https://doi.org/10.33451/florafauna.v32i1pp39-46
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 39-46 ISSN 0971 - 6920 (Print)

Invasive Alien Plants of Bhagalpur Diara Lands, Bihar (India)

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Received **:** 28.03.2026; ***Accepted*** : 08.05.2026

How to cite : Rani M, Singh CB. Invasive Alien Plants of Bhagalpur Diara Lands, Bihar (India). *Flora and Fauna* 2026. 32(1) : 39-46

ABSTRACT

This paper deals with 72 invasive alien plant species, under 65 genera and 38 families, from Bhagalpur diara lands. These Species are annual (51.39%) and perennial (48.61%) which exist as herbs (63.89%), shrubs (15.28%), trees (12.50%), grasses (5.55%) and climbers (2.78%). Their nativity pertains to 32 geographical regions – 7 major (45 species/62.50%) and 25 minor (27 species/37.50%). The major regions of origin include Tropical America (27.77%), Tropical Africa (8.33%), South America (6.94%); Central-South America/Mediterranean region (4.56% each) and China/Europe (4.17% each). The common wild invasive alien plant species *in diara* lands are *Alternanthera sessilis*, *Amaranthus spinosus*, *Argemone mexicana*, *Blumea lacera*, *Calotropis gigantea*, *Calotropis procera*, *Cannabis sativa*, *Chenopodium album*, *Croton bonplandianus*, *Cynodon dactylon*, *Eclipta prostrata*, *Euphorbia hirta*, *Ipomoea carnea*, *Oxalis corniculata*, *Phyla nodiflora*, *Pistia stratiotes*, *Pontederia crassipes*, *Portulaca quadrifolia*, *Saccharum spontaneum*, *Solanum nigrum*, *Sonchus oleraceus*, *Tridax procumbens*, *Urena lobata* and *Xanthium strumarium*. Unlike their native regions possessing the largest family Asteraceae, *diara* lands are with the largest family Fabaceae and also emerging as repository of invasive alien plants due to introduction through flood water.

Figure : 01 References : 36 Table : 01
KEY WORDS : Bhagalpur *Diara* lands, Invasive alien plants.

Introduction

Alien plants are those plants that have been introduced to a new region and become non-native or exotic there. The introduction of these plants to a recent habitat is primarily done by anthropogenic activities like mobility, tourism, horticulture, global trade and others[9,12,18,19]. The introduced alien plants become invasive after acquisition of ability to create self-sustaining population and to proliferate themselves in the invaded region to an extent of surpassing the native flora particularly in habitat occupation and use of water together with nutritional resources[22]. Later on, the invasive alien plant species spread to the newer ecosystems by different agents as wind, flood, animals and human beings[3,4,6,12,35]. Out of these agents, flood seems to play a crucial role in spreading the invasive alien plants to diverse newer habitats like lakes, ponds, riverine *diara* lands.

Diara lands are landscapes/ islands in the riverbed and its tributaries. These are formed due to meandering and course changing behaviour of the river itself[2,11,13,14,24]. These are characterised by yearly inundation during monsoon season, emergence after recession of flood water and mostly undulating topography. These lands are found in the northern and eastern regions of India mainly in the states like Assam, Bihar, Odisha, Uttar Pradesh and West Benga[10,16]. Among these unique lands, Bhagalpur *diara* lands are considered to be typical due to their successive annual submergence by flood water, moderately to highly fertile alluvial soils and richness in biodiversity cum biological resources[2,20,24,32]. The submergence of Bhagalpur *diara* lands is usually caused by the flood water that comes from other states (*viz.,* Uttarakhand, Uttar Pradesh, Madhya Pradesh) of our country and a neighbour country Nepal bordering Bihar in the north.The arriving water passes through various vegetational belts and reaches *diara* lands through the river Ganga and its tributaries mostly Yamuna, Ghaghara, Sone, Gandak and Kosi. Obviously, flood water brings the propagules of several new plants including invasive alien plants into the Gangetic *diara* lands of Bihar and introduces them in the newhabitats, *i.e., diara* lands. These invasive alien plants are unexplored due to the lack of taxonomic work on the flora of *diara* lands. Therefore, a taxonomic

TABLE-1 : Enumeration of Invasive Alien Angiosperms of Bhagalpur *Diara* Land, Bihar

S. No.	Name of the species	Family	Life span/ Habit	Nativity
1	*Abelmoschus esculentus*	Malvaceae	A/H	East Africa
2	*Alternanthera philoxeroides*	Amaranthaceae	P/H	Tropical America
3	*Alternanthera sessilis*	Amaranthaceae	A/H	Tropical America
4	*Amaranthus spinosus*	Amaranthaceae	A/H	Tropical America
5	*Amaranthus viridis*	Amaranthaceae	A/H	Central & South America
6	*Anagalis arvensis*	Primulaceae	A/H	Europe
7	*Argemone mexicana*	Papaveraceae	A/H	Tropical South America
8	*Blumea lacera*	Asteraceae	A/H	Tropical America
9	*Boerhavia diffusa*	Nyctaginaceae	P/H	Tropical America
10	*Borassus flabellifer*	Arecaceae	P/T	Tropical Africa
11	*Brassica campestris*	Brassicaceae	A/H	China
12	*Calotropis gigantea*	Asclepiadaceae	P/S	Tropical Africa
13	*Calotropis procera*	Asclepiadaceae	P/S	Tropical Africa
14	*Cannabis sativa*	Cannabaceae	A/H	Central Asia
15	*Capsicum annuum*	Solanaceae	A/H	Central & South America
16	*Carica papaya*	Caricaceae	P/T	Tropical America
17	*Cascabela thevetia*	Apocynaceae	P/S	Mexico & Central America
18	*Chenopodium album*	Chenopodiaceae	A/H	Europe
19	*Cicer arietinum*	Fabaceae	A/H	South East Turkey
20	*Citrullus lanatus*	Cucurbitaceae	A/H	Africa
21	*Cleome viscosa*	Cleomaceae	A/H	Tropical America
22	*Clerodendrum petasites*	Lamiaceae	P/S	Vietnam

S. No.	Name of the species	Family	Life span/ Habit	Nativity
23	*Clitoria ternatea*	Fabaceae	P/C	Indonesia
24	*Coriandrum sativum*	Apiaceae	A/H	Mediterranean region & South West Asia
25	*Croton bonplandianus*	Euphorbiaceae	A/H	Temperate South America
26	*Cucurbita maxima*	Cucurbitaceae	A/H	South America
27	*Cuscuta reflexa*	Cuscutaceae	P/C	Mediterranean region
28	*Cynodon dactylon*	Poaceae	P/G	Africa & Eurasia
29	*Datura metel*	Solanaceae	A/H	Tropical America
30	*Delonix regia*	Caesalpiniaceae	P/T	Madagascar
31	*Eclipta prostrata*	Asteraceae	A/H	Tropical America
32	*Euphorbia hirta*	Euphorbiaceae	A/H	Tropical America
33	*Hibiscus rosa-sinensis*	Malvaceae	P/S	East Asia
34	*Hibiscus sabdariffa*	Malvaceae	A/H	Tropical Africa
35	*Impatiens balsamina*	Balsaminaceae	A/H	Tropical America
36	*Ipomoea carnea*	Convolvulaceae	P/S	Tropical America
37	*Lablab purpureus*	Fabaceae	P/H	Africa
38	*Lathyrus sativus*	Fabaceae	A/H	South Europe & West Asia
39	*Lepidium sativum*	Brassicaceae	A/H	Egypt & West Asia
40	*Lippia alba*	Verbenaceae	P/S	South & Central America
41	*Litchi chinensis*	Sapindaceae	P/T	South China
42	*Ludwigia adscendens*	Onagraceae	P/H	Tropical America
43	*Mimosa pudica*	Mimosaceae	P/H	Brazil
44	*Mirabilis jalapa*	Nyctaginaceae	A/H	Peru
45	*Morus alba*	Moraceae	P/T	China

S. No.	Name of the species	Family	Life span/ Habit	Nativity
46	*Nicotiana plumbaginifolia*	Solanaceae	A/H	Tropical America
47	*Opuntia elatior*	Cactaceae	P/S	Tropical America
48	*Oxalis corniculata*	Oxalidaceae	A/H	Europe
49	*Oxalis corymbosa*	Oxalidaceae	P/H	South America
50	*Phyla nodiflora*	Verbenaceae	P/H	South America & United States
51	*Pistia stratiotes*	Araceae	P/H	South America
52	*Pisum sativum*	Fabaceae	A/H	Mediterranean region
53	*Pithecellobium dulce*	Caesalpiniaceae	P/T	Tropical & Subtropical America
54	*Pontederia crassipes*	Pontederiaceae	P/H	South America
55	*Portulaca oleracea*	Portulacaceae	A/H	Tropical South America
56	*Portlaca quadrifolia*	Portulacaceae	A/H	Tropical America
57	*Psidium guajava*	Myrtaceae	P/T	Tropical America
58	*Ricinus communis*	Euphorbiaceae	P/S	Tropical Africa
59	*Saccharum spontaneum*	Poaceae	P/G	Tropical West Asia
60	*Solanum nigrum*	Solanaceae	A/H	Tropical America
61	*Solanum lycopersicum*	Solanaceae	A/H	South America
62	*Sonchus oleraceus*	Asteraceae	A/H	Mediterranean region
63	*Swietenia macrophylla*	Meliaceae	P/T	Central & South America
64	*Tamarindus indica*	Caesalpiniaceae	P/T	Tropical America & Madagascar
65	*Tecoma stans*	Bignoniaceae	P/S	America
66	*Tridax procumbens*	Asteraceae	P/H	Tropical Central America
67	*Trigonella foenum-graecum*	Fabaceae	A/H	Mediterranean region, South Europe & West Asia

S. No.	Name of the species	Family	Life span/ Habit	Nativity
68	*Triticum aestivum*	Poaceae	A/G	South Western Asia
69	*Urena lobata*	Malvaceae	P/S	Tropical Africa
70	*Vicia faba*	Fabaceae	A/H	Mediterranean region
71	*Xanthium strumarium*	Asteraceae	A/H	Tropical America
72	*Zea mays*	Poaceae	A/G	Mexico & Central America

Symbols: Lifespan - A = Annual, P = Perennial

Habit - H = Herb, S = Shrub, T = Tree, C = Climber, G = Grass

investigation was conducted in Bhagalpur diara lands with sole aim of cataloguing the invasive alien angiosperms that flourish in the new regions.

Study Area

The investigation area was comprised of three popular *diara* lands namely Bairya *diara*, Chauwania *diara* and Shankarpur *diara* (Fig. 1) of Bhagalpur district. This district is situated in the southern part of the Bihar, India. It is bordered in the east by Godda – Sahebganj districts of Jharkhand state and in the rest sides by own districts of Bihar state, *viz.,* west – Munger and Khagaria; north – Purnea, Madhepura and Katihar; south – Banka. It experiences a tropical monsoon type climate with three distinct seasons namely summer, rainy and winter in a year.

Materials and Methods

The different habitats of selected *diara* lands (Bairya *diara*, Chauwania *diara* and Shankarpur *diara*) were visited frequently in every season during January 2022 – December 2024 and thereafter as and when for solving the problems related to research work. The specimens of plants growing in the selected *diara* lands of Bhagalpur were collected and pressed in the fields. Subsequently, the pressed specimens were dried properly and the herbaria were prepared from the dried plant materials following the standard methods. The plant specimens were critically studied and properly identified with the help of available floras[1,7,8,33]. The identified specimens were deposited in the Herbarium, University Department of Botany, Bhagalpur (Bihar), India (BHAG). The nativity of invasive alien plants was ascertained from available literature[15,17,21,23,29,34].

Results and Discussion

Seventy two species of invasive alien angiosperms growing in Bhagalpur *diara* lands of Bihar (India) have been catalogued (Table-1). These species, under 65 genera and 38 families, exhibit much diversity in their occurring families, life span, habit and nativity. Their maximum number (7) is reported in the family Fabaceae followed bytwo families (Asteraceae and Solanaceae), three families (Amaranthaceae, Malvaceae and Poaceae), two familes (Caesalpiniace and Euphorbiaceae) and seven families (Asclepiadaceae , Brassicaceae, Cucurbitaceae, Nyctaginaceae, Oxalidaceae, Portulacaceae and Verbenaceae) having 5, 4, 3 and 2 species respectively. Each of the remaining families is represented by only one species. The common wild invasive alien plant species are *Alternanthera sessilis*, *Amaranthus spinosus*, *Argemone mexicana*, *Blumea lacera*, *Calotropis gigantea*, *Calotropis procera*, *Cannabis sativa*, *Chenopodium album*, *Croton bonplandianus*, *Cynodon dactylon*, *Eclipta prostrata*, *Euphorbiahirta*, *Ipomoeacarnea*, *Oxalis corniculata*, *Phyla nodiflora*, *Pistia stratiotes*, *Pontederia crassipes*, *Portulaca quadrifolia*, *Saccharum spontaneum*, *Solanum nigrum*, *Sonchus oleraceus*, *Tridax procumbens*, *Urena lobata* and *Xanthium strumarium*.

The investigated invasive alien plant species display their life span as annual (51.39%) and perennial (48.61%) with a narrow difference in proportions. With respect to habit, the herbs (63.89%) dominate over all other forms (36.11%), *viz.,* shrubs (15.28%), trees (12.50%), grasses (5.55%) and climbers (2.78%). Further their nativity belongs to 32 different geographical regions spread over the world. The major regions (7) contribute 45 species (62.50%) exceeding the minor ones (25) giving only 27 species (37.50%). Amongst the major regions, Tropical America (27.77%) outpaces

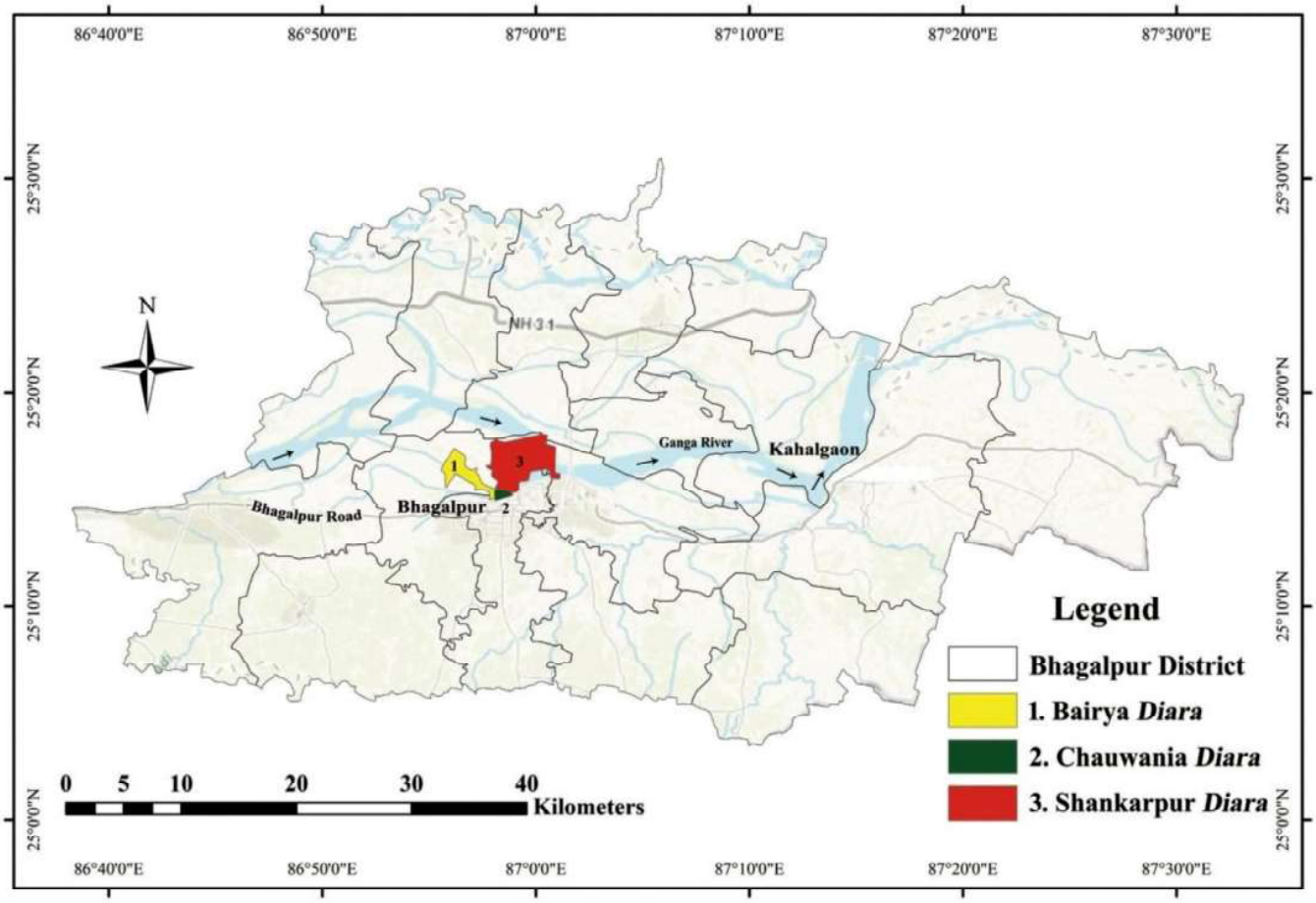

Fig. 1 : Map of Bhagalpur disctrict (Bihar) showing location of study *diara* lands

others, namely Tropical Africa (8.33%), South America (6.94%), Central-South America/Mediterranean region (4.56% each) and China/Europe (4.17% each) in providing invasive alien plant species.

The invasive alien plant species have been expanding their horizon since the time of origin. Now their spread is driven and accelerated by the prevailing environmental factors and climatic changes[3-6,12,30,35,36]. In this context, it seems that the flood is playing a decisive role in the introduction of alien/invasive alien plant species from their source territories (Uttarakhand, Uttar Pradesh, Madhya Pradesh and Nepal) to the destination areas, *i.e., diara* lands. This becomes evident from the natural growth of invasive alien plant species in *diara* lands of Bhagalpur districts and their sourceregions[23,25,27,28,31,34]. *Diara* lands are unique in having Fabaceae as the largest family unlike source regions with the largest family Asteraceae[23,25-28].

Conclusion

Bhagalpur *diara* lands undergo submergence every year in monsoon season by flood water which brings propagules of invasive alien plants. Due to introduction of these plant species, "Bhagalpur *diara* lands are emerging as repository of invasive alien plants."

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https://doi.org/10.33451/florafauna.v32i1pp47-55
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 47-55 ISSN 0971 - 6920 (Print)

Identification of the Ethnobotanical Plants in Pantar Lanao del Norte, Philippines

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Received **:** 20.02.2026; ***Accepted*** : 08.04.2026

How to cite : Macaraya JC, Macaraya JC. Identification of the Ethnobotanical Plants in Pantar Lanao del Norte, Philippines. *Flora and Fauna* 2026. 32(1) : 47-55.

ABSTRACT

This ethnomedicinal survey documented the traditional plant knowledge of the Maranaos in Pantar, Lanao del Norte. Findings indicate that older women, particularly those aged 61 and above, are the primary custodians of medicinal knowledge, acquired mainly from elders and parents, while men rely more on the broader community. A total of 75 medicinal plant species from 38 families were recorded, with leaves being the most frequently used part (56%), followed by bulbs, fruits, roots, stems, and bark, reflecting both therapeutic efficacy and sustainability considerations. Six main preparation methods were identified, with infusions (30%) and decoctions (27%) most common, demonstrating adaptive techniques aligned with plant properties and targeted ailments. Informant Consensus Factor (ICF) analysis revealed high agreement among respondents, particularly for asthma, labor pain, and promoting healthy hair (ICF = 0.98), highlighting the coherence and reliability of traditional knowledge and identifying plants with potential for further pharmacological investigation. These findings underscore the importance of preserving intergenerational ethnomedicinal knowledge and prioritizing high-ICF species for future research.

Figure : 01 References : 16 Tables : 05
KEY WORDS : Ethnobotanical plants, Maranaos, Pantar Lanao del Norte, Philippines

Introduction

Despite the increasing dominance of biomedical healthcare, many indigenous communities continue to rely on traditional herbal medicine as a primary source of health care. Among the Maranao of Lanao del Norte, this reliance is shaped by limited access to formal medical services, economic constraints, and deeply rooted cultural traditions. Traditional medicinal knowledge remains robust and culturally embedded; however, rapid modernization and shifting healthcare preferences threaten its continuity. Consequently, there is an urgent need to bridge indigenous healing practices with scientific documentation frameworks to support their validation, preservation, and intergenerational transmission.

Maranao traditional medicine reflects not only practical healthcare strategies but also spiritual beliefs and ancestral knowledge passed down through oral tradition. Medicinal plants are used for both therapeutic and ritual purposes, with healers demonstrating detailed understanding of plant selection, preparation, dosage, and contraindications. Previous ethnobotanical studies have documented this knowledge across several Maranao communities, including Madalum and urban Iligan City, highlighting the resilience of ethnomedicinal practices despite increasing exposure to Western medicine.

However, these studies collectively reveal a notable research gap in Pantar, Lanao del Norte—a municipality situated between Lanao del Sur and Iligan

ACKNOWLEDGEMENTS : The researchers sincerely extend their deepest gratitude to the community of Pantar, Lanao del Norte, for their generous support and warm cooperation throughout this study. Their thoughtful insights, stories, and shared knowledge were invaluable, enriching both the depth and meaning of this research. The encouragement, openness, and trust they provided were not only essential to the successful completion of this work but also deeply inspiring, leaving a lasting impression on the researchers' hearts.

TABLE-1 : Age of the Respondents Categorized by Gender

Gender	Population	Age			
		30-40	*41-50*	*51-60*	*61+*
Female	305	54	43	56	152
Male	81	9	33	18	21

City, where rural traditions and urban influences may intersect. No ethnobotanical research has yet focused on this area, limiting comparative understanding of how Maranao medicinal knowledge is maintained or transformed across different settings. Thus, this study aims to document the ethnobotanical use of medicinal plants in Pantar by identifying treated health conditions, plant parts used, methods of preparation, and associated bioactive constituents based on existing literature. Additionally, the study calculates the Informant Consensus Factor (ICF) to assess agreement among community informants, contributing to indigenous knowledge preservation and sustainable use of natural resources in Northern Mindanao.

Methods

2.1 Research Design

This study employed a qualitative–descriptive research design complemented by quantitative analysis to document and evaluate the ethnomedicinal knowledge of the Maranao community in Pantar, Lanao del Norte, Philippines. Data were collected through key informant interviews, focus group discussions, and field observations involving community elders aged 30 years and above, selected for their extensive indigenous knowledge of medicinal plants. The Informant Consensus Factor (ICF) was calculated to assess the level of agreement among informants regarding medicinal plant use, providing a quantitative measure of knowledge reliability[7,14]. Ethical protocols, including informed consent and respect for indigenous intellectual property rights, were strictly observed throughout the study[5,13].

2.2 Research Instruments

The study utilized an institutional informed consent form approved by Mindanao State University–Iligan Institute of Technology (MSU-IIT) to ensure ethical compliance, voluntary participation, confidentiality, and respondents' right to withdraw at any time. Data were collected using an adapted semi-structured interview questionnaire, which was modified to suit the cultural and environmental context of Pantar, Lanao del Norte. This instrument enabled the systematic and culturally sensitive documentation of medicinal plant species, their therapeutic uses, and preparation methods among the Maranao community.

2.3 Data Gathering Procedure

Data were collected using a triangulated mixed-methods approach that integrated qualitative ethnobotanical documentation and quantitative analysis. Semi-structured interviews were conducted with 386 Maranao informants from Pantar, Lanao del Norte, following informed consent and translation of the interview guide into the Maranao dialect to ensure cultural appropriateness, complemented by informal conversations and focus group discussions[3,9]. Information gathered included medicinal plant species, treated ailments, plant parts used, preparation methods, routes of administration, and reported bioactive constituents. Plant species were identified and validated using Co's Digital Flora of the Philippines, the Philippine Traditional Knowledge Digital Library on Health, and the World Flora Online Plant List, with verification conducted by a botanist from MSU-IIT. The Informant Consensus Factor (ICF) was computed to assess the level of agreement among informants on medicinal plant use, while ethical standards on informed consent, confidentiality, and protection of indigenous intellectual property were strictly observed[5,7,13].

RESULTS

3.1. Demographic profile of the respondents

The age and gender distribution of respondents provides insight into patterns of ethnomedicinal knowledge transmission within the community. As shown in Table-1, female respondents constituted the majority of participants, with nearly half belonging to the 61 years and above age group, indicating that traditional medicinal knowledge is largely retained by older women, while male respondents were fewer and more evenly distributed

TABLE-2 : Source of Traditional Knowledge

Gender	Parents	Elders	Community	Doctors
Female	109	136	59	1
Male	25	23	33	0

across age categories. These patterns underscore the significance of both age and gender in shaping the custodianship of ethnobotanical knowledge.

The sources of traditional knowledge further highlight gender-based differences in knowledge acquisition (Table-2). Among female respondents, elders and parents were the primary sources of ethnomedicinal knowledge, emphasizing strong intergenerational and familial transmission, whereas male respondents most frequently cited the community as their main source. Reliance on medical professionals was negligible for both genders, reinforcing the continued importance of indigenous knowledge systems in local healthcare practices.

3.2 Plant species recorded

In this ethnomedicinal survey, Table-3 shows the 75 plant species used for medicinal purposes were documented in various areas of Pantar, Lanao del Norte. The plant families represented include Acanthaceae (1 species), Acoraceae (1), Amaranthaceae (3), Annonaceae (1), Apiaceae (1), Arecaceae (1), Asparagaceae (1), Asphodelaceae (1), Asteraceae (9), Athyriaceae (1), Boraginaceae (2), Caricaceae (1), Clusiaceae (1), Combretaceae (1), Convolvulaceae (1), Crassulaceae (1), Cucurbitaceae (2), Euphorbiaceae (4), Fabaceae (4), Lamiaceae (5), Lauraceae (1), Lythraceae (1), Malvaceae (5), Menispermaceae (1), Moraceae (1), Musaceae (2), Myrtaceae (2), Orthotrichaceae (1), Pinaceae (1), Piperaceae (2), Poaceae (2), Portulacaceae (1), Primulaceae (1), Rosaceae (1), Rubiaceae (1), Rutaceae (2), Sapotaceae (1), Solanaceae (3), Verbenaceae (1), and Zingiberaceae (2). This taxonomic variety reflects the rich ethnomedicinal plant resources within the study area.

3.3 Plant parts utilized as medicine

The Maranaos of Pantar Lanao del Norte utilize various plant parts in herbal medicine, demonstrating extensive ethnobotanical knowledge. Figure 1 shows that the leaves are the most frequently used plant part (56%), largely due to their ease of collection, renewability, and high concentration of bioactive compounds, making them both effective and sustainable. Bulbs and fruits follow at 15%, valued for their rich nutrients and secondary metabolites, while roots account for 13% and are recognized for their strong medicinal potency despite sustainability concerns. Stems and stalks (8%) and bark (5%) are used less often, typically for decoctions containing potent phytochemicals, and the use of the whole plant is rare (3%).

3.4 Veriety of preparations

The study documented (Table-4) six main methods for preparing and administering medicinal plants among the Maranaos of Pantar, Lanao del Norte, reflecting a highly adaptive traditional knowledge system. Infusion was the most common method (30%), typically using leaves or flowers steeped in hot water to extract active compounds. Decoction (27%) involved boiling tougher plant parts such as roots, bark, or stems. Poultices (22%) were applied externally to treat localized conditions like wounds, inflammation, or skin irritations. Less frequent methods included consuming raw plant material (13%), extract preparation (5%) for concentrated bioactive compounds, and heating over fire (3%) to activate or modify chemical properties. Each method corresponded to specific health conditions: infusions were used for ailments such as toothache, high blood pressure, urinary issues, and menstrual stimulation; decoctions for postnatal recovery, coughs, fevers, and rheumatism; poultices for skin, hair, and wound treatments; raw consumption for digestive, reproductive, and inflammatory conditions; extracts for hair health, cough, and postpartum strength; and heating over fire for wounds, sprains, and flatulence. This variety demonstrates the Maranaos' empirical knowledge in selecting preparation techniques that optimize therapeutic efficacy according to both the plant's properties and the ailment being treated.

3.5 The Informant Consensus Factor (ICF)

In this study, ICF values revealed a high level of agreement among the Maranaos of Pantar, Lanao del Norte. The highest consensus was observed for treating asthma, reducing labor pain, and promoting healthy hair (ICF = 0.98), followed closely by arthritis, insect bites, and menstrual cramps (ICF = 0.97). Even ailments with comparatively lower ICF values, such as bleeding (0.76) and urinary tract infections (0.77), showed notable

TABLE-3 : Over-all distribution of species across different families of medicinal plants used by the Maranaos in Pantar LDN

Family	Species	Family	Species	Family	Species	Family	Species
Acanthaceae	1	**Caricaceae**	1	**Menispermaceae**	1	**Rubiaceae**	1
Acoraceae	1	**Clusiaceae**	1	**Moraceae**	1	**Rutaceae**	2
Amaranthaceae	3	**Combretaceae**	1	**Musaceae**	2	**Sapotaceae**	1
Annonaceae	1	**Convolvulaceae**	1	**Myrtaceae**	2	**Solanaceae**	3
Apiaceae	1	**Crassulaceae**	1	**Orthotrichaceae**	1	**Verbenaceae**	1
Arecaceae	1	**Cucurbitaceae**	2	**Pinaceae**	1	**Zingiberaceae**	2
Asparagaceae	1	**Euphorbiaceae**	4	**Piperaceae**	2	**Rubiaceae**	1
Asphodelaceae	1	**Fabaceae**	4	**Poaceae**	2	**Rutaceae**	2
Asteraceae	9	**Lamiaceae**	5	**Portulacaceae**	1	**Sapotaceae**	1
Athyriaceae	1	**Lauraceae**	1	**Primulaceae**	1	**Solanaceae**	3
Boraginaceae	2	**Lythraceae**	1	**Rosaceae**	1	**Verbenaceae**	1

agreement. These results highlight the coherence and reliability of traditional medicinal knowledge in the community and suggest that plants associated with high ICF values may hold significant therapeutic potential for further pharmacological study and integration into primary healthcare.

DISUSSIONS

The findings that ethnomedicinal knowledge is predominantly retained by older women, particularly those aged 61 and above, align with recent research indicating that age significantly influences the retention and depth of traditional medicinal knowledge, with older community members serving as primary custodians[1]. Furthermore, the observation that knowledge acquisition is gendered—where women learn mainly from elders and parents while men rely more on the broader community—is supported by studies showing that women typically possess greater medicinal plant knowledge due to household and caregiving roles, highlighting the strong intergenerational and familial transmission of ethnobotanical knowledge[2].The ethnomedicinal survey documented 75 plant species used by the Maranaos in Pantar, Lanao del Norte, representing a diverse range of 38 plant families, with Asteraceae (9 species), Lamiaceae (5), Malvaceae (5), Euphorbiaceae (4), Fabaceae (4), and Solanaceae (3) among the most represented. This wide taxonomic variety highlights the richness and diversity of the community's medicinal plant resources in the study area.

The Maranaos of Pantar, Lanao del Norte, primarily use leaves (56%) in herbal medicine due to their accessibility, renewability, and high concentration of bioactive compounds. Other plant parts include bulbs and fruits (15%), roots (13%), stems and stalks (8%), bark (5%), and rarely the whole plant (3%), reflecting both medicinal efficacy and sustainability considerations. Several recent ethnobotanical studies corroborate that **leaves are the most frequently used plant part** in traditional medicinal practices due to their accessibility, renewability, and high concentrations of bioactive compounds, followed by other parts like fruits, roots, stems, and bark, reflecting both therapeutic efficacy and sustainability considerations in community herbal medicine use. For example, a 2025 survey in the Philippines found that leaves were the dominant plant part used in herbal remedies (62.3%), with other parts

TABLE-4 : Method of preparation of medicinal plants used by the Maranaos in Pantar LDN, Philippines

Method of Preparations	Percentage	
Infusion	33	30%
Decoction	30	27%
Poultice	24	22%
Raw/Eat Directly	15	13%
Extract	5	5%
Heating over fire	3	3%
Total	110	100%

used to a lesser extent, highlighting similar patterns of plant part preference in ethnomedicinal traditions[6]. Likewise, a 2024 study in Benguet Province documented that leaves were the most utilized plant part (61.5%) among indigenous communities, followed by whole plants, stems, bark, and flowers[16].

The Maranaos of Pantar use six main methods to prepare medicinal plants, with infusions (30%) and decoctions (27%) being the most common, followed by poultices (22%), raw consumption (13%), extracts (5%), and heating over fire (3%). Each method is tailored to specific ailments, reflecting the community's adaptive knowledge in optimizing therapeutic efficacy based on both plant properties and the conditions treated. Recent ethnobotanical studies similarly document a range of preparation techniques for medicinal plants, with infusion, decoction, poultices, and raw consumption among the most commonly reported methods, reflecting how traditional communities tailor preparation to optimize therapeutic benefit based on plant properties and ailments treated. For example, a study among indigenous communities in Mizoram, India found decoction, crushing, chewing, poultice, and heating were widely used for remedy preparation, demonstrating diverse, adaptive traditional knowledge in medicinal plant use[15] and another recent survey reported multiple preparation methods including decoction, raw food, poultice, and heating in a similar ethnobotanical context[12].

The Informant Consensus Factor (ICF) analysis showed a high level of agreement among the Maranaos of Pantar regarding the use of medicinal plants, with the highest consensus for treating asthma, labor pain, and promoting healthy hair (ICF = 0.98), followed by arthritis, insect bites, and menstrual cramps (ICF = 0.97). Even conditions with lower ICF values, such as bleeding (0.76) and urinary tract infections (0.77), demonstrated notable agreement, indicating the community's coherent and reliable traditional medicinal knowledge and highlighting plants with potential for further pharmacological study. Recent ethnobotanical research has similarly documented high Informant Consensus Factor (ICF) values, highlighting strong agreement among informants on the use of specific medicinal plants for particular ailments, which underscores the reliability of traditional knowledge and suggests priority species for further pharmacological study. For example, a study among urban and rural communities in Khyber Pakhtunkhwa, Pakistan reported a high ICF value of 0.97 for the use of plants to treat kidney disorders, indicating consistent community agreement on plant use[8], and another investigation in northeast India found ICF values ranging from 0.97 to 0.98 for several ailment categories, demonstrating a robust shared knowledge base regarding medicinal plant applications[4].

4.1 Summary of Findings

The ethnomedicinal survey of the Maranaos in Pantar, Lanao del Norte, shows that older women, particularly those aged 61 and above, are the primary holders of traditional knowledge, learning mainly from elders and parents, while men rely more on the broader community, highlighting strong intergenerational and gendered knowledge transmission. A total of 75 medicinal plant species from 38 families were documented, with leaves being the most frequently used plant part (56%), followed by bulbs, fruits, roots, stems, and bark, reflecting both efficacy and sustainability considerations. Six main preparation methods were identified—infusions (30%) and decoctions (27%) were the most common, followed by poultices, raw consumption, extracts, and heating over fire—demonstrating adaptive selection of techniques based on plant properties and targeted ailments. Informant Consensus Factor (ICF) analysis revealed high agreement among community members, especially for asthma, labor pain, and promoting healthy hair (ICF = 0.98), indicating coherent and reliable traditional medicinal knowledge and identifying plants with potential for further pharmacological research.

4.2 Conclusions

The ethnomedicinal knowledge of the Maranaos in Pantar is predominantly held by older

TABLE-5 : The level of agreement among the respondents in Pantar Lanao del Norte, Philippines

Indications	ICF	ICF Interpretations
Allergy	0.85	High Consensus
Arthritis	0.97	High Consensus
Arthritis	0.93	High Consensus
Asthma	0.98	High Consensus
Bleeding	0.76	High Consensus
Body pain	0.94	High Consensus
Boils and pus	0.75	High Consensus
Boosts sperm count	0.98	High Consensus
Cleanses toxins	0.92	High Consensus
Constipation	0.80	High Consensus
Cough	0.94	High Consensus
Dengue	0.96	High Consensus
Diabetes	0.89	High Consensus
Fever	0.95	High Consensus
Flatulence	0.86	High Consensus
Gives strength to a weak body	0.88	High Consensus
Healthy hair	0.98	High Consensus
High blood pressure	0.93	High Consensus
Inflammations inside the body	0.94	High Consensus
Insect bites	0.97	High Consensus
Kidney stones	0.86	High Consensus
LBM	0.94	High Consensus
Lessens labor pain	0.98	High Consensus

Indications	ICF	ICF Interpretations
Menstrual cramps	0.97	High Consensus
Migraine/headache	0.87	High Consensus
Myoma	0.91	High Consensus
Nosebleed	0.83	High Consensus
Indications	ICF	ICF Interpretations
Overfatigue	0.80	High Consensus
Rheuma	0.80	High Consensus
Skin irritation	0.85	High Consensus
Sprain	0.92	High Consensus
Stimulates period	0.92	High Consensus
Stomachache	0.93	High Consensus
Toothache	0.94	High Consensus
Ulcer	0.91	High Consensus
UTI	0.77	High Consensus
Vomiting	0.92	High Consensus
Wound	0.89	High Consensus

women and transmitted intergenerationally, with 75 plant species from 38 families documented. Leaves were the most used plant part, and six preparation methods—primarily infusions and decoctions—reflect adaptive practices tailored to specific ailments. High Informant Consensus Factor values indicate reliable traditional knowledge and highlight plants with potential for further pharmacological study.

4.3 Recommendations

1. Further research should document and preserve the ethnomedicinal knowledge of older women, particularly those aged 61 and above, to safeguard intergenerational transmission of traditional practices.
2. Medicinal plants with high Informant Consensus Factor (ICF) values, such as those used for asthma, labor pain, and promoting healthy hair, should be prioritized for phytochemical and pharmacological investigations to validate their therapeutic potential.
3. Studies should explore sustainable harvesting and utilization of plant parts, especially leaves and roots, to ensure long-term availability and conservation of local medicinal plant resources.
4. Efforts to engage younger generations and male community members in ethnomedicinal practices should be encouraged, promoting wider knowledge transfer and integration into community healthcare systems.

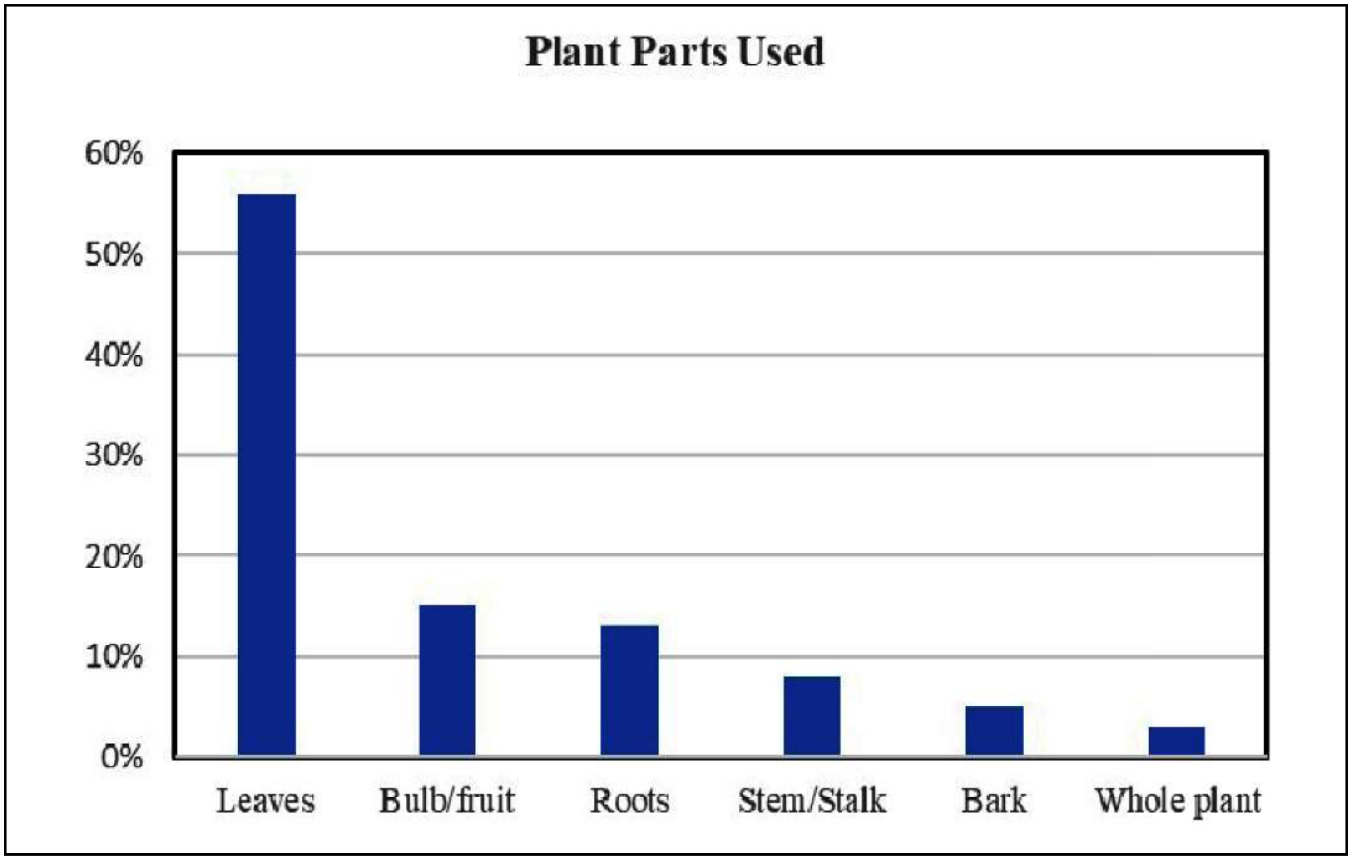

Fig. 1 : Plant parts used in herbal preparations by the Maranaos in Pantar LDN, Philippines

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https://doi.org/10.33451/florafauna.v32i1pp56-64
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 56-64 ISSN 0971 - 6920 (Print)

Role of Plant-Based Antimicrobial Agents in Controlling Multidrug-Resistant Bacteria: A Mini Review

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Received **:** 25.03.2026; ***Accepted*** : 08.05.2026

How to cite : Soni S, Srivastava S. Role of Plant-Based Antimicrobial Agents in Controlling Multidrug-Resistant Bacteria: A Mini Review. *Flora and Fauna* 2026. 32(1) : 56-64.

ABSTRACT

The rapid escalation of multidrug-resistant (MDR) bacteria, particularly the virulent "ESKAPE" pathogens, has compromised conventional antibiotic efficacy and necessitated the search for alternative therapeutic strategies. Plant-derived antimicrobial agents have emerged as critical candidates due to their immense chemical diversity and multi-target modes of action, which impose lower selective pressure for resistance compared to synthetic drugs. This review synthesizes current research on medicinal plant extracts.such as those from *Curcuma longa, Allium sativum*, and *Azadirachta indica* and isolated phytochemicals including alkaloids, polyphenols, and terpenoids. These bioactive compounds exert their effects through diverse mechanisms, including the disruption of cell membrane integrity, inhibition of bacterial efflux pumps, interference with quorum sensing pathways, and the degradation of biofilm matrices. Furthermore, their role as antibiotic adjuvants facilitates the restoration of standard drug activity and potential dose reduction against resistant strains. Despite their potential, significant research gaps remain regarding standardized extraction methodologies, clinical validation, and the optimization of bioavailability. Future progress depends on leveraging nanotechnology-based delivery systems and advanced omics technologies to bridge the gap between laboratory findings and effective clinical applications.

Figure : 00 References : 30 Tables : 02

KEY WORDS : Antimicrobial activity, Biofilm inhibition, Efflux pump inhibitors, Multidrug-resistant bacteria, Phytochemicals, Quorum sensing, Secondary metabolite

Introduction

Antimicrobial resistance (AMR) represents one of the most critical challenges to global public health, compromising the efficacy of modern medical treatments and leading to increased morbidity and mortality[3,14]. This phenomenon occurs when microorganisms transform over time and no longer respond to conventional pharmacological agents, rendering standard infections difficult or impossible to treat[3,14]. Driven primarily by the irrational use and over-prescription of antibiotics in human medicine, veterinary settings, and agriculture, AMR was associated with approximately 4.95 million deaths in 2019, with estimates suggesting this could rise to 10 million annual deaths by 2050[12,14]. Beyond clinical impacts, AMR imposes a severe economic burden, with healthcare costs and productivity losses expected to reach trillions of dollars globally[3,18].

The clinical severity of this crisis is underscored by the emergence of multidrug-resistant (MDR) pathogens, defined as bacterial strains resistant to at least one agent in three or more antimicrobial classes[3,11]. Of particular concern are the "ESKAPE" pathogens. *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. which are frequently implicated in healthcare-associated infections[14,18,25]. The World Health Organization (WHO) has categorized several of these, such as carbapenem-resistant Gram-negative bacilli, as "critical priority" pathogens that necessitate the urgent development of alternative therapeutic strategies[8,24].

The stagnation of the antibiotic discovery pipeline further complicates the management of MDR infections[2,24]. In the past 50 years, only two new classes of synthetic antibiotics fluoroquinolones and oxazolidinones have been developed, while bacterial resistance mechanisms continue to evolve rapidly[15,24]. These mechanisms include the enzymatic degradation of drugs (such as swech-lactamases), the alteration of antibiotic target sites, and the over expression of energy-

driven efflux pumps that extrude drugs from the cell[11,21,24]. Furthermore, the high doses often required to treat resistant infections can lead to severe host toxicity, such as the nephrotoxicity associated with colistin[2,7].

Medicinal plants have emerged as promising reservoir for novel antimicrobial agents, serving as "natural laboratories" for the production of chemically diverse secondary metabolites[15,25]. Phytochemical classes, including alkaloids, polyphenols, and terpenoids, exhibit broad-spectrum antimicrobial activity through multi-target modes of action[3,15]. Unlike conventional antibiotics, these plant-derived compounds often target non-growth-related processes like quorum sensing and biofilm formation, which reduces the selective pressure for resistance development[15]. Furthermore, these agents can function as antibiotic adjuvants or potentiators, restoring the efficacy of existing drugs by inhibiting bacterial efflux pumps and increasing membrane permeability[15].

Despite this therapeutic potential, significant research gaps remain. Current studies are limited by a lack of standardization in extraction methodologies and inconsistencies in the concentrations used across different laboratories[10,20]. Furthermore, the vast majority of research is based on *in vitro* evidence, with a significant deficiency in animal models and clinical trials to evaluate the safety, bioavailability, and pharmacokinetics of plant-based antimicrobials in humans[7,11,20].

This mini-review aims to provide a comprehensive analysis of the role of plant-based antimicrobial agents in controlling MDR bacteria. It evaluates the mechanisms of action of key phytochemicals, their effectiveness against high-priority pathogens, and their potential for synergistic use as antibiotic adjuvants[11,15,20]. Finally, the review identifies current challenges and future directions, such as the integration of nanotechnology for enhanced drug delivery, to advance the clinical utility of plant-derived compounds in the fight against antimicrobial resistance[10].

Plant-Based Antimicrobial Agents: Diversity and Phytochemical Characterisation

The therapeutic utility of medicinal plants is derived from their capacity to synthesise a vast library of secondary metabolites (SM), which evolved as chemical defences against microbial predation and environmental stressors[3,27]. These bioactive compounds exhibit immense structural diversity and are distributed unevenly across specific plant anatomical parts, including leaves, roots, bark, seeds, and flowers[11,27]. For instance, antimicrobial constituents are concentrated in the rhizomes of *Curcuma longa* and *Curcuma caesia,* the bulbs of *Allium sativum,* the bark of *Cinnamomum cassia,* and the flower buds of *Syzygium aromaticum*[9,13,23,27]. Systematic research into species such as *Hagenia abyssinica* and *Azadirachta indica* underscores the selection of plant parts is non-trivial, as specific tissues may harbour significantly higher concentrations of active principles[11,30].

The efficacy of these agents is heavily influenced by the extraction methodology and solvent polarity, which determine the profile of the isolated metabolites[13]. Organic solvents, particularly methanol and ethanol, are generally superior to aqueous extraction for recovering moderately polar constituents such as polyphenols and flavonoids[9,30]. Furthermore, sequential extraction techniques using solvents of increasing polarity.ranging from non-polar n-hexane to polar water.allow for the fractionated isolation of bioactive classes according to their solubility profiles[13]. Volatile essential oils, predominantly composed of hydrophobic monoterpenes and sesquiterpenes, represent a distinct class of antimicrobials often isolated *via* distillation or solvent extraction to treat persistent infections[19,27].

Phytochemical classes are broadly categorised into nitrogenous compounds, such as alkaloids, and nitrogen-free metabolites, including phenolics and terpenoids[3,27]. Alkaloids like berberine and sanguinarine are among the most potent agents, frequently acting as efflux pump inhibitors (EPIs) or DNA intercalators[2]. Phenolics, encompassing flavonoids, tannins, and phenolic acids (*e.g.*, gallic acid and quercetin), constitute the largest group of antimicrobial SMs and are ubiquitous in traditional phytotherapies[3,27,29]. Terpenoids and their derivatives exhibit broad-spectrum activity through their lipophilic nature, which facilitates interaction with microbial lipid bilayers[2,10,27]. The synergistic presence of multiple phytochemical classes within a single extract often enhances biological activity and reduces the selective pressure for the development of resistance in multidrug-resistant pathogens[3,15].

Antimicrobial Activity against Multidrug-Resistant Pathogens

The antimicrobial efficacy of plant-based agents is quantitatively evaluated using the diameter of the zone of inhibition (ZOI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC)[5]. As summarized in Table-1, these metrics reveal a broad spectrum of activity against "ESKAPE" pathogens, with potency varying significantly based on plant species and extraction methods.

Comparative analysis highlights exceptional inhibitory activity in certain species. One of the strongest

TABLE -1 : Antimicrobial activity of plant extracts against multidrug-resistant bacteria

Plant Species	Extraction Method	Target MDR Bacteria	ZOI (mm)	MIC	MBC	Reference
Cirsium arvense	Ethanol	*P.aeruginosa*	72	2 µg/mL	4 µg/mL	5
Avena fatua	Ethanol	*P.aeruginosa*	65	1 µg/mL	2 µg/mL	5
Chenopodium murale	Ethanol	*P.aeruginosa*	52	1 µg/mL	2 µg/mL	5
Curcuma caesia	n-hexane (Rhizome)	*A.baumannii*	23	3.12 µg/mL	6.25 µg/mL	13
Curcuma caesia	n-hexane (Leaf)	*S. aureus*	24	3.12 µg/mL	6.25 µg/mL	13
Curcuma caesia	Chloroform (Leaf)	*K.pneumoniae*	32	6.2 µg/mL	12.5 µg/mL	13
Phyllanthus emblica	Ethanol (70%)	*A.baumannii*	Not reported	125 µg/mL	250 µg/mL	16
Nigella sativa	Ethyl Acetate	*S. aureus*	20	5 µg/mL	Not reported	4
Curcuma amada	Methanol	*E.coli*	22	10 µg/mL	Not reported	4
Arnica montana	Ethanol	*A.baumannii*	Not reported	234.4 µg/mL	Not reported	1
Curcuma longa	Methanol	*S. aureus*	18	Not reported	Not reported	20
Curcuma longa	Methanol	*E.coli*	15	Not reported	Not reported	20
Curcuma longa	Methanol	*K.pneumoniae*	16	Not reported	Not reported	20
Opuntia ficus-indica	Aqueous	*P.aeruginosa*	Not reported	0.05 mg/mL	Not reported	20
Azadirachta indica	Ethanol	*E.coli* (MDR)	20	Not reported	Not reported	26
Allium sativum	Ethanol	*S. aureus* (MRSA)	18	Not reported	Not reported	26

Plant Species	Extraction Method	Target MDR Bacteria	ZOI (mm)	MIC	MBC	Reference
Ocimum sanctum	Ethanol	*K.pneumoniae*	14	Not reported	Not reported	26
Roemeria refracta	Alkaloidal ext.	*S. aureus*	Not reported	0.065 µg/mL	Not reported	11
Hagenia abyssinica	Ethanol (Flower)	*E.coli*	5	Not reported	Not reported	30
Hagenia abyssinica	Ethanol (Leaf)	*S. aureus*	3	Not reported	Not reported	30

Note : *Values for ZOI are generally reported at specific extract concentrations (e.g., 75 µg/mL for C. caesia or 20 mg/mL for crude extracts) as per individual study methodologies.*

results recorded involves ethanol extracts of *Cirsium arvense*, which exhibited a ZOI of 72 mm against *Pseudomonas aeruginosa*, outperforming the standard antibiotic ciprofloxacin (66 mm) under identical conditions[5]. Similarly, *Avena fatua* and *Chenopodium murale* demonstrated superior absolute potency, achieving MIC values as low as 1 µg/mL and MBC values of 2 µg/mL against *P. aeruginosa* 5. In the Gram-positive category, alkaloidal extracts from *Roemeria refracta* showed remarkable sensitivity against *Staphylococcus aureus* with an MIC of 0.065 µg/mL[11].

Conversely, some plants demonstrated relatively weak or standalone activity. Extracts from *Hagenia abyssinica* produced minimal ZOI values of only 5 mm against *Escherichia coli* and 3 mm against *S. aureus*[30]. Furthermore, *Linum usitatissimum* (flaxseed) extracts were reported to show no inhibition, and in some instances, actually enhanced bacterial growth, suggesting they are ineffective as standalone treatments[20].

The data in Table 1 also underscore the influence of solvent polarity on antimicrobial outcomes. Research on *Curcuma caesia* indicates that non-polar sequential extracts (n-hexane and chloroform) are significantly more effective than aqueous versions, with MICs as low as 3.12 µg/mL against *Acinetobacter baumannii* and *K. pneumoniae*[13]. Similarly, *Phyllanthus emblica* (amla) required higher concentrations (MIC 125 µg/mL) to inhibit MDR *A. baumannii*, yet it remained effective where standard drugs failed[16]. These findings, systematically detailed in Table-1, emphasize that while many plants possess antimicrobial properties, clinical relevance is highly dependent on achieving specific sub-microgram inhibitory thresholds.

Molecular and Cellular Mechanisms of Action

Plant-based antimicrobial agents employ multi-target mechanisms that significantly diminish the probability of resistance development compared to conventional synthetic monotherapies[15]. As systematically presented in Table 2, a primary mode of action is the disruption of bacterial cell membrane integrity. Lipophilic terpenoids and essential oil components, such as thymol, carvacrol, and eugenol, interact with microbial lipid bilayers, causing membrane depolarization, loss of ion homeostasis, and the leakage of vital intracellular components including ATP and proteins[10,22]. Similarly, indole alkaloids isolated from *Rhazya stricta* and polyphenolic compounds from *Phyllanthus emblica* increase membrane permeability in MRSA and Gram-negative rods, facilitating the entry of standard antibiotics[3,10,16].

Efflux pump inhibition (EPI) represents another critical mechanism for controlling multidrug-resistant (MDR) bacteria[24]. Phytochemicals such as resveratrol, curcumin, and piperine act as potent EPIs by downregulating resistance-associated genes, such as adeJ in *Acinetobacter baumannii* and MexAB-OprM in *Pseudomonas aeruginosa,* or by interfering with pump proteins to prevent the extrusion of antibiotics[6,10,20,24]. This restores therapeutic concentrations of drugs within the bacterial cell[20,22].

Interference with quorum sensing (QS) and biofilm formation further distinguishes these natural agents as "antipathogenic" therapies[7,15,22]. Quorum-quenching (QQ) compounds, including naringenin and

TABLE-2. Molecular mechanisms of action of plant-derived antimicrobial agents against MDR bacteria

Plant Compound/ Extract	Target Mechanism	Specific Molecular Action	Target Pathogen(s)	Reference
Thymol / Carvacrol	C Disruption	Membrane depolarization, increased permeability, and leakage of ATP and proteins.	*S. aureus, E. coli, P. aeruginosa*	10, 11
Resveratrol	Efflux Pump Inhibition (EPI)	Downregulation of *adeJ* gene (AdeABC pump) and inhibition of CmeABC efflux systems.	*A. baumannii, C. jejuni*	2, 6
Curcumin	Multi-target (QS & EPI)	Inhibition of Mex AB-Opr M and Nor A pumps; reduction of virulence factors (prodigiosin) via QS interference.	*P. aeruginosa, S. aureus, S. marcescens*	3, 15, 24
Quercetin	EPI & Biofilm Inhibition	High affinity binding to *AdeJ* and Acr B proteins; interference with rhl transcription to reduce EPS synthesis.	*A. baumannii, K. pneumoniae, P. aeruginosa*	6, 10
Hamamelitannin	Quorum Sensing Inhibition	Antagonism of the Tra P receptor and downregulation of regulator involved in QS and motility.	*S. aureus* (MRSA)	10, 15
Allicin (Garlic)	Enzyme Inhibition	Inhibition of DNA gyrase, preventing the synthesis of RNA, DNA, and proteins.	*General MDR bacteria*	22
Conessine	Efflux Pump Inhibition	Restored levofloxacin activity by blocking Mex AB-Opr M, Mex CD-Opr J, and Mex EF-Opr N pumps.	*P. aeruginosa*	11

Plant Compound/ Extract	Target Mechanism	Specific Molecular Action	Target Pathogen(s)	Reference
Naringenin	Quorum Sensing Inhibition	Downregulation of las and rhl family genes, reducing AHL-mediated virulence.	*P. aeruginosa*	10, 15
Baicalein	Efflux Pump Inhibition	Restoration of tetracycline and lactam activity by blocking Nor A and other MDR pumps.	*MRSA, E. coli, Salmonella enteridis*	2, 11
Luteolin	EPS & Gene Inhibition	Reduction of polysaccharide and eDNA synthesis; down regulation of oqx A RND pump gene.	*E. coli, Enterobacter cloacae*	6, 10
Andrographolide	Quorum Quencing	Downregulation of las R gene expression, attenuating protease activity and swarming motility.	*P. aeruginosa*	15
Gallotannin (PGG)	Metal Chelation	Sequestration of iron from the extracellular matrix, destabilizing the biofilm structure.	*E. coli*	10

hamamelitannin, disrupt intercellular communication by inhibiting signal synthesis (e.g., AHLs in Gram-negatives) or by antagonizing QS receptors[10,15]. This process suppresses virulence factors like pyocyanin production and swarming motility without imposing lethal selective pressure[15,28]. Furthermore, flavonoids like luteolin and quercetin inhibit the synthesis of extracellular polymeric substances (EPS), thereby destabilizing the biofilm matrix and rendering protected microbial communities vulnerable to standard pharmacological treatments[7,10,17].

Comparative Insights and Critical Synthesis of Findings

Comparative analysis identifies *Cirsium arvense* as exhibiting the largest zone of inhibition (72 mm) against *P. aeruginosa*, yet this does not universally translate to the lowest minimum inhibitory concentrations across all pathogens[5]. Instead, species such as *Avena fatua* and *Chenopodium murale* demonstrate superior absolute potency with MICs as low as 1 µg/mL against the same resistant strains[5]. This highlights a significant contradiction in reporting metrics; large inhibition zones frequently reflect high agar diffusion rates rather than intrinsic bactericidal efficacy, necessitating standardized MIC and MBC evaluations for clinical relevance[5,13].

Regarding extraction, a consistent pattern confirms the superiority of organic solvents particularly methanol, ethanol, and chloroform over aqueous methods for isolating bioactive phenolics and flavonoids[9,13,30]. In species like *Curcuma caesia*, non-polar sequential extraction using n-hexane and chloroform yields significantly more potent antimicrobial activity against MRSA and *K. pneumoniae* than aqueous fractions, which often show negligible effects[13]. Essential oils also consistently outperform crude extracts due to their concentrated volatile terpenes like carvacrol and

thymol, which facilitate immediate membrane disruption[19].

Critical synthesis of spectrum activity reveals that while plant extracts are often described as broad-spectrum, their standalone efficacy is structurally biased toward Gram-positive pathogens due to the lack of an outer membrane barrier[11,22,25]. However, structure-activity relationship (SAR) analysis suggests that while individual compounds may vary, those containing catechol or gallol motifs exhibit the highest synergy rates (80.9%) and up to an 8-fold reduction in antibiotic MICs[17,26]. Paradoxically, species such as *Linum usitatissimum* exhibit concentration-dependent contradictions where sub-optimal levels may enhance rather than inhibit bacterial growth, underscoring the critical need for precise standardization to avoid unintended growth promotion in MDR isolates[20].

Conclusion

Plant-derived antimicrobial agents, specifically alkaloids, phenolics, and terpenoids, demonstrate significant potential in controlling multidrug-resistant (MDR) pathogens through multi-target mechanisms[3,24,25,27]. Key findings highlight their ability to disrupt cell membrane integrity, inhibit biofilm maturation, and deactivate energy-driven efflux pumps[3,10,22,24]. These secondary metabolites are critical because they function as potent antibiotic adjuvants, restoring the efficacy of conventional drugs and allowing for dose reductions that mitigate host toxicity[14,20,26].

Despite these promising findings, several research gaps limit the clinical translation of phytochemicals. Current literature is characterized by a lack of standardized extraction methodologies and a significant deficiency in human clinical trials to validate safety and efficacy[12,20,30]. Furthermore, the poor aqueous solubility and rapid metabolism of many bioactive compounds remain substantial hurdles[7,11,15]. Future directions must prioritize the development of nanotechnology-based delivery systems to enhance bioavailability and stability[15,18]. Additionally, the integration of artificial intelligence and advanced omics technologies will be essential for the high-throughput discovery and molecular characterization of next-generation plant-based therapies[14,15]. Addressing these challenges is vital to successfully integrating medicinal plant compounds into the global strategy against antimicrobial resistance.

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https://doi.org/10.33451/florafauna.v32i1pp65-72
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 65-72 ISSN 0971 - 6920 (Print)

Seasonal Variation and Distribution Pattern of Copepod Diversity in Pravara River Dist. Ahilyanagar (MS) India

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Received **:** 29.03.2026; ***Revised*** **:** 15.04.2026; ***Accepted*** : 08.05.2026

How to cite : Khemnar RS, Shinde LV, Thete KD, Parkhe SB. Seasonal Variation and Distribution Pattern of Copepod Diversity in Pravara River Dist. Ahilya nagar (MS) India. *Flora and Fauna* 2026. 32(1) : 65-72.

ABSTRACT

This study investigates the seasonal variation and distribution pattern of copepod diversity in the Pravara River, Maharashtra, India, from June 2023 to May 2024. Seven sampling sites representing varied ecological zones were examined monthly. A total of 842 copepod individuals belonging to eight species were identified. The highest abundance was recorded during winter 42.99 % (362individuals) and summer 42.40 % (357 individuals), with the lowest during the monsoon season 14.61% (123 individuals). *M. aspericornis* was the dominant species, followed by *M. edax* and *M. leuckarti*. Diversity indices revealed a highly balanced community (Simpson's 0.87; Shannon 1.98; Evenness 0.95). These findings suggest that copepod populations in the Pravara River are strongly influenced by seasonal hydrological fluctuations, with post-monsoon stability favouring higher species richness and abundance. The study underscores the potential of copepods as effective bioindicators for assessing freshwater ecosystem health and productivity

Figures : 04 References : 30 Tables : 05

KEY WORDS : Copepods, Distribution, Diversity, Pravara River, Seasonal Variation.

Introduