

This continuous input from multiple sources ensures that microplastic contamination is perpetual and annually increasing as global plastic production and usage continue to expand. The particles become distributed across all environmental compartments - air, water, and soil - confirming their ubiquitous presence and creating multiple pathways for human exposure (Kochanek *et al.,* 2025)(Wang *et al.,* 2020).The identification of toxic tire-derived compounds like 6PPD-quinone, which can cause acute mortality in salmon at concentrations as low as 1 microgram per liter, demonstrates the environmental significance of automotive sources (Coffin *et al.,* 2022)(Tian *et al.,* 2020).

The chemical complexity of these sources adds another dimension to the contamination problem. Plastic materials contain more than 10,000 added chemicals, many of which may drive toxicity in both humans and ecological systems. Over 2,400 of these substances are identified as substances of potential concern, meeting persistence, bioaccumulation, and toxicity criteria, yet many remain poorly studied or inadequately regulated (Wiesinger *et al.,* 2021). This creates a situation where microplastics serve not only as direct contaminants but also as vectors for transporting additional harmful substances, including pathogens and chemical pollutants.

PATHWAYS OF HUMAN EXPOSURE

Microplastics can enter the human body through three distinct exposure routes: inhalation, ingestion, and dermal exposure (Ghosh *et al.,* 2023) (Emenike *et al.,* 2023) (Pramaningsih *et al.,* 2023) (Karak *et al.,* 2025). Of these pathways, ingestion represents the most common route for microplastic exposure in humans.

INGESTION PATHWAY

Human exposure through ingestion occurs via multiple contaminated sources. Microplastics are present in contaminated seafood and drinking water, as well as in food packaging and utensils made of plastic. Aquatic species ingest microplastics, which subsequently transfer up the food chain to humans through consumption of seafood that contains microplastics (Pramaningsih *et al.,* 2023) (Karak *et al.,* 2025). Additional dietary sources include contaminated beverages, honey, sugar, and other food items (Sulong *et al.,* 2024). Humans are estimated to ingest tens of thousands to millions of microplastic particles annually, equivalent to several milligrams daily. Evidence shows that feeding bottles and medical devices can contribute to microplastic exposure in newborns and infants (Kannan *et al.,* 2021).

INHALATION PATHWAY

Inhalation represents another significant exposure pathway, particularly for individuals who work in industries involving plastic production or handling. Airborne microplastics have been identified in both indoor and outdoor atmospheres, with indoor microplastic abundance generally higher than outdoor levels. Textiles serve as a primary source of indoor airborne microplastics, while traffic-derived particles, agricultural plastic mulch, marine microplastics, and landfill sites contribute to outdoor atmospheric pollution (Zhang *et al.,* 2023).

Microplastics can be present in air as a result of their release during manufacturing processes, transportation, and disposal. These minute particles can potentially be inhaled and settle within the respiratory tract (Karak *et al.,* 2025).

DERMAL CONTACT PATHWAY

Dermal exposure occurs through the use of personal care products that contain microbeads, such as facial scrubs and body washes (Ghosh *et al.,* 2023). Microplastics are also present in clothing and textiles made of synthetic materials, which can shed microfibers during use and washing (Enyoh *et al.,* 2020). This dermal pathway requires equal attention as the other two main exposure routes (Sun *et al.,* 2023).

BIOACCUMULATION AND DISTRIBUTION

Once ingested, microplastics can potentially accumulate in the digestive tract and other organs (Ghosh *et al.,* 2023) (Campanale *et al.,* 2020). Biomonitoring studies have provided direct evidence of microplastic presence in human stool, fetus, and placenta, demonstrating exposure in infants and children (Kannan *et al.,* 2021). Microplastics smaller than 20 μm have been reported to cross biological membranes (Kannan *et al.,* 2021). These particles have been found in human stool, blood, and even placental tissue, suggesting they can be absorbed into the human body and may accumulate in organs over time (Grzelak, 2024).

Human exposure occurs through multiple environmental compartments - water, air, and soil - with each serving as a significant source of microplastic exposure (Kochanek *et al.,* 2025). Available information suggests that inhalation of indoor air and ingestion of drinking water bottled in plastic are the major sources of microplastic exposure (Kannan *et al.,* 2021).

HUMAN HEALTH EFFECTS AND TOXICOLOGICAL IMPACTS

The growing presence of microplastics in human biological specimens has raised significant concerns about their potential health impacts. Microplastics have been found in various human biological samples such as faeces, sputum, saliva, blood, Broncho alveolar lavage fluid, placenta, and other organs, suggesting that these particles may induce detrimental effects on human health (Osman *et al.,* 2023). Evidence shows these particles have been detected in human stool, blood, and even placental tissue, suggesting they can be absorbed into the human body and may accumulate in organs over time (Grzelak, 2024).

CELLULAR AND MOLECULAR EFFECTS

At the cellular level, microplastics induce toxic effects on humans including cytotoxicity, immune response, oxidative stress, barrier attributes, and genotoxicity, even at minimal dosages of 10 μg/mL (Osman *et al.,* 2023). Studies demonstrate that microplastics potentially trigger inflammation, cellular impairment, and genotoxicity in human cells (Palaniappan *et al.,* 2021) (Roursgaard *et al.,* 2022). Research shows that microplastics may increase the production of reactive oxygen species (ROS), leading to DNA and cellular damage, oxidative stress, alterations in gene expression, and decreased cell viability (Karak *et al.,* 2025).

Laboratory studies reveal that exposure to microplastics activates inflammatory pathways including NFkB/MyD88/NLRP3, with cells exposed to microplastics showing lower metabolic activity rates compared to unexposed cells (Ghosh *et al.,* 2023). Additionally, nanoplastics from real-life food containers can cause genotoxicity in cell cultures, with concentration-dependent increases in DNA strand breaks observed (Ghosh *et al.,* 2023) (Roursgaard *et al.,* 2022).

SYSTEMIC HEALTH EFFECTS

The potential health risks associated with microplastic exposure include cancer, immunotoxicity, intestinal diseases, pulmonary diseases, cardiovascular disease, inflammatory diseases, and adverse effects on pregnancy and maternal exposure to progeny (Osman *et al.,* 2023). Microplastics have been linked to immunotoxicity, neurotoxicity, metabolic disorders, and reproductive toxicity (Sulong *et al.,* 2024). Research suggests that microplastics may affect the immune system, potentially leading to inflammation or other health issues (Karak *et al.,* 2025).

Specific organ systems appear to be particularly vulnerable to microplastic exposure. Energy homeostasis, intestinal microflora, and the reproductive, immune, and nervous systems were regarded as targets of microplastics (Sun *et al.,* 2023). Microplastics can accumulate in the gastrointestinal tract, potentially leading to inflammation, disruption of gut microbiota, and even the release of hazardous chemicals (Pramaningsih *et al.,* 2023).

MATERNAL AND DEVELOPMENTAL EFFECTS

Evidence from laboratory animals demonstrates maternal transfer of microplastics to the developing fetus, with maternal exposure altering energy and lipid metabolism in offspring

and subsequent generations (Sun *et al.,* 2023) (Kannan *et al.,* 2021). Microplastic exposure during pregnancy and maternal periods represents a particular concern for human health (Osman *et al.,* 2023).

TOXICITY MECHANISMS AND CHEMICAL CONCERNS

The toxicity of microplastics extends beyond the physical presence of particles themselves. As carriers, microplastics have the potential to magnify the toxicity of other contaminants in the environment, including plasticizers, metals, antibiotics, and microorganisms (Sun *et al.,* 2023). These particles can serve as carriers of other harmful substances, including pathogens and chemical pollutants (Kochanek *et al.,* 2025). Exposure to microplastic additives such as phthalates, bisphenols, and organotin causes adverse effects through the activation of nuclear receptors, leading to oxidative stress, cytotoxicity, immunotoxicity, thyroid hormone disruption, and altered adipogenesis and energy production (Kannan *et al.,* 2021).

The size, shape, chemical composition, surface charge, and hydrophobicity of microplastics influence their toxicity. Their widespread distribution poses significant threats to human health, including oxidative stress, inflammation, cellular damage, and potential carcinogenic effects

Despite growing awareness of microplastic contamination, substantial research gaps persist that limit our understanding of their full impact on human health. Studies of microplastics at environmentally realistic conditions are still in their infancy with many unsolved questions to predict their risks on human health. The field requires more comprehensive research to fully understand the long-term health consequences of microplastics in the human body (Grzelak, 2024).

DISCUSSION

A major limitation in current research is that most toxicological studies of microplastics on humans have been based on laboratory rodents and human-derived cells rather than comprehensive human studies. This reliance on in vitro and animal models creates uncertainty about how findings translate to real-world human exposure scenarios. Additionally, little is known about the potential risks of microplastics to human health despite their status as a globally emerging environmental contaminant.

The complexity of microplastic exposure presents particular research challenges. Dermal penetration as an exposure route needs equal attention as ingestion and inhalation pathways, yet it remains understudied. Furthermore, the role of microplastics as carriers that potentially magnify the toxicity of other environmental contaminants including plasticizers, metals, antibiotics, and microorganisms requires more investigation (Sun *et al.,* 2023).

FUTURE RESEARCH PRIORITIES

Several key areas require immediate research attention to address current knowledge gaps. Long-term epidemiological studies are needed to establish causal relationships between microplastic exposure and adverse health outcomes in human populations. Standardized methods for detecting and quantifying microplastics in biological samples must be developed to enable consistent research findings across different studies.

Research should focus on understanding dose-response relationships under environmentally relevant exposure conditions, as current studies often use concentrations that may not reflect real-world scenarios. Additionally, investigations into the mechanisms by which microplastics cross biological barriers and accumulate in specific organs will be crucial for risk assessment

REGULATORY AND POLICY NEEDS

The development of regulatory frameworks and safety standards for microplastic exposure represents another critical research need. This includes establishing acceptable daily intake levels and developing monitoring protocols for environmental and food safety agencies. Research into effective remediation strategies and prevention methods will be essential for developing comprehensive policy responses to microplastic contamination.

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MORPHOLOGICAL AND COLOUR CHARACTERIZATION OF WING SCALES OF *Colotis amata* AND *Acraea terpsicore*

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ABSTRACT: This study examines the wing scale morphology and coloration mechanisms in *Colotis amata* and *Acraea terpsicore*, emphasizing the distinction between structural and pigmentary coloration. Three scale types dentate, obovate, and obtuse-round were identified and quantified in both orange and black wing regions. In *C. amata*, the orange color was pigment-based, while the black regions showed a combination of pigmentary and structural mechanisms. In *A. terpsicore*, both orange and black regions displayed mixed mechanisms involving pigment and structural coloration. Refractometric analysis with clove oil and chemical bleaching with ammonium hydroxide effectively differentiated pigmentary from structural coloration. These findings highlight species- and region-specific differences in butterfly coloration strategies.

KEY WORDS: Scales, Morphology, Microstructure, Pigmentation and Coloration.

INTRODUCTION

Butterfly wings are among the most remarkable natural examples of optical nanostructures, generating vibrant structural colours and striking iridescence through intricately organized scale morphology (Saba *et al.,* 2014; Zhang *et al.,* 2014). Each scale typically consists of a flat basal lamina, longitudinal ridges, crossribs, and an internal hollow region known as the lumen, all supported by nanoscale pillars called trabeculae (Ghiradella *et al.,* 1985). This hierarchical architecture enables precise interaction with light, resulting in optical phenomena such as interference, diffraction, and polarization. Beyond structural coloration, butterfly scales may also contain pigments such as melanin, ommochromes, and papiliochromes, which contribute to or modify the observed colours (Stevenga *et al.,* 2006). The interplay between pigmentary and structural elements leads to complex and species- specific colour patterns that serve various ecological functions, including camouflage, thermoregulation, and sexual signaling (Wang *et al.,* 2016). These scales, typically measuring 0.1–0.2 mm in length, densely cover both dorsal and ventral wing surfaces, making butterflies not only visual marvels but also models of biological photonic engineering. The convergence of biology and

physics in butterfly wings exemplifies nature's sophisticated command over light manipulation at the nanoscale.

The study aims to catalogue and characterize the various scale types present in these butterflies, with a focus on scale profiling and distinguishing between structural, pigmentary, and combined coloration mechanisms. Pigmentary scales will be identified using a bleaching method that removes chemical pigments such as melanin and ommochromes. Structural coloration, on the other hand, will be confirmed using an index-matching liquid technique that neutralizes optical interference effects, allowing accurate detection of nanostructure-based colour contributions. This integrated approach helps reveal the physical and chemical basis of wing coloration, offering deeper insight into the diversity, function, and evolution of butterfly scale nanostructures.

METHODOLOGY

Sample Preparation and Microscopic Observation

Specimens of *C.altoris amata* and *Atrophaneura terpsichore* were selected based on the diversity and vibrancy of wing coloration. Scales were carefully collected from various colored regions of the wings using a fine-tipped brush and dusted onto clean glass microscope slides. The slides were initially observed without a mounting medium under a Labomed LX400 optical light microscope at magnifications of 4X and 10X. Photomicrographs were taken at each magnification to document scale morphology. Preliminary identification and nomenclature of scale types were carried out with reference to established literature.

Differentiation of Structural and Pigmentary Scales

To distinguish between structural and pigmentary coloration, two optical techniques were employed. First, index matching was conducted using clove oil (refractive index ≈ 1.5), which closely approximates the refractive index of chitin. A drop of clove oil was applied to the slides, allowing it to penetrate the microstructures of the scales and reduce refractive index contrast. This treatment rendered structural scales more transparent, facilitating their identification by the suppression of iridescence or structural colour (Matsuoka *et al.,* 2008). Second, chemical bleaching was carried out using a 10% aqueous solution of ammonia. Small portions of butterfly wings were immersed in the solution and left undisturbed for several hours. The ammonia gradually decolorized pigment-based scales, while structurally colored scales remained largely unaffected (Giraldo *et al.,* 2008). The combined use of these methods

enabled the reliable differentiation between scales producing colour through structural interference and those containing chemical pigments.

RESULTS AND DISCUSSION

The present study examined wing scale morphology in two butterfly species, *Colotis amata* (Small Salmon Arab) and *Acraea terpsichore* (Tawny Coster), with emphasis on scale types across different coloured regions. These species were selected for their distinctly vibrant wing colours and their representation of two ecologically and morphologically diverse butterfly families. *Colotis amata* is noted for its delicate salmon-pink to orange coloration, whereas *Acraea terpsichore* displays bright orange wings bordered with dark brown to black, a feature often linked to aposematic signaling (Mile *et al.,* 2013). The common scale types identified were dentate, obovate, and obtuse round, each attached to the wing membrane by a pedicel (Wilts *et al.,* 2017). In *C. amata*, orange wing regions predominantly contained dentate (one) and obtuse round (three) scales, while black regions exhibited greater morphological diversity with dentate (three), obovate (one), and obtuse round (one) scales. Similarly, *A. terpsicore*'s black regions featured dentate (three), obovate (one), and obtuse round (one) scales, whereas the orange regions were mainly composed of obtuse round (three) scales. This distribution highlights species- and region-specific variation in scale morphology (Table.1 and 2).

To further characterize these scales, refractometric analysis using clove oil a medium with refractive index (~1.53) closely matching chitin (~1.5) was employed to reduce light scattering and reveal structural features. Additionally, aqueous ammonium solution was used as a bleaching agent to selectively degrade organic pigments. In *C. amata*, orange scales showed no colour change with clove oil but bleached under ammonium treatment, indicating pigment predominance. Black region scales partially dulled with clove oil and showed minor bleaching, suggesting a combination of structural and pigmentary components. In *A. terpsicore*, both orange and black scales exhibited partial responses to clove oil and ammonium, reflecting mixed scale composition. These findings demonstrate that scale morphology and chemical properties vary between species and wing regions, and that combined refractive index matching and chemical bleaching effectively distinguish scale characteristics (Fig.1a, 1b and 2). These findings demonstrate that scale morphology and chemical composition vary between species and wing regions. Moreover, the combined use of refractive index matching and chemical bleaching proves effective in distinguishing scale characteristics. Beyond their biological relevance, such studies hold considerable promise in biomimetics particularly in the development of non-toxic, biodegradable textile dyes that

mimic natural pigment degradation. Additionally, insights into structurally derived colours could inform the creation of colourfast, light-responsive fabrics. Understanding butterfly scale architecture may thus inspire innovation in sustainable fashion, eco-friendly coloration technologies, and advanced optical materials (Sharmila *et al.,* 2022; Thayer *et al.,* 2023).

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Table 1. Scale Morphology Distribution in Wing Regions of *Colotis amata* and *Acraea terpsicore*

Species	Wing Region	Dentate Scales	Obovate Scales	Obtuse Round Scales
Colotis amata	Orange	1	0	3
	Black	3	1	1
Acraea terpsicore	Orange	0	0	3
	Black	3	1	1

Table 2. Optical and Chemical Responses of Wing Scales to Clove Oil and Ammonium Treatment

Species	Wing Region	Clove Oil Response	Ammonium Bleaching Response	Inference
Colotis amata	Orange	No change	Bleached	Pigment-based coloration
	Black	Partial dulling	Minor bleaching	Mixed structural and pigmentary coloration
Acraea terpsicore	Orange	Partial response	Partial bleaching	Mixed scale composition
	Black	Partial response	Partial bleaching	Mixed scale composition

Fig.1 Microscopic differentiation of structural and pigmentary wing Scales in *Colotis amata*

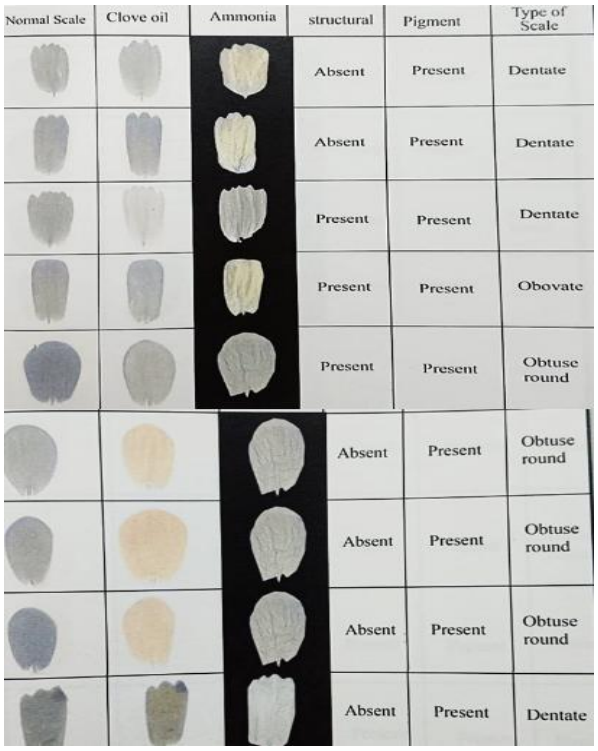
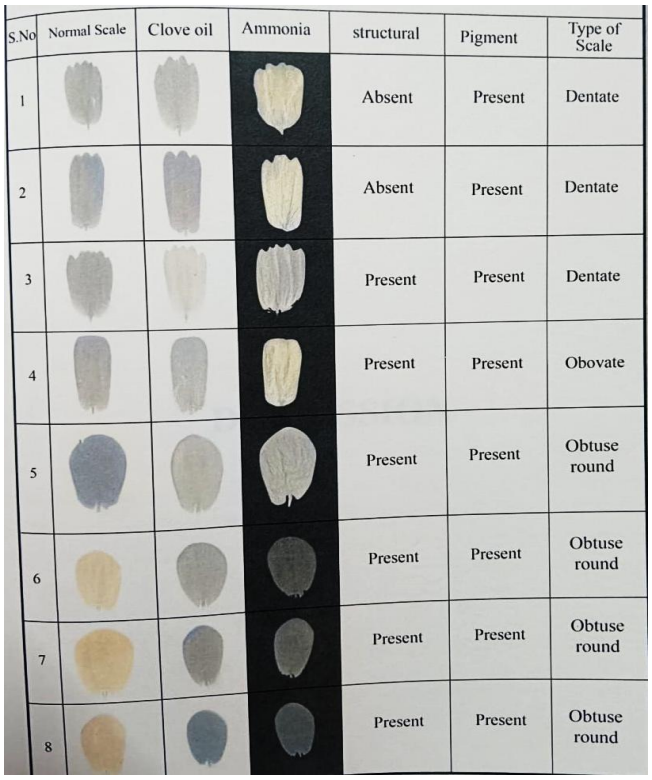


Fig. 2. Microscopic differentiation of structural and pigmentary wing scales in Acraea terpsicore



DIVERSITY OF BIRDS OF THE IRUKKANKUDI RESERVOIR, VIRUDHUNAGAR DISTRICT, TAMIL NADU, INDIA

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ABSTRACT: Wetlands serve as vital environments for both resident and migratory bird species, providing essential feeding, resting, and breeding sites. The survey was carried out within a calendar year, March to September 2025 and observations were conducted across weekly twice on Morning and Evening. The Irukkankudi water reservoir attracts both local and migratory bird species, with the number of birds fluctuating seasonally due to factors like water availability, vegetation, and food supply. Current research emphasizes the avian diversity and conservation importance of wetland birds at the Irukkankudi water reservoir, a significant freshwater habitat in southern India. This investigation aimed to document species richness, seasonal fluctuations, and the conservation relevance of the bird community within this reservoir. Conventional ornithological survey techniques, such as point counts, were utilized throughout different seasons to record bird presence and abundance. During this study, a total of 10 wetland-associated bird species were recorded, indicating a moderate degree of species richness compared to larger wetland systems. These included both permanent residents and migratory visitors, highlighting Irukkankudi's ecological role as a crucial habitat within a semi-arid region. The presence of migratory species underscores its function as a temporary sanctuary along migration routes, while the detection of species listed under conservation concern underscores its ecological significance. Seasonal trends revealed that variations in water levels and vegetation coverage markedly influenced bird communities and their use of various microhabitats. Hence, this study highlights the urgency for effective conservation measures, including habitat preservation, community-led management, and ongoing monitoring programs, to safeguard the persistent survival of wetland avifauna in the Irukkankudi reservoir.

KEYWORDS: Avifaunal diversity, Species richness, Seasonal variations, Ecological significance, Conservation measures.

INTRODUCTION

Biological diversity includes the variety of living organisms across terrestrial, marine, and aquatic ecosystems. Water is essential for life, with wetlands serving as transitional zones

rich in diverse flora and fauna. Birds play a vital ecological role in aquatic systems, contributing to food webs and adding aesthetic value. India hosts about 243 water bird species, many dependents on wetlands. Reservoirs are biologically productive, with marshy areas providing critical feeding, breeding, and nesting habitats. Globally, around 9,000 bird species exist, known for their ecological importance and role as sensitive environmental indicators. Birds also help control insect populations, especially in tropical regions with high bird diversity. However, studies on bird communities in South India's natural and artificial habitats are limited. Birds act as ecological indicators, reflecting changes and disturbances in ecosystems. Conserving habitat diversity is vital to protect endangered bird species. This study highlights bird diversity at Irukkankudi Reservoir, Virudhunagar District, Tamil Nadu, where 26 species were recorded, underlining the reservoir's ecological significance.

Study area description:

The Irukkankudi reservoir in Virudhunagar, Tamil Nadu, built between 1993-2004, holds 500 million cubic feet of water for irrigating over 11,700 acres. However, poor maintenance, damaged canals, upstream reservoirs, and irregular rainfall have caused underutilization, disrupting irrigation for local farmers.

MATERIALS AND METHODS

Fixed-radius point counts at Irukkankudi water reservoir involved 10-minute surveys within 20-meter radius circles. Surveys were conducted in morning and evening to maximize bird detections. When habitat obstacles limited full circle coverage, the surveyed area was noted. Birds seen or heard within the radius were identified and counted 40x60 monocular provided detailed, bright views by magnifying birds 40 times with a large 60mm lens. Its narrow field of view required steady hands or a tripod. Stopwatch with countdown timers ensured precise 10-minute counts. Clipboards with data sheets were organized, weather-resistant field recording was done. Together, these tools enabled accurate observation, consistent timing, and systematic record-keeping critical for reliable point count bird surveys.

RESULTS

The Irukkankudi Reservoir in Virudhunagar District, Tamil Nadu, presents a diverse wetland habitat characterized by marshy zones that support rich plant and animal life. This area serves as an essential ecological niche for feeding, breeding, and nesting bird species. During the survey, 26 bird species were observed, including notable wetland and terrestrial species such

as Indian Spot-billed Duck, Little Egret, Painted Stork, White-throated Kingfisher, and Red-wattled Lapwing (Table 1). Counts were conducted during morning and evening hours to optimize detection, and all individuals within a 20-meter radius at each fixed point were recorded systematically. The variety and abundance of bird species highlight the ecological importance of this reservoir as a habitat supporting avian biodiversity (Table 2). The presence of diverse species indicates a healthy environment and underscores the need for continued monitoring and habitat conservation to maintain this biodiversity hotspot. Finally, the reservoir's habitat complexity and bird diversity signify its vital role in regional biodiversity conservation and reflect the health of wetland ecosystems in South India.

Table 1: Diversity of birds observed at Irukkankudi Water Reservoir,

Virudhunagar district

S.No	Family	Common Name	Scientific Name
1	Anatidae	Indian Spot-billed Duck	*Anas poecilorhyncha*
2	Ardeidae	Little Egret	*Egretta garzetta*
3	Threskiornithidae	Red-naped Ibis	*Pseudibis papillosa*
4	Alcedinidae	White-throated Kingfisher	*Halcyon smyrnensis*
5	Charadriidae	Red-wattled Lapwing	*Vanellus indicus*
6	Anhingidae	Oriental Darter	*Anhinga melanogaster*
7	Ciconiidae	painted Stork	*Mycteria leucocephala*
8	Ardeidae	Indian Pond-Heron	*Ardeola grayii*
9	Laniidae	Brown Shrike	*Lanius cristatu*
10	Pycnonotidae	Red-vented Bulbul	*Pycnonotus cafer*
11	Ploceidae	Baya Weaver	*Ploceus philippinus*
12	Artamidae	Ashy Woodswallow	*Artamus fuscus*
13	Coraciidae	Indian Roller	*Coracias benghalensis*
14	Oriolidae	Indian Golden Oriole	*Oriolus kundoo*
15	Phalacrocoracidae	Indian Cormorant	*Phalacrocorax fuscicollis*
16	Rallidae	Grey-headed Swamphen	*Porphyrio poliocephalus*
17	Picidae	Black-rumped Flameback	*Dinopium benghalense*
18	Corvidae	Rufous Treepie	*Dendrocitta vagabunda*
19	Muscicapidae	Indian Robin	*Copsychus fulicatus*
20	Motacillidae	White-browed Wagtail	*Motacilla maderaspatensis*
21	Nectariniidae	Purple Sunbird	*Cinnyris asiaticus*
22	Cisticolidae	Common Tailorbird	*Orthotomus sutorius*
23	Laridae	Whiskered Tern	*Chlidonias hybrida*

24	Columbidae	Rock Pigeo	*Columba livia*
25	Cuculidae	Asian Koel	*Eudynamys scolopaceus*
26	Dicruridae	Black Drongo	*Dicrurus macrocercus*

Table 2: Relative abundance of bird families in Irukkankudi Water Reservoir

S.No	Family	No. of Species	Relative Abundance(%)
1	Anatidae	1	3.85%
2	Ardeidae	2	7.69%
3	Threskiornithidae	1	3.85%
4	Alcedinidae	1	3.85%
5	Charadriidae	1	3.85%
6	Anhingidae	1	3.85%
7	Ciconiidae	1	3.85%
8	Laniidae	1	3.85%
9	Pycnonotidaer5	1	3.85%
10	Ploceidae	1	3.85%
11	Artamidae	1	3.85%
12	Coraciidae	1	3.85%
13	Oriolidae	1	3.85%
14	Phalacrocoracidae	1	3.85%
15	Rallidae	1	3.85%
16	Picidae	1	3.85%
17	Corvidae	1	3.85%
18	Muscicapidae	1	3.85%
19	Motacillidae	1	3.85%
20	Nectariniidae	1	3.85%
21	Cisticolidae	1	3.85%
22	Laridae	1	3.85%
23	Columbidae	1	3.85%
24	Cuculidae	1	3.85%
25	Dicruridae	1	3.85%
	Total	26	100%

Relative abundance of bird families was calculated by using the following formula Abundance % = Total number of individuals of the species / Total number of all species* 100

DISCUSSION

The study from March to September 2025 at Irukkankudi reservoir, Virudhunagar district, recorded 26 bird species from 25 families, showing high avian diversity with no dominant family. The reservoir supports varied ecological niches, enabling multiple bird families to

occupy distinct roles in the freshwater ecosystem. Both resident and migratory birds were observed, with habitat selection influenced by seasonal food availability, mainly aquatic invertebrates. Birds serves as reliable bio-indicators due to their ecological sensitivity, with population changes reflecting ecosystem health. During the dry season, migratory species like *Pelecanus onocrotalus* and *Threskiornis aethiopicus* withdrew, indicating habitat shifts linked to water quality. In winter, increased food, water inflow, and suitable nesting attracted the highest diversity and abundance of birds. These findings highlight the reservoir's ecological importance as a critical feeding, roosting, and breeding habitat throughout different seasons.

CONCLUSION

The present study highlights the rich avian diversity of Irukkankudi reservoir, with 26 species from 25 families recorded between March and September 2025. The reservoir supports both resident and migratory birds, reflecting its ecological importance as a feeding, breeding, and roosting habitat. Seasonal variations in bird populations were closely linked to prey availability, water quality, and habitat conditions, demonstrating the sensitivity of birds as bio-indicators. The observed patterns emphasize the need for continued monitoring and conservation of this freshwater ecosystem to maintain its biodiversity. Protecting such habitats is essential for sustaining both native and migratory avifaunal communities.

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A STUDY ON THE MORPHOMETRIC AND MERISTIC CHARACTERS OF FISHES AVAILABLE IN THE MARKET OF THANJAVUR, TAMIL NADU

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ABSTRACT: Aquaculture is one of the fastest-growing sectors in India, contributing significantly to food security and nutrition. Fish serve as rich sources of high-quality protein, essential fatty acids, vitamins, and minerals, playing a vital role in human health. The present study focused on the comparative analysis of morphometric and meristic characters of freshwater and marine fishes available in the commercial market of Thanjavur. A variety of fish samples were collected and identified through detailed morphometric measurements and meristic counts. The freshwater species identified includes *Labeo rohita, Mystus cavasius, Oreochromis mossambicus, Channa marulius, Channa striata, Cyprinus carpio, Mugil cephalus* while the marine species comprised *Elops machnata, Rastrelliger kanagurta, Poecilia reticulata, Sphyraena barracuda, Alectis indica, Lutjanus campechanus, Solea solea, Siganus sp., Elopidae, Carangoides malabaricus, Caranx ignobilis.* The study revealed distinct variations influenced by environmental and genetic factors, highlighting the importance of morphometric and meristic analysis in fish taxonomy, stock identification, and biodiversity documentation. These findings contribute to baseline data for fish systematics and aid in sustainable fishery resource management.

KEYWORDS: Aquaculture, Morphometric, Meristic, Fishes, Baselin

INTRODUCTION

Aquaculture is an industrial process of raising aquatic organisms up to final commercial production with in properly portioned aquatic areas, controlling the environmental factors and administering the life history of the positively and it has to be considered as an independent industry from the fisheries. One has to gain familiarity and control water quality to enhance it biological productivity One has understand fish nutrition so as to be able to formulate nutritionally balanced fish died; One has to develop deep into fish genetics as to be so able to evolve new varieties and strains which bestow nutritionally balanced advantages to the product in terms of surprise growth rate , nutritive value, boneless , taste, colour, etc…culture

fisheries are usually carried out in small water bodies which can be manipulated , pre-prepared for stocking; which are often manured and /are fertilized before during and after stocking and /or where fish are fed from extraneous sources. All shades of intermediate stages between true capture and culture fisheries exist such as in man-made lakes, which are stocked extraneously.

Fish plays a vital role as one of the most affordable and accessible sources of high- quality animal protein worldwide, contributing significantly to food security and nutrition (Patel *et al.,* 2023). In India, inland and marine fisheries provide livelihood opportunities for millions of people, and fish biodiversity supports ecological balance and sustainable aquaculture practices (Ramesh *et al.,* 2023). With growing consumer demand and expanding aquaculture activities, understanding the biology and stock structure of fishes has become increasingly important.

Morphometric and meristic analyses are widely used tools in fish biology for species identification, population differentiation, and stock assessment. Morphometric characters refer to measurable traits such as length, depth, and fin dimensions, which are influenced by environmental factors, ecological conditions, and growth patterns. Meristic characters, on the other hand, include countable traits such as fin rays, scales, and gill rakers, which are generally stable and species-specific (Sharma *et al.,* 2024). Together, these parameters provide valuable insights into taxonomy, systematics, and fisheries management. Recent studies have emphasized the importance of morphometric and meristic traits for assessing fish populations under different ecological and aquaculture systems. Ravichandran *et al.,* (2025) reported that morphometric traits of native freshwater fishes in Tamil Nadu varied significantly across environments, while meristic counts remained consistent, thereby confirming their taxonomic value. Similarly, Kumar *et al.,* (2025) highlighted that integrating morphometric and meristic characters allows effective monitoring of commercially important fish species in landing centers. In addition, Patel *et al.,* (2023) demonstrated that length–weight relationships derived from morphometric data are essential to evaluate growth patterns and condition factors, which serve as indicators of fish health.

The reason for the superior feed conversion efficiency of fish is that it assimilates diets with higher percentage environment than worm- blooded animals. The Asian Fisheries Society (AFS) was established in 1984 to promote greater interactions, research and development (R&D) in the Asian Pacific (AP) region; it aims to encourage and facilitate research activity.

The marine state of Tamil Nadu is blessed with 1,076 Km long coastline and Tamil Nadu are enriched with marine, brackish water and inland fisheries resources amenable for capture and culture. Fisheries substantial focuses are being given on the economic and social dimensions of fisheries resources by the government of Tamil Nadu. The Indian Fisheries Act 1897 enacted by the Madras Presidency paved the way for the formulation of fisheries legislation across India. The fisheries sector places an important role in the socio-economic development of the County, by providing livelihood to large number of fishers, generating employment opportunities in allied sectors and ensuring nutritional security. Dwivedi (2019), Performed the future detecting Natural hybridization among Indian major carp's bio river. Gonzalex *et al.,* (2020) morphometric and meristic characterization of native chime fish in Ecuador using multivariate analysis. Sanjay and Shital (2021) studied of morphometric and meristic study of five freshwater fish species of Song River, Uttarakhand.

MATERIALS AND METHODS STUDY AREA

The samples were collected from Kamarajar fish market Thanjavur. Thanjavur lies between 78045' and 70025' of and 905' and 11025'. The samples were collected for a period of 2 weeks (plate1). The samples were brought to the Laboratory and morphometric measurements were performed (Jayaram 1981). The meristic characters were performed (Bengal 1978).

MORPHOMETRIC MEASUREMENT (JAYARAM 1981, PAULIN, 1988)

Morphometric measurements, such as length parameters which quantify axial growth of various parts of the fresh fish samples, were carried out with the aid of a measuring board, ruler, and divider. All measurements were conducted with the head of the fish specimen positioned to the left flank. The following measurements were recorded: total length (TL), standard length (SL), head length (HL), body depth (BD), eye diameter, pre-pelvic fin length, fork length, snout length, dorsal fin base length, pre-pectoral length, upper jaw length, lower jaw length, post-orbital length, lateral line scales, and anal fin length.

MERISTIC CHARACTERS (Bengal, 1978) Meristic character count represents the number of lateral line fin count, dorsal fin count, anal fin count, caudal fin count and pelvic fin count.

RESULTS

In the present study the morphometric and meristic analysis of freshwater and marine sample collected from Kamarajar fish market revealed the following observation. In the study totally 8 freshwater fishes and 10 marine fishes were studied.

DISCUSSION

Kamboj and Kamboj (2019) conducted a morphometric and meristic analysis of four freshwater fish species from the River Ganga. They observed that morphometric traits, such as total length, head length, and body depth, varied significantly among populations, reflecting ecological and environmental influences. However, meristic traits like fin ray counts and scale numbers were stable across all populations, confirming their reliability for species identification. These results align with the notion that morphometric variation is sensitive to environmental and habitat conditions, whereas meristic characters are conservative and taxonomically informative. Kumar et al. (2020) conducted a morphometric and meristic analysis of Wallago attu from the Bhadar Reservoir, West Coast of India. Their study revealed that morphometric traits exhibited positive allometric growth, indicating that the fish's body length increased at a faster rate than its body weight. Additionally, meristic counts, such as fin ray numbers, were consistent across different size classes, underscoring their reliability for species identification. These findings align with the notion that while morphometric traits can reflect environmental influences and growth patterns, meristic characters remain stable and are valuable for taxonomic studies.

Paunikar and Panwar (2021) conducted a morphometric and meristic analysis of five freshwater fish species from the Song River in Dehradun, Uttarakhand. Their study revealed that while morphometric traits such as total length, fork length, and standard length exhibited significant variations among species, meristic characters like fin ray counts and scale numbers remained consistent within species.

Singh *et al.,* (2022) studied fish populations from the Ganga and Gomti rivers of Uttar Pradesh and reported that morphometric traits differed significantly between river systems, whereas meristic counts remained stable within species. They concluded that morphometric variation is influenced by environmental factors, and meristic traits provide consistent taxonomic markers.

Patel *et al.,* (2023) analyzed the length– weight relationship and condition factor of freshwater fishes and demonstrated that the regression exponent (b value) varied among species, indicating differences in growth patterns (isometric vs. allometric). These findings confirmed that morphometric and LWR studies are essential for evaluating fish health, growth, and population dynamics.

Similarly, Ramesh *et al.,* (2023**)** investigated morphometric characteristics of freshwater fishes in Tamil Nadu and observed that length–weight relationships (LWR) and condition factors varied significantly between species and locations.

Table :1 Morphometric characters of fishes Available in commercial market

S.No	CHARACTER (CM)	S1−$\bar{x}$ ± SD	S2−$\bar{x}$ ± SD	S3−$\bar{x}$ ± SD	S4−$\bar{x}$ ± SD	S5−$\bar{x}$ ± SD	S6−$\bar{x}$ ± SD	S7−$\bar{x}$ ± SD	S8−$\bar{x}$ ± SD	S8−$\bar{x}$ ± SD	S8−$\bar{x}$ ±SD	S8−$\bar{x}$ ±SD	S8−$\bar{x}$ ± SD
1	Total Length *(cm)*	8.2 ± 0.21	11.0 ± 2.26	14.7 ± 5.55	18.8 ± 2.32	15.9 ± 1.57	17.8 ± 2.28	18.1 ± 2.30	8.8 ± 2.32	19.1 ± 2.94	8.9 ± 0.86	22.8 ± 3.72	28 ± 3.55
2	Standard Length *(cm)*	6.4 ± 1.47	8.0 ± 1.57	11.8 ± 3.52	16.7 ± 2.02	12.9 ± 1.69	15.1 ± 2.18	15.8 ± 2.60	16.3 ± 3.15	17.8 ± 3.08	6.8 ± 1.27	18.9 ± 3.43	24.8 ±3.86
3	Fork Length *(cm)*	7.5 ± 1.39	9.7 ± 2.53	13.7 ± 4.85	18.2 ± 2.30	15.2 ± 1.95	17.3 ± 2.86	5.8 ± 16.3	20. ± 2.69	19.1 ± 2.47	8.2 ± 2.36	22.1 ± 2.85	26.2 ± 2.92
4	Head Length *(cm)*	1.7 ± 1.09	1.7 ± 1.29	3.7 ± 13.6	0.8 ± 0.88	4.1 ± 1.50	5.3 ± 1.87	3.2 ± 1.46	8.9 ± 1.87	2.3 ± 0.89	1.8 ± 0.87	1.8 ± 0.48	1.8 ± 1.56
5	Snout Length *(cm)*	1.0 ± 1.01	0.9 ± 0.99	0.9 ± 0.97	1.5 ± 1.09	1.9 ± 1.23	2.7 ± 0.33	5.0 ± 1.73	3.8 ±1.47	2.8 ± 0.55	1.2 ± 0.92	1.7 ± 0.95	1.6 ± 0.86
6	Eye Diameter *(cm)*	0.5 ± 0.25	0.8 ± 0.93	2.9 ± 1.84	1.5 ± 1.13	3.9 ± 1.41	3.9 ± 1.66	6.1 ± 1.97	6.3 ± 1.98	2.4 ± 0.89	1.2 ± 0.88	0.9 ± 0.86	1.9 ± 0.56
7	Post Orbital Length *(cm)*	3.2 ± 1.29	2.6 ± 1.39	2.7 ± 1.79	3.8 ± 1.40	3.3 ± 1.29	5.0 ± 0.34	12.7 ± 255	8.0 ±1.82	5.3 ± 1.58	2.2 ± 1.36	5.9 ± 2.37	7.7 ± 2.94
8	Dorsal Finbase Length *(cm)*	3.2 ± 1.28	2.8 ± 1.50	7.6 ± 1.88	7.9 ± 1.40	9.1 ± 3.67	11.4 ± 0.46	13.4 ± 2.64	13.2 ±2.34	12.1 ± 2.36	3.5 ± 1.85	12.8 ± 2.54	17.9 ± 2.88
9	Pre-Pectoral Length *(cm)*	9.0 ± 1.73	7.7 ± 1.95	3.8 ± 1.40	19.8 ± 2.36	5.0 ± 2.72	12.1 ± 0.46	8.9 ± 1.87	12.2 ± 1.95	25.2 ± 2.29	8.2 ± 2.48	24.7 ± 3.98	28.3 ± 3.86
10	Pre-Pelvic Length *(cm)*	4.0 ± 1.39	3.9 ±1.76	4.1 ± 2.20	8.0 ± 3.11	2.8 ± 1.30	7.2 ± 0.37	6.1 ± 1.68	11.1 ± 1.80	23.6 ± 3.87	3.7 ± 0.89	12.8 ± 0.85	18.2 ± 2.98
11	Upper Jaw Length *(cm)*	2.3 ± 1.35	2.3 ± 1.31	1.8 ± 1.35	2.9 ± 1.42	7.9 ±1.96	5.4 ± 1.88	18.1 ± 2.60	8.1 ± 2.59	4.8 ± 0.58	3.7 ± 0.86	4.8 ± 2.30	5.3 ± 0.56
12	Maximum Body Depth *(cm)*	4.5 ± 1.36	4.1 ± 1.60	6.6 ± 2.08	12.4 ± 3.14	14.9 ± 4.49	9.9 ± 1.75	12.1 ± 2.05	13.2 ± 2.34	30.2 ± 2.54	3.4 ± 0.95	16.1 ± 0.46	47.8 ± 0.86
13	Lateral Line Scales Length *(cm)*	12.9 ± 1.69	13.9 ± 1.91	12.9 ± 4.02	17.1 ± 5.32	14.9 ± 1.55	19.1 ± 2.62	20.9 ± 2.11	23.1 ± 2.79	19.8 ± 2.45	9.8 ± 2.76	19.7 ± 2.36	21.7 ± 3.08
14	Caudal Line Scales Length *(cm)*	22.1 ± 2.43	24.0 ± 5.64	25.7 ± 2.60	29.8 ± 3.56	32.2 ± 2.38	34.9 ± 3.19	37.1 ± 2.42	38.1 ± 2.92	35.1 ± 3.56	18.8 ± 1.56	39.7 ± 3.96	45.3 ± 3.89
15	Anal Fine Length *(cm)*	5.0 ± 2.07	5.7 ± 2.05	11.6 ± 3.80	14.8 ± 1.93	17.1 ± 2.26	19.1 ± 3.77	21.8 ± 2.42	22.6 ± 2.15	11.8 ± 1.87	8.8 ± 0.83	19.9 ± 2.33	22.5 ± 2

CHARACTER	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Lateral Fin Count	11.8 ± 2.03	12.8 ± 2.38	12.7±3.99	15.9 ± 1.77	15.9 ± 1.77	21.3 ± 3.96	18.9 ± 2.64	21.1 ± 2.71	15.1 ± 1.94	14.7 ± 2.41	21.3 ± 3.96	18.9 ± 2.64
Dorsal Fin Count	12.8 ± 2.32	13.7 ± 5.45	14.8 ± 3.95	11.8 ± 1.66	11.8 ± 1.66	24.8 ± 2.20	22.8 ± 2.45	24.9 ± 2.20	10.9 ± 1.82	11.2 ± 2.04	24.8 ± 2.20	22.8 ± 2.45
Anul Fin Count	11.3 ± 5.62	12.7 ± 3.99	14.1± 4.36	12.9 ± 1.89	12.9 ± 1.89	27.6 ± 3.60	25.0 ± 0.29	22.9 ± 2.78	11.7 ± 2.26	12.1 ± 2.08	27.6 ± 3.60	25.0 ± 0.29
Caudal Fin Count	22.2 ± 2.49	23.5 ± 6.47	22.9 ± 2.28	21.8 ± 2.42	21.8 ± 2.42	29.7 ± 2.65	32.1 ± 3.14	32.9 ± 2.05	25.1 ± 1.94	24.7 ± 3.32	29.7 ± 2.65	32.1 ± 3.14
Pelvic Fin Count	11.9 ± 1.50	14.7 ± 4.91	17.9 ± 3.28	12.8 ± 1.69	12.8 ± 1.66	16.1 ± 2.50	18.8 ± 2.32	22.9 ± 2.90	13.7 ± 1.58	13.8 ± 2.13	16.1 ± 2.50	18.8 ± 2.32

Their results emphasized the importance of morphometric studies in fisheries biology and stock assessment, particularly in distinguishing cultured and wild populations. Sharma *et al.,* (2024) carried out morphometric and meristic analysis of Ompok pabda from two different environments and reported that morphometric traits varied considerably between populations due to ecological differences, while meristic characters remained constant. This study highlighted that morphometric traits reveal population-level plasticity, whereas meristics confirm taxonomic identity.

Kumar *et al.,* (2025) characterized morphometric and meristic traits of commercially important fishes from a landing centre in India and found that most morphometric ratios showed significant interspecific variations, while meristic counts were consistent within species. They concluded that combining morphometric and meristic data provides an effective tool for stock identification and biodiversity monitoring. Ravichandran *et al.,* (2025) studied the morphometric analysis of native freshwater fishes from Manur Pond, Tirunelveli District, Tamil Nadu and reported that significant variation was observed in body proportions of the examined species, while meristic counts such as dorsal fin rays, anal fin rays and scale counts remained stable across individuals. The authors concluded that morphometric traits are strongly influenced by environmental conditions, whereas meristic characters are conservative and reliable for species identification.

In the present study, the fishes collected from Thanjavur markets showed patterns consistent with these previous findings. Morphometric traits such as body depth, head length, and fin measurements varied among species and even within species, likely due to environmental differences, aquaculture practices, or size-selective harvesting. Conversely, meristic characters like dorsal and anal fin rays and scale counts remained consistent across specimens, confirming their reliability for species identification. These findings emphasize the dual importance of morphometric and meristic analyses in fish population studies. Morphometric characters provide insights into environmental adaptation, growth, and ecological plasticity, while meristic characters serve as stable taxonomic markers. Such integrative approaches are essential for monitoring fish diversity in markets like Thanjavur, assessing the origin of cultured versus wild specimens, and informing fisheries management and conservation strategies.

CONCLUSION

Aquaculture is a rapidly growing fisheries sector in India with an annual rate of over 7%. Fish water Aquaculture contributes over 95% of the total annual Aquaculture production of 5.77 million t. Fish are the rich sources of protein, essential fatty acids, vitamins and minerals. The fat and fatty acids in fish, particularly omega-3 fatty acids, are highly beneficial and difficult to obtain from other food sources. In many developing countries, fish is the cheapest source, of animal protein to the poor and fish protein superior quality as it contains all the essential amino acids for body building than plant protein. Geometric increases in population have shortage of food stuff from land mean ratio is decreasing day by day. Hence, an attempt was made to collect the available fish sample in commercial market of Thanjavur. To categorize the fish sample, the perform the morphometric and meristic characters of a collected fish sample, to identify the fishes.

The morphometric study was conducted on freshwater fishes, including *Labeo rohita* (Rohu), *Mystus cavasius* (Gangetic Mystus), *Oreochromis mossambicus* (Tilapia), *Channa marulius* (Great Snakehead), *Channa striata* (Striped Snakehead), *Cyprinus carpio* (Common Carp), and *Mugil cephalus* (Flathead Grey Mullet). Similarly, morphometric analysis was performed on marine fishes, including *Elops machnata* (Ladyfish), *Rastrelliger kanagurta* (Indian Mackerel), *Poecilia reticulata* (Guppy), *Sphyraena barracuda* (Barracuda), *Alectis indica* (Indian Threadfish), *Lutjanus campechanus* (Red Snapper), *Solea solea* (Common Sole), *Siganus canaliculatus* (Rabbitfish), *Carangoides malabaricus* (Malabar Trevally), and *Caranx ignobilis* (Giant Trevally). Thus, the present study revealed that diversity of freshwater and marine fishes available in the market.

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BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM ENDOPHYTIC BACTERIA *Serratia odorifera* ISOLATED FROM THE BROWN ALGA *Dictyota dichotoma*

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ABSTRACT

The field of nanotechnology has revolutionized modern medicine, particularly through the development of eco-friendly biosynthesis methods for metallic nanoparticles. This study focuses on the biogenic synthesis of silver nanoparticles (So-AgNPs) using the endophytic bacterium *Serratia odorifera*, isolated from the marine brown seaweed *Dictyota dichotoma*. The extracellular synthesis was initiated by treating the cell-free culture filtrate with silver nitrate ($AgNO_3$), resulting in a distinct color change from milky white to pale brown, indicating the formation of So-AgNPs. Characterization via UV-Vis spectroscopy, Fourier-Transform Infrared (FTIR) spectroscopy, and Scanning Electron Microscopy (SEM) confirmed the successful production and stabilization of these nanoparticles. The findings suggest that marine-derived endophytes serve as a potent, sustainable "bio-factory" for producing functional nanomaterials with significant biomedical potential.

KEYWORDS: Silver Nanoparticles, Biosynthesis, *Serratia odorifera*, *Dictyota dichotoma*, Nanobiotechnology.

INTRODUCTION

Nanoparticles, often defined as structures ranging from 1 to 1000 nm, have gained immense intrest due to their high surface-area-to-volume ratio, which enhances their functional interactions in medical and industrial applications (Thakkar *et al.,* 2010). Among these, silver nanoparticles (So-AgNPs) are particularly valued for their antimicrobial properties, finding utility in everything from water purification to wound healing (Krishnaraj *et al.,* 2010).

While chemical and physical methods for So - AgNPs synthesis exist, they often involve toxic reagents and high energy consumption. Consequently, biological synthesis using

bacteria, fungi, or algae has emerged as a rapid, non-toxic, and "green" alternative (Zhang *et al.,* 2016). Marine macroalgae, such as the brown seaweed *Dictyota dichotoma*, are rich in bioactive secondary metabolites that contribute to their survival and pharmacological potential (Wang *et al.,* 2019). These seaweeds host unique communities of endophytic bacteria—symbiotic microorganisms that live within the host tissues and aid in growth and disease resistance. Recent studies have begun to explore these endophytes as sustainable mediators for nanoparticle formation (Swetha *et al.,* 2020).

This research aims to isolate *Serratia odorifera* from *D. dichotoma* and utilize its metabolic bioactive compounds to synthesize and characterize So-AgNPs for future biomedical applications.

MATERIALS AND METHODS

The endophytic bacterium *Serratia odorifera* was isolated from the marine brown macroalgae *Dictyota dichotoma* and maintained as a pure culture on Zobel marine Agar (ZMA) with 2% NaCl. For the biosynthesis of silver nanoparticles (So-AgNPs), an overnight culture was inoculated into LB broth and incubated for 48 hours to reach the exponential growth phase. The culture was then subjected to centrifugation to obtain a cell-free filtrate, which served as the reducing and stabilizing medium. This filtrate was treated with a silver nitrate ($AgNO_3$) solution and incubated for 96 hours, during which a visual transition from a milky white to a pale brown color confirmed the extracellular reduction of silver ions. The resulting So-AgNPs were harvested via high-speed centrifugation, purified, and dried. Structural and morphological characterization was performed using UV-Vis spectroscopy (200–800 nm) using UV-Visible Spectrophotometer 2550 (Shimadzu, Japan), Fourier-Transform Infrared (FTIR) spectroscopy to identify bioactive functional groups specifically at peaks 3333.36cm^{-1} (amide), 1369.21 cm^{-1} (amine), and 1028.84 cm^{-1} (alkyl amine) and Scanning Electron Microscopy (SEM) to analyze nanoparticle surface topography. (done at the Madurai Kamaraj university).

RESULTS AND DISCUSSION

The successful isolation of *Serratia odorifera* was confirmed by the growth of distinct colonies after 48 hours of incubation.

Plate 1: Growth of endophytic bacteria after 48 hrs

Upon adding $AgNO_3$ to the cell-free culture filtrate, a visible color change occurred within 96 hours. This change is a hallmark of silver ion reduction and the excitation of surface plasmon resonance (SPR), typical for metallic nanoparticles.

Plate 2: Change of color from milky white to pale brown after 96hrs of incubation

0hr 96h

FTIR analysis revealed several key peaks, notably at 3333.36 cm^{-1} (N-H stretching of amides) and 1369.21cm^{-1}(C-N and C-C stretching of amines). These results indicate that proteins and other biomolecules act as stabilizing and capping agents, preventing aggregation.

Figure 1. FT-IR spectra of silver nanoparticles synthesized from *Seratia odorifera*

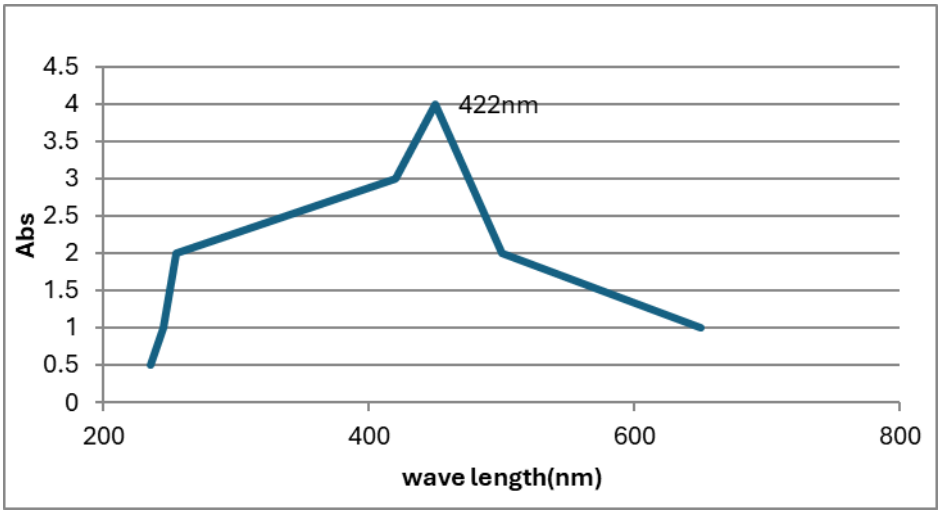

Table 1: FT-IR Frequency and Functional group responsible for the formation of So-AgNPs

Peak (cm−1)	Bond	Functional Group
1028.84	C=C	Alkyl amine
1369.21	C-N, C-C	Amines
3333.36	N-H	Stretch amide

SEM micrographs further illustrated the morphology of the biosynthesized So-AgNPs, showing the formation of distinct pellets after centrifugation of the reduced solution. The use of *S. odorifera* highlights the versatility of the *Serratia* genus, which is known for secreting various functional factors. By leveraging these biological pathways, we can produce stable So-AgNPs without hazardous chemical reducers.

CONCLUSION

The present study successfully demonstrated a "green" and sustainable route for the synthesis of silver nanoparticles using the marine endophytic bacteria *Serratia odorifera*. The characterization data confirmed that the nanoparticles are well-stabilized by bacterial biomolecules. Given the growing demand for eco-friendly nanomaterials, these marine-

derived So-AgNPs offer a promising foundation for applications in medicine, biotechnology, and environmental sciences.

Plate 3-5: SEM micrograph of silver nanoparticles synthesized from *Serratia odorifera*

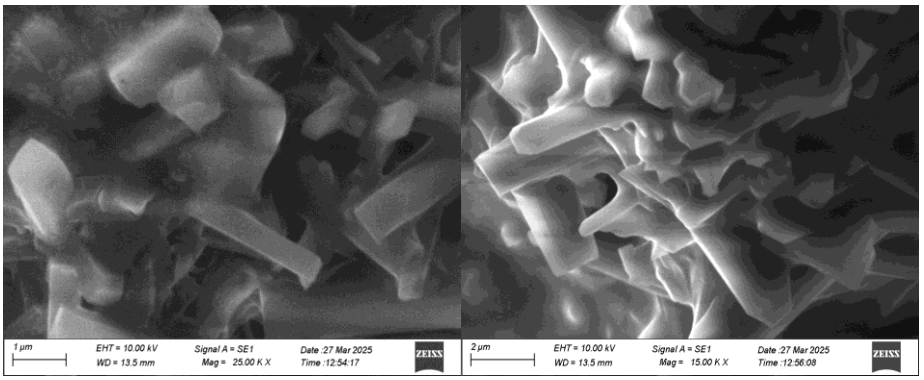

Plate 3 Plate 4

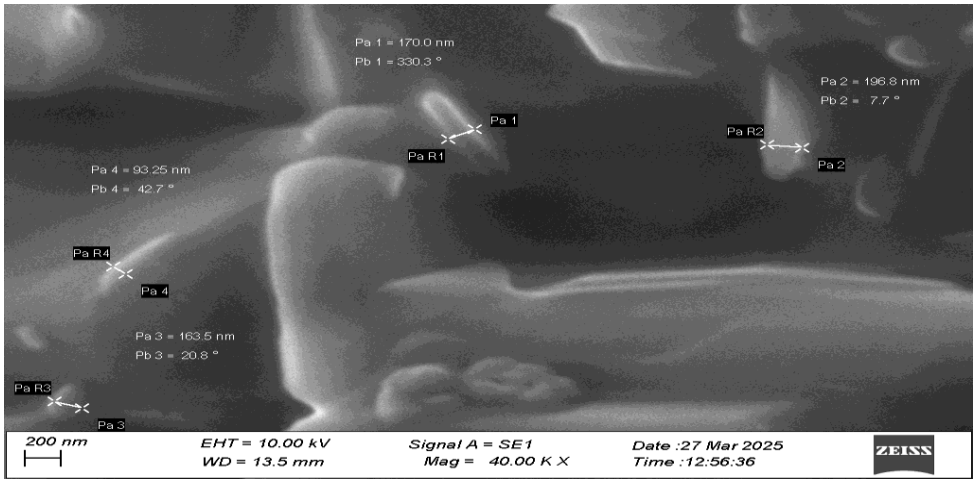

Plate 5

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THE PLASTIC-EATING MUSHROOMS

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ABSTRACT: Plastic pollution has emerged as one of the most pressing environmental challenges of the 21st century, with synthetic polymers accumulating in landfills, oceans, and ecosystems at alarming rates. Traditional recycling methods have proven insufficient, prompting the search for innovative, sustainable solutions. Among these, the discovery of plastic-degrading fungi commonly referred to as plastic-eating mushrooms—offers a promising avenue for bioremediation. Notably, species such as *Pestalotiopsis microspora* and *Aspergillus tubingensis* have demonstrated the ability to metabolize polyurethane and other synthetic polymers, even in anaerobic conditions, making them suitable for landfill environments. This research explores the biological mechanisms underlying fungal plastic degradation, focusing on the enzymatic pathways involving esterases, laccases, and peroxidases. These enzymes facilitate the breakdown of complex polymer chains into simpler, non-toxic compounds. Laboratory studies have shown that certain fungi can degrade plastics within weeks, a stark contrast to the centuries required for natural decomposition. The potential applications of these fungi extend beyond waste management to include integration into recycling systems, development of bio-reactors, and even consumer-level composting technologies. However, challenges remain in scaling these processes for industrial use. Factors such as environmental stability, degradation efficiency, and ecological safety must be addressed. Moreover, genetic engineering and synthetic biology offer opportunities to enhance fungal capabilities, potentially accelerating degradation rates and expanding the range of target plastics. In conclusion, plastic-eating mushrooms represent a novel and ecologically sound strategy for mitigating plastic pollution. By harnessing the power of fungal metabolism, we may pave the way toward a more sustainable future—where nature itself contributes to healing the damage caused by human consumption.

INTRODUCTION

Plastic pollution has become one of the most pressing environmental challenges of our time. With millions of tonnes of plastic waste accumulating in oceans, land and landfills every year, the search for sustainable solutions is more urgent than ever. Faced with this problem, a

surprising discovery has attracted the attention of scientists and the public: certain mushrooms have the ability to “eat” plastic. This article explores this potential game-changing solution, detailing current scientific research, challenges and possible future applications.

THE DEVASTATING IMPACT OF PLASTIC ON THE ENVIRONMENT

Global plastic production has exceeded 400 million tonnes per year, and the majority of these plastics end up as waste after often very brief use. Plastics, due to their chemical composition, take centuries to decompose naturally, leading to a massive accumulation of plastic waste in the environment. The oceans, for example, today contain more than 150 million tons of plastic, creating "islands" of floating trash, such as the infamous "Great Pacific Garbage Patch. "Plastics do not completely degrade in nature; they fragment into microplastics, tiny particles that infiltrate ecosystems and the food chain. These microplastics have been found in fish, shellfish and even drinking water. They pose a danger not only to wildlife, which may ingest them by mistake, but also to human health.

MUSHROOMS: POWERFUL NATURAL DECOMPOSERS

Fungi play a crucial role in natural ecosystems as decomposers. Unlike plants, which use photosynthesis, fungi break down organic matter using enzymes they secrete into their environment. This process recycles essential nutrients into the soil. But recently, researchers have discovered that certain fungi are capable of breaking down much tougher materials, including plastic. (Khan, S., et al. (2017). “Biodegradation of Polyester Polyurethane by *Aspergillus tubingensis.”* Environmental Pollution, 225, 469-480)

These decomposer fungi produce enzymes such as peroxidases and laccases, which can break down the long polymer chains found in plastics. This process is similar to the one they use to break down complex materials like wood.

This unique ability has generated considerable interest in the scientific community, which seeks to harness these fungi to process plastic waste.

PLASTIC-EATING MUSHROOMS: A REVOLUTIONARY SOLUTION

Two species of fungi have particularly attracted attention for their ability to break down plastic: *Pestalotiopsis microspora* and *Aspergillus tubingensis.*

Pestalotiopsis microspore: Originally discovered in the rainforest of Ecuador, this fungus has the unique ability to break down polyurethane, a type of plastic widely used in products ranging from clothes to refrigerators. Yale University researchers, who conducted studies on

Pestalotiopsis microspora, discovered that this fungus could not only decompose polyurethane in the absence of oxygen, but could also convert it to biomass, making it a potential solution for anaerobic landfills.

Aspergillus tubingensis: Discovered in a dump site in Pakistan, *Aspergillus tubingensis* was revealed be able to break down polyester in just a few weeks. This fungus works by secreting enzymes that break the chemical bonds in plastic, thus facilitating its degradation. Research by scientists at the Kunming Institute of Botany showed that this process could be accelerated by adjusting environmental conditions, such as pH and temperature.

HOW DOES THE PLASTIC DECOMPOSITION PROCESS BY FUNGI WORK

The process of plastic decomposition by fungi is based on enzymatic activity. Fungi secrete enzymes such as hydrolases, which break down plastic polymers into smaller monomers. Here are the main steps of the process:

- Hydrolysis: Fungal enzymes attack ester bonds in plastic polymers, initiating the breakage of long, complex chains into smaller, soluble units.
- Biodegradation: Once polymers are broken down into monomers, fungi use them as a source of carbon and energy. This process not only breaks down plastic, but also converts it into biomass and other organic compounds.

  Research has shown that certain species of mushrooms can reduce the weight of plastic waste by 40-60% in just a few weeks. However, the rate of decomposition depends on many factors, such as the type of plastic, enzyme concentration, and environmental conditions like temperature and humidity.

PRACTICAL APPLICATIONS AND FUTURE POTENTIAL

The use of plastic-eating mushrooms has enormous potential for waste management. Among the practical applications, we can cite:

- Landfill bioremediation: Mushrooms could be used to treat plastic waste in landfills, reducing their volume and the risk of environmental contamination.
- Wastewater treatment systems: Fungi could be integrated into wastewater treatment systems to degrade microplastics, thereby reducing plastic pollution in waterways. water and oceans.
- Sustainable manufacturing: By harnessing fungi to produce plastic-degrading enzymes, it may be possible to develop plastic materials that are more easily biodegradable.

Biotech startups such as Biohm in the UK and Fungi Mutarium in Austria are already working on commercial applications of fungi for plastic degradation and sustainable materials development. However, there are still many challenges to overcome for large-scale implementation.

THE CHALLENGES AND LIMITATIONS OF USING PLASTIC-EATING MUSHROOMS

Although promising, plastic-eating fungi present significant limitations and challenges:

- Specific growing conditions: Fungi require specific growing conditions, such as optimal pH and humidity levels, which can be difficult to reproduce on a large scale. ladder.
- Variable decomposition rate: Not all types of plastics are as easily broken down by fungi. For example, high-density plastics like high-density polyethylene (HDPE) are stronger than low-density plastics.
- Potential environmental impacts: Introducing decomposer fungi into uncontrolled environments could have unforeseen effects on local ecosystems.

  Despite these challenges, continued research and technological improvements could help overcome these obstacles. Mushrooms offer a potential biological solution to the global plastics crisis, but they should only be seen as part of a broader, integrated approach to plastic waste management.

CONCLUSION

MUSHROOMS, A HOPE FOR A PLASTIC-FREE FUTURE

Plastic-eating mushrooms represent an innovative and natural approach to addressing the global plastic waste crisis. By breaking down complex plastic polymers into their basic components, these fungi could offer a sustainable solution for waste management and bioremediation. However, their large-scale use requires further research, testing and technological development.

It is essential to continue exploring natural solutions like mushrooms while integrating plastic reduction, reuse and recycling strategies. With increased collaboration between scientists, governments, and businesses, it is possible to hope for a future where plastics are no longer a burden on our planet.

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IRS-1 (INSULIN RECEPTOR SUBSTRATE-1) GENE POLYMORPHISM AND OXIDATIVE STRESS MARKERS IN POLYCYSTIC OVARIAN SYNDROME AMONG WOMEN IN MADURAI -A CASE CONTROL STUDY

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Abstract: Polycystic ovary syndrome (PCOS) is considered to be a multifaceted disease affecting not only women of childbearing age, but also adolescents and postmenopausal women. The clinical manifestation of PCOS includes oligomenorrhoea, acne, hair loss and psychiatric disturbances such as anxiety and depression. PCOS appears to be associated with an increased risk of metabolic aberrations, with consequences that include impaired glucose tolerance, type 2 diabetes, obesity and cardiovascular diseases. This study aimed at finding the association of genetic predisposition of polymorphism in *IRS-1*(Insulin Receptor Substrate-1) gene in pathogenesis of PCOS and the related biochemical marker of oxidative stress in controls and cases. Blood samples (n=50) each form healthy controls and cases were collected from Ashirvatham specialty clinic, Madurai, with basic details using a consent form. Genomic DNA isolated from blood was subjected to PCR based RFLP analysis. Plasma obtained was used for lipid peroxidation and total antioxidant assays and the data were subjected to Mann Whitney Rank Sum Test using the Sigma Plot software (v 11.0). The results of the study showed that the mean age was 20.4 ± 0.2 and 20.1 ± 0.6 years in controls and cases respectively however; the incidence of PCOS was high among the adolescents (34%) and young adults (38%). Incidence of PCOS was 78% in urban locality and that of rural was 64%. The differences in the body mass index between the controls 22.6 [CI: 19.8-24] and cases 24.9[CI: 22.3- 28.4] was statistically significant ($p<0.001$). Estimation of biochemical markers revealed that the median value of lipid peroxidation was higher (97.5 nM/mL) in cases than controls (90 nM/mL). The total antioxidant status was found to be

lower (458.5µM/L; CI: 183-717) in cases than controls (500µM/L; CI: 333-667). However, the differences in the median values were statistically not significant (*p = 0.906*). The SNP analysis of IRS-1 gene revealed that the variant genotype was absent in study subjects and distribution of genotypes was not in accordance to Hardy Weinberg equilibrium. Thus, the study showed no association of IRS1 gene polymorphism with PCOS and need to extend to a large sample size and explore the influence of life style modifications.

Keywords: PCOS, Oligomenorrhoea, Polymorphism, Lipid Peroxidation, Total Antioxidant

INTRODUCTION

The change in life style habits towards sedentary to less active mode and consumption of unhealthy food habits has pushed women to acquire PCOS a disease of concern. A report in daily (2019) projected an estimate of one in five (20%) Indian women suffer from PCOS. Polycystic ovary syndrome (PCOS) affects not only women of childbearing age, but also adolescents and postmenopausal women.

PCOS, is defined by the presence of any two of the three features: (1) Oligo/amenorrhea: absence of menstruation for 45 days or more and/or ≤8 menses per year. (2) Clinical hyperandrogenism: score of 6 or higher. (3) Polycystic ovaries usually combined with increased ovarian volume of >10 cm^3, and an echo-dense stroma in pelvic ultrasound scan (Ganie *et al.,* 2019).

The clinical manifestation of PCOS includes oligomenorrhoea, hirsutism, excessive acne and hair loss. In adolescents, it causes significant psychiatric disturbances such as anxiety and depression. PCOS is the leading cause of anovulatory infertility in women and even endometrial carcinoma PCOS appears to be associated with an increased risk of metabolic aberrations. A meta-analysis study by Murri *et al.,* (2013) reported that circulating markers of oxidative stress are abnormal in women with PCOS, suggesting that oxidative stress may participate in the pathophysiology apart from several characteristics and

associations, such as androgen excess, abdominal adiposity, insulin resistance and obesity, may contribute to the development of local and systemic oxidative stress

The metabolic consequences include impaired glucose tolerance, type 2 diabetes, obesity and increased risk of cardiovascular diseases. Metabolic complications, including insulin resistance and hyperinsulinism, type 2 diabetes mellitus, dyslipidemia, (Azziz *et al.,* 2004) increased cardiovascular disease, and endometrial carcinoma were found to be more in the classic PCOS compared to other phenotypes (Diamanti-Kandarakis and Panidis, 2007). Polycystic ovary syndrome is associated with hyperinsulinemia and insulin resistance that affects the insulin receptor substrate-1 (IRS-1) protein, an important intermediate in insulin signaling which also plays a key role in maintaining the basic function of the cell.

The IRS-1 gene is located on chromosome 2q36 and encodes a 1,242-amino acid protein with a molecular weight of 131.6 kDa. The most common variant, Gly972Arg (rs1801278), was reported to be associated with insulin resistance, type 2 diabetes and PCOS (Balaji *et al.,* 2015; Joshi *et al.,* 2014). Hence any polymorphism in IRS-1 genes acts as a competitive inhibitor of the insulin receptor. IRS-1 (rs2943641) polymorphism may be associated with the susceptibility to insulin resistance.

Hence, this study aimed at finding the association of genetic predisposition due to IRS-1 gene polymorphism in the pathogenesis and epidemiology of PCOS and the related oxidative stress marker among women in Madurai. The objectives of the study are to analyse the oxidative stress ratio (a biochemical marker) in control and cases and to predict the relative risk of the disease due to *IRS-1* gene polymorphisms.

MATERIALS AND METHODS

Blood samples of 2 ml was obtained from normal healthy women **(**control) and women with PCOS (cases) (n=50 each) between the age group of 20–40 years. Inclusive criteria for cases with at least two of the following signs: 1) chronic oligoanovulation 2) hirsutism or

increased serum total testosterone levels; and 3) polycystic ovarian morphology at ultrasound, according to the Rotterdam consensus.

Controls: Individuals with normal menstrual cycle since adolescence, asymptomatic, normo-androgenic, non-medicated, consenting women of reproductive age in whom PCOS was objectively excluded by clinical, biochemical and ultrasound assessment, were recruited as controls.

The Ethical approval for the study was obtained from the Institutional Review Board, of the Ashirwatham Specialty Hospital, Madurai. A written informed consent was obtained from the individuals during sample collection.

Plasma separated from whole blood was subjected to the Lipid peroxidation assay Estimation of lipid peroxidation using thiobarbituric acid reactive substance assay (TBARS) (Ohkawa *et al.,* 1979) and total antioxidant status (Benzie and Strain *et al.,*1999) to assess the oxidative stress (Suresh *et al.,* 2009). Genomic DNA was isolated from the whole blood by the method of Iranpur and Esmailizadeh, (2010). The polymorphism of IRS-1 gene was detected by PCR based RFLP method using the following primers FP-5'GCTTTCCACAGCTCACCTTC-3' and RP-5'GGTAGGCCTGCAAATGCTA-3' respectively with the PCR product size of 198 bp. The conditions of PCR include, initial denaturation for 3 min at 94°C, followed by second denaturation at 94 °C for 30 s, annealing at 56 °C for 45 s, extension at 72° C, for 55 s and final extension at 72° C for 5 minutes. The amplicon was confirmed by resolving in 1.5% agarose gel at 70 V for 60 mins. The PCR amplicon was subjected to restriction analysis using *SmaI*. The digested products were resolved in 2.5% agarose gel with the expected band size of 198bp, 171bp and 37bp.The data obtained was expressed as mean ± SD. The data was analyzed for its significance using Sigmaplot statistical software (11.0).

RESULTS AND DISCUSSION

The results of the present study showed that the mean age of the control group and patients were 20.4± 0.2 years and 20.1 ± 0.6 years respectively. The age of the individuals among control ranged from 15-25 years of age and that of cases ranged from 12-32 years. Among the cases 34% of individuals were in the age group of 15-20 years and 38% were in the age group of 20-25 years. The incidence of PCOS in the age group of 25-30 years was 10% (Table 1). It reflects that the incidence of PCOS was more among the young adults and 10% more among the cases in the reproductive age group of 25-30 years. The mean age of the controls and cases were more or less the same 20.4± 0.25 years and 20.1 ±0.64 years respectively (Table 2).

In contrast to the present study, a report from Lucknow, showed that the calculated prevalence with menstrual irregularity and hirsutism, using the NIH criteria among college-going women of age 18-25 yrs, was only 3.7 percent (Ganie *et al.,* 2019). It strongly suggests a drastic change in food habits at the regional level and during the pandemic period reflecting the higher incidence of PCOS in the post Covid pandemic scenario. It is observed that patient walk-in usually complaining of irregular periods, obesity or acne. It is believed that the heritability and lifestyle adaptations are the major cause for this disorder

Table 1- Demographic parameters of the study group

Parameters	**Controls (n= 50)**		**Cases (n= 50)**	
	(n)	Percentage (%)	(n)	Percentage (%)
Age(years)				
less than 15	-	-	8	16
15-20	28	56	17	34
20-25	22	44	19	38
25-30	-	-	5	10
more than 30	-	-	1	2
Place of Residence				

Rural	11	22	16	32
Urban	39	78	34	68
Food habits				
Vegetarian Diet	3	6	3	6
Non-Vegetarian Diet	47	94	47	94
BMI (Kg/m^2)				
Below 18.5	6	12	5	10
Normal 18.5 -24.9	36	72	20	40
Overweight 25 - 29.9	6	12	17	34
Obese 30 or over	2	4	8	16

Table 2 - Estimation of Oxidative stress related parameters among the study subjects

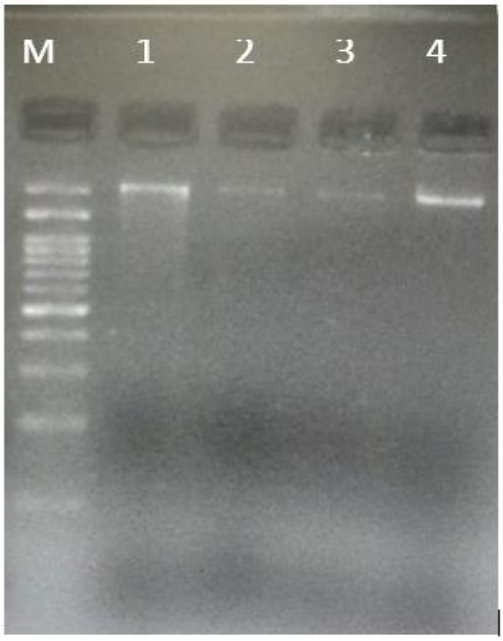
Parameters	**Controls (n=50)**	**Cases (n=50)**	**U- Statistics**
Age (yrs)	20.4± 0.25	20.1 ±0.64	-
BMI * (Median) CI	22.6 [CI: 19.8-24]	24.9 [CI:22.3- 28.4]	352.000 (P = 0.001)
MDA# nmol/ml	90.0 [CI:50-160]	97.5 [CI: 50-160]	1178.000 (P = 0.621)
TAS# µmols/L	500.0 [CI: 333-667]	458.5 [CI:183-717]	1232.500 (P = 0.906)
OS	0.226 [CI:0.090- 0.360	0.210 [CI:0.103-0.398]	1180.500 (P = 0.634)

**Statistically significant #Statistically not significant*

**The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)*

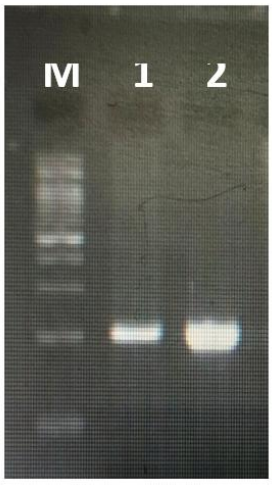
#The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; the differences between the groups are not statistically significant (P = 0.621)

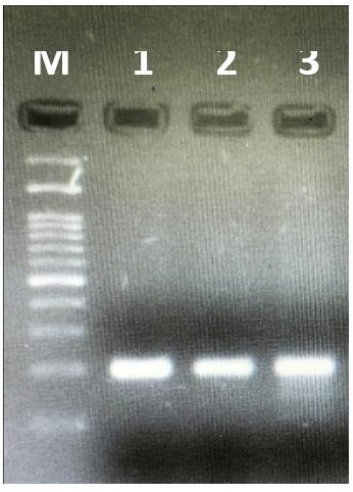
Fig1: Genomic DNA resolved in 0.7% Agarose



M: 1000Bp DNA Ladder
Lane 1-4: Genomic DNA Samples

Fig 2: IRS-1 PCR amplicon (a) and Restricted Fragments (b) in 2.5 % agarose





M: 100 Bp DNA Ladder
Lane 1-2: PCR Amplicon (198bp)

M: 100 Bp DNA Ladder
Lane 1-3: *Sma I* Restricted Samples (198bp)

In contrast to the present study, a report from Lucknow, showed that the calculated prevalence with menstrual irregularity and hirsutism, using the NIH criteria among college-

going women of age 18-25 yrs, was only 3.7 percent (Ganie *et al.,* 2019). It strongly suggests a drastic change in food habits at the regional level and during the pandemic period reflecting the higher incidence of PCOS in the post Covid pandemic scenario. It is observed that patient walk-in usually complaining of irregular periods, obesity or acne. It is believed that the heritability and lifestyle adaptations are the major cause for this disorder.

Around 78% and 64% of controls and cases respectively were from the urban locality which suggests that the incidence of PCOS among women from rural areas is either low or they are ignorant of the seriousness of the issues or hesitant to report the condition. The higher percentage of individuals among the controls belong to urban settings were young adults of age group 20-25 years (Table 1). A cross-sectional pilot study conducted in Tamil Nadu assessed young adolescent females and reported a prevalence of 18 percent for PCOS and concluded that the proportion of PCOS was higher in urban women in comparison to the rural women (Ganie *et al.,* 2019). According to the study by Barathi *et al.,* (2017) the percentage of girls who knew about PCOS in urban was comparatively higher than that of the rural population.

Regarding the food habits, the preference for non-vegetarian diet was highly pronounced both in controls and cases respectively accounting to 94 % (Table 1). The major proportion of the individuals belong to the urban when compared to rural substantiating the influence of junk food in the urban settings. The consumption of junk food was double in the metropolitan areas as reported by Bharathi *et al.,* (2017).

The body mass index of the study subjects recorded was classified as 12% underweight, 12 % overweight and 2% obese among the controls and that of the cases, 10% were underweight and 34% were overweight and 16 % were obese respectively (Table 1). The percentage of overweight and obese subject among the cases was more than the controls reflecting the manifestation of the disease. In the present study, the median BMI among the control was 22.6 [CI: 19.8-24] and that of cases was 24.9[CI:22.3- 28.4] ($p < 0.001$) which

showed statistically significant (Table 2). However, the prevalence of obesity in the PCOS patient group was tilted towards overweight, contributed by the factors such as food habit and urban life style setting. A similar state was reported by Diamanti-Kandarakis *et al.*, (1999) among Greek women. Higher percentages of women reported oligomenorrhea as the major symptoms for PCOS. In a study by Deswal *et al.,* (2020) infertility was significantly higher in women with polycystic ovary morphology (21.70%) using the Androgen Excess Society (AES criteria), while Rotterdam criteria reported the presence of the same in minority of women (6%).Krishna *et al.,* (2015) demonstrated for the first time that the immunosuppressive action of CD4+CD25+CD 127−Treg cells was reduced in PCOS, and this might influence the reproductive function of these women adversely.

The results of the biochemical parameters (Table 2) such as the level of Melondialdehyde (MDA) denotes the extent of lipid peroxidation/ oxidative damage in an individual. Among the study group the median and inter quartile range of MDA was high among cases with 97.5 nM/mL [CI:50-160 nM/mL] when compared to the controls which amounts to 90 nM/mL and [CI: 50-160 nM/mL] respectively, by the Mann Whitney test U= 1178.000 (P = 0.621). The oxidative damage due to disease was clear from the levels of MDA among patients which was higher than in controls. However, the differences between the level were not statistically significant. Increased MDA level also points to the hypothesis of possible ROS accumulation which in turn might result in increased lipid peroxidation at cellular and molecular levels (Bandyopadhyay and Chattopadhyay, 2006). MDA, a low-molecular weight aldehyde produced by free radical attack on polyunsaturated fatty acid, binds covalently to proteins, thereby altering their function resulting in enzyme inhibition, alteration of the structure of cellular receptors and cellular damage (Halliwell and Gutteridge, 2007). In recent years, there has been a growing interest, in using MDA as a marker of oxidative stress for its role in oxidative modification of LDL leading to cellular damage related to the disease state.

The total antioxidant status (TAS) was 458.5 µM/L [CI:183-717 µM/L] in patients and that of controls was 500 µM/L [CI:333-667 µM/L] respectively, according to Mann Whitney test 1232.500 (P = 0.906). The TAS level in the present study was low among the patients when compared to controls. However, there is no statistical difference between cases and controls with respect to TAS level (Table 2). Total Antioxidant Capacity (TAC) reflects the collective power of reducing agents to neutralize free radicals in each individual. Total antioxidant status (TAS) measures peroxyl- scavenging capacity of the extracellular antioxidant system comprised of sulfhydryl groups (mostly albumin), urate, ascorbate, carotenoids, retinols, α–tocopherol, bilirubin and proteins (Zowczak- Drabarczyk *et al.,* 2007). A single test which denotes the antioxidant power of blood was established and estimated as the ferric reducing ability of plasma (FRAP) to give more biologically relevant information than the measurement of individual antioxidants by Benzie and Strain, (1996). FRAP assay assesses the 'antioxidant power' and summarizes the overall activity of antioxidant vitamins and enzymes. There are numerous studies carried out reporting that the level of TAS in PCOS patients were found to be remarkably lower than those in healthy controls. Low total antioxidant capacity could be indicative of oxidative stress or increased susceptibility to oxidative damage (Selvi *et al.,* 2011).

The median value of the oxidative stress ratio in controls and cases was 0.226 and (CI: 0.090-0.360); 0.210 and (CI: 0.103-0.398) respectively by the Mann-Whitney test U=1180.500 (P = 0.634) (Table 2). In the present study, the increase in MDA level, was well reflected in the reduction in the amount of TAC (Total Antioxidant Capacity) by the free radicals generated due to oxidative stress which utilized the endogenous as well as exogenous antioxidants in the system. The result of the present study is in compliance with similar reports of Kolanjiappan *et al.,* (2002); Sinha *et al.,* (2009); Prabasheela *et al.,* (2011) and Li *et al.,* (2022).

An association between oxidative stress and of age was correlated by estimating the ratio of malondialdehyde: total antioxidant capacity (MDA: TAC ratio) as novel indicator of

oxidative stress in age related changes among healthy individuals (Suresh *et al.,* 2010). The elevated lipid peroxidation and decline in enzymatic antioxidant status were also reported in many diseases such as in cervical cancer (Manju *et al.,* (2002). Deepika *et al.,* (2014) studied genomic instability and cytotoxicity due to oxidative stress, assessed by estimating the frequency of micronucleated cells in epithelial samples and serum malondialdehyde levels, respectively, and found a positive correlation in patients of PCOS as compared to controls, which suggested high oxidative stress in PCOS women. Ganie *et al.,* (2019) have specifically demonstrated elevated oxidative stress markers in lean PCOS patient.

The variant genotype was completely absent in the present study as the restriction digestion of the amplified product (198bp) by *Sma I* was not obtained. Hence the distribution of genotypes was not in accordance to Hardy Weinberg equilibrium. Similar to the results of the present study on the possible association between the single nucleotide polymorphisms (SNPs) (rs2943641) of the IRS1 gene and susceptibility to PCOS in Iraqi women, the frequencies of SNP rs2943641 variant observed were not significantly different from healthy controls (Fadhil *et al.,* 2021). The Gly972Arg polymorphism of *IRS-1* reduces its phosphorylation and allows IRS-1 to act as an inhibitor of the INSR kinase, thereby impairing insulin signaling (Nisar *et al.,* 2012). This polymorphism has shown significant association with PCOS in Italian (Kumar *et al.,* 2017). However, contradictory results of no association have also been reported in studies with South Indian women with PCOS (Dasgupta *et al.,* 2012 and Shaikh *et al.,* 2014).

The present study revealed that the IRS-1 polymorphism (rs2943641) is not associated with PCOS among women in Madurai. However, one cannot exclude the possibility that other genetic polymorphisms of the IRS-1 family, may be associated with PCOS and might be clinically useful as markers to assess the disease risk. The polymorphism of Gly972Arg could play a contributory role in the pathophysiology and risk of PCOS, and the C allele of IRS-I is reported to be associated with increased hyperinsulinemia and

impaired insulin sensitivity but not polycystic ovary syndrome in Iraqi women as reported by Fadhil *et al.,* (2021).

The management of PCOS is as complex as the condition itself. The management and treatment of PCOS include a healthy diet, regular physical activity, and medications, which address the associated manifestations and co-morbidities. PCOS management strategies mainly aim at resolving the four major components of PCOS including regularity of menstrual periods, control of hyperandrogenism (acne and hirsutism), management of infertility and insulin resistance along with its associated risk factors (type 2 diabetes mellitus, hyperlipidaemia, and obesity). Both non-pharmacological and pharmacological management strategies are important in the overall management of PCOS (Ganie *et al.,* 2019).

As insulin resistance enhances hyper androgenemia as well as metabolic dysfunctions in PCOS, identification of candidate genes can help to assign predisposition factors and establish genetic makeup of affected women. This would further help to understand complex phenotypes of PCOS and advance the design of therapeutic approaches which would ameliorate major comorbidities like T2DM, metabolic syndrome, CVD, endometrial cancer, and so forth in later life (Shaikh *et al.,* 2014).

In contrast with present results, a study from the Japanese population reported significantly more IRS-1 Gly972Arg carriers among PCOS women compared to healthy controls (10.6% versus 4.8%, P = 0.029), and had a significantly increased risk of developing PCOS (OR: 3.31, 95% CI: 1.49–7.35). They also found that the IRS1 polymorphism was not associated with its phenotypes such as BMI, insulin resistance or androgen levels in PCOS women (Baba *et al.*, 2007). The variant rs1801278 (P=0.002; OR = 2.88; 95% CI = 1.43, 5.80) showed an association with PCOS in south Indian women (Thangavelu *et al.,* 2017). Genotyping is now an important diagnostic means for elucidating most diseases. Among the advanced techniques available to study single nucleotide polymorphisms (SNPs), only a few

are applicable for routine disease screening (Branavan *et al.,* 2018). The exact aetiology and pathogenesis of PCOS are still an area of active research, although genetic susceptibility to environmental exposure need to be analysed (Deswal *et al.,* 2020).

CONCLUSION

The results of the study indicate that incidence of PCOS was high in early adolescents and young adults. The preference in diet showed a shift towards Non- vegetarianism and the Prevalence of PCOS was high among Urban women. The Oxidative stress was pronounced in patients than the controls. As there was no variant genotype observed in the study there is no association of IRS1 gene polymorphism with PCOS among the study subjects.

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A REVIEW ON DEATH ANALYSIS DUE TO ACTIVE AND PASSIVE SMOKING (TOBACCO USAGE) IN INDIA

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ABSTRACT: Tobacco use has severe health effects, significantly increasing the risk of cancers (especially lung cancer), cardiovascular diseases like heart attack and stroke, chronic respiratory illnesses (COPD), and negative pregnancy outcomes as it contains nearly 250 chemicals which are very harmful to the human health and environmental health. It also impairs vision, weakens bones, causes oral and skin issues. Nicotine, the highly toxic substance present in the tobacco leaves leads to the addiction too. Age, sex, education, wealth status, and alcohol consumers are also associated with tobacco use in India. Smoking harms almost every part of our body and increases the risk of several diseases. Beedi is the most popular product to the smokers. Smoking not only affecting the smoker's health but also affect the passive smokers. Tobacco uses among males was higher compared to females, The present review depicts the deaths rates in world level and in India due to tobacco use especially among beedi rollers. This project also brings important awareness related to smoke death and urgent need implementation of alternative livelihood to the tobacco manufacturing industry workers as well.

KEY WORDS: human health, nicotine, passive smoking, awareness, alternative livelihood

INTRODUCTION

Tobacco is a plant grown for its leaves in the forest, and they are dried and fermented before being put in tobacco products. Tobacco contains nicotine, an ingredient that can lead to addiction, which is why so many people who use tobacco find it difficult to quit. Cigarette smoke contains a mix of over 7000 chemicals, including nicotine. Nicotine is a highly addictive chemical present in the tobacco plant. There are also many other potentially harmful chemicals found in tobacco or created by burning it. (Centers for Disease Control

and Prevention, 2008). People can smoke, chew, or sniff tobacco. Smoked tobacco products include cigarettes, cigars, bidis, and kreteks. Some people also smoke loose tobacco in a pipe or hookah (water pipe).

The nicotine in any tobacco product readily absorbs into the blood when a person uses it. Upon entering the blood, nicotine immediately stimulates the adrenal glands to release the hormone epinephrine (adrenaline). Epinephrine stimulates the central nervous system and increases blood pressure, breathing, and heart rate. As with drugs such as cocaine and heroin, nicotine activates the brain's reward circuits and also increases levels of the chemical messenger *dopamine*, which reinforces rewarding behaviors. Studies suggest that other chemicals in tobacco smoke, such as acetaldehyde, may enhance nicotine's effects on the brain (Giovino *et. al.,* 1995; Rani *et. al*., 2003; Subramanian *et. al*., 2004; Glynn *et. al.,* 2010; Gajalakshmi 2010; MOHFW 2010; Giovino *et. al.,* 2012). The tobacco epidemic continues to be a major public health concern with nearly1.4 billion tobacco users worldwide. It is one of the most important preventable causes of premature death in the world claiming more than 8 million lives each year (WHO 2019, Drope Schluger 2018).

TOBACCO PRODUCTION IN INDIA

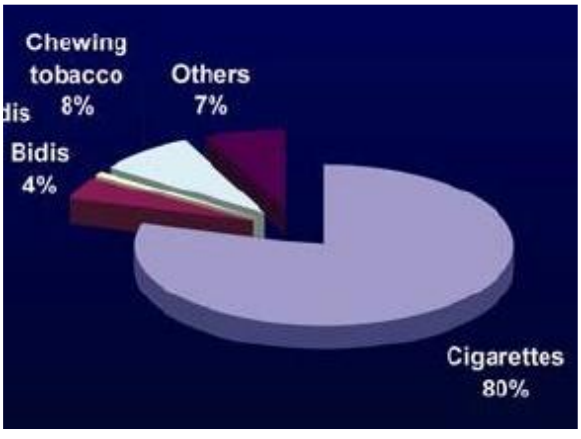
India is the third largest producer and sixth largest exporter of tobacco. It is estimated from the annual survey of industries (1997 -1998) data that almost 85% of employees of tobacco manufacturing industries are employed in the beedi industry (Humair 2009). Moreover, in India, many of these tobacco products are manufactured in cottage and small-scale industries (Gupta & Ray 2004). India is the second-largest tobacco producing country in the world (Pednekar *et. al.,* 2016). Tamil Nadu, West Bengal and Andhra Pradesh were the largest beedi manufacturer in India. In India beedi making is an age-old industry and one of the largest jobs provided for women in the unorganized sector. The Indian market for smoking is dominated by beedi. A beedi is a thin South Asian cigarette and called poor man's smoke or poor cigarette. A large number of workers were employed in the tobacco farming, beedi rolling and curing.

TOBACCO PRODUCTION IN TAMIL NADU

Beedi rolling is a popular small-scale industry in Tamil Nadu. Tirunelveli and Tenkasi district is considered the largest center for beedi industry in the state, with a significant number of home-based workers, particularly women. Specific districts like Thoothukudi, Tirunelveli, Kanniyakumari and Tenkasi have been cited as having a large workforce, with one report mentioning over six lakh workers in these regions alone. Trade unions in India, including thosein Tamil Nadu, have claimed the number could be significantly higher, possibly in the millions, especially when including unregistered workers. Tirunelveli and Tenkasi district has the highest concentration of beedi workers in Tamil Nadu. Especially in this area town and nearby rural areas, with a strong tradition of home-based workers. While other district like Tiruchirappalli and Vellore also significant centers for beedi production.

SMOKERS IN INDIA

In India, 275 million adults use tobacco in some form, primarily smokeless tobacco products (Ministry of Health and Family Welfare, 2010).There are approximately 120 million smokers in India. According to the World Health Organization (WHO), India is home to 12% of the world's smokers. More than 10 million die each year due to tobacco in India. According to a 2002 WHO estimate, 25% of adult's males in India smoke. Among adult females, the figure is much lower at between 13-15%. About 90% of children under the age of 16 years (10th standard) have used some form of tobacco in the past and 70% are still using tobacco products.



A survey conducted by the International Institute of Population Science and The Ministry of Health and Family Welfare, reveals that 56.6% of the peoples in Kolkata, the highest rate in India. 82% of the men and 23.5% of the women smoke in Kolkata. The highest number of beedi smokers is in Uttarakhand. The findings of GATS was that majority of people in India smoked beedis and not cigarettes. (Giovino *et. al.,* 2012) which is different from another observation majority of men smoked cigarettes (Kolappan *et. al.,* 2013).

Tobacco Consumption in India (Million Kgs)						
Legal Cigarettes			Other Tobacco forms*		Total*	
Year	Million Kg.	Share	Million Kg.	Share	Million Kg.	Share
1981-82	86	***21%***	320	***79%***	406	*100%*
2023-24	62	***10%***	543	***90%***	605	*100%*
Difference	**-24(-28%)**		**+223 (+70%)**		**+199 (+49%)**	

Source: USDA; Tobacco Board; FAO; Industry estimates * Other tobacco products include illegal cigarettes

Many adult smokers initiate the smoking habit during adolescence or as young adults (Rachiotis *et. al.,* 2008). While adults were the initial targets of tobacco companies, in order to increase profits, the industry sought out to target adolescents, age 15-19, Furthermore; adolescents believed that smoking would promote their social status as a member of the modern generation (Duangdao, 2012). Tobacco use among adolescents has been found to be a major public health concern. Despite the widespread awareness of the short and long -term

consequences of smoking, recent studies have revealed that even if there has been a decrease, the incidence of smoking among adolescents remains high (Karimy *et. al.,* 2012a)

SMOKERS IN TAMIL NADU

Tamil Nadu is the sixth largest state by population with about 72million people (Registrar General of India 2011). It has 32 administrative districts. With nearly half of the population residing in urban areas, it has a high literacy rate of 80%. A cross-sectional household tobacco survey, Tamil Nadu Tobacco Survey (TNTS), was conducted in 2015/2016 in the state of Tamil Nadu, South India, by the Cancer Institute (Women's India Association), Chennai, India, to provide reliable state and district-wise estimates tobacco use (Resource Centre for Tobacco Control, Cancer Institute (WIA, 2016) .

The survey covered nearly 100 000 adults (> 15 years) in all 32 districts across the state. The results of the survey showed that 5.2% were current tobacco users reported to have intention to quit tobacco use in the next one month. But how many of them actually quit and how many of those who made of quit attempt were successful, is unknown. Tobacco use among males was higher (4.3%) compared to females (0.9%), a small difference in tobacco use between urban (2.5%) and rural areas (2.6%) of Tamil Nadu. 47% of current tobacco users consumed tobacco within half an hour of waking up and Pudukottai district is most tobacco users than that of other districts of Tamil Nadu (Tamil Nadu Tobacco Survey 2015-2016).

HEALTH EFFECTS OF TOBACCO USE

Although nicotine is addictive, most of the severe health effects of tobacco use comes from other chemicals. Tobacco smoking can lead to lung cancer, chronic bronchitis, and emphysema. It increases the risk of heart disease, which can lead to stroke or heart attack. Smoking has also been linked to other cancers, leukemia, cataracts, Type 2 Diabetes, and pneumonia. All of these risks apply to use of any smoked product, including hookah tobacco

and Smokeless tobacco. Tobacco use leading a public health problem all over the world with82% of the world's 1.1 billion smokers residing in low- and middle- income countries and where, in contrast to the declining consumption in high- income countries, tobacco consumption is on the rise (Gajalakshmi *et. al.,* 2000).

WHO Report on the Global Tobacco Epidemic, 2023

Beedi is the most popular product among smokers, consumed by the majority of those belonging to lower socio- economic backgrounds (Sarit K Rout *et. al.,* 2017; Giovino *et. al.,* 1995; WHO-2011; Ministry of Health and Family Welfare 2010). Gupta (2003) has attempted to focus on the health hazards of the women Beedi rollers in Karnataka. Through the study author has found that, the women Beedi rollers have faced many health problems like lung infection and bronchial problems, contact dermatitis and other health hazards.

Author has observed that the employers or contractors are not providing any health services to these beedi rollers. Ranjith singh and Padmalatha (1995), have found that, women beedi rollers were affected by respiratory disorder, gastrointestinal illness and gynecological issues and susceptible to fungal diseases, peptic ulcer, diarrhea and they also have a high degree of the leucorrhoea.

Nakkeeran, Pugalendhi (2010) investigated respiratory, gas and astrological problems among beedi rolling workers in four districts of Tamil Nadu. Yasmin *et. al.,* (2010) highlighted some important facts on the work- related health issues of female beedi rollers in Patna, Bihar. The study identified lower hemoglobin levels and SGPT (ALT) enzyme concentration among beedi rollers. Dikshit and Kanhere 2000; Mittal *et. al*., (2008), found that postural pains, eye problems and burning sensation in the throat are common ailments in women beedi rollers. J.K. Singh *et. al*., (2014) in their empirical study have stated that, beedi rolling workers facing numerous health problems due to direct inhalation of tobacco flakes. Monica Munjail Singh *et. al.,* (2018) conducted a study on the socio-economic health and working conditions of child beedi rollers in beedi industry in Solapur cities of Maharashtra state in India. The study revealed that, majority of the child labours are very poor and they are facing various diseases due to direct inhalation of tobacco dust.

DEATH RATIO IN INDIA

Tobacco usage is one of the biggest problems of the public health and environment in the world, killing more than 7 million people a year (Praveena Raman and Raghuram Pitty (2020). Tobacco use kills nearly six million people worldwide each year (Mishra *et. al*., 2012). Tobacco causes 8 million deaths every year worldwide (Alebshehy, 2019, Elisabeth *et. al.,* 2011). Gupta *et. al.,* (2005) have estimated the tobacco attributable mortality among Indian men and women from their Mumbai cohort study. Based on these estimates, nearly 23.7% of the deaths among men (527,500) and 5.7% of the deaths among women (83000) aged 35-69 years are due to tobacco attributable illnesses. (Chaly, 2007).

Tobacco smoking caused one million deaths in India in 2010, 70% occurring among smokers in the 30-69 age groups (John *et. al*., 2010). According to the National Family Health Survey (NFHS)-3 Global Adult Tobacco Survey 2016/2017 (GATS) in India, more than half of the current tobacco users were planning or thinking of quitting tobacco use (Ministry of Health & Family Welfare, GOI WHO and T. GATS-2 (2016). Dr. Anbumanni Ramadoss, Former Ministry of Health and Family Welfare said 40% of deaths occur in India due to tobacco related diseases while two-third of deaths occur due to smoking, junk foods and usage of drugs. Tobacco continues to kill 6 million people each year globally, including more than 600000 non-smokers who die from exposure to tobacco smoke (WHO, 2011).

CONCLUSION AND SUGGESTIONS

This review explains the death rates of tobacco usage in the world and India. The continuous use of tobacco products or smoking to increase the adult death and premature death also were identified from several studies, WHO report, NFHS report. Alternative livelihood to the tobacco manufacturing industry workers should be implemented. Also, awareness should be given to the society regarding ill effects of tobacco usage especially active and passive smoking. Sanat Kumar P and B.K. Sardar (2005) have portrayed the causes and also the remedial measures like awareness programme, health education, proper implementation of various schemes to mitigate the health problems of women beedi rollers. Through the study, a large number of health problems have been observed by the author among the women beedi rollers. To overcome the health problems, author have suggested that, health dispensaries should be settled in beedi workers concentrated blocks, imparting proper health education and increase the awareness among the women beedi rollers about their health care.

Beedi workers are poor and mostly unorganized. They face innumerable problems such as poor working conditions, low wages, fraudulent actions by the contractors, health

hazards, etc. Burman (2018). The results observed were similar to those found by Dharmalingam (1993) who observed a high occurrence of numbness in arms and fingers of beedi workers. So the government should take necessary steps to stop the production of tobacco materials and bring the alternative livelihood to the beedi manufacturing workers related to the residential lands/areas. Government should provide funds to the workers for improving their life and residential work. And regularly watch their work through any government organization. It leads to country and world into tobacco free and TB free country and world. Alternative livelihoods for beedi rollers include skills training in tailoring, coir handicraft, wig making, and farming, along with initiatives like skill development programs supported by government bodies and NGOs. These programs offer training, stipends to offset lost wages, and support for lodging and travel. Despite challenges like limited education and financial constraints, many beedi workers are willing to switch to healthier, more sustainable options due to growing awareness of occupational health hazards. The beedi workers welfare department should conduct regular health check-up to reduce occupational health problems as well.

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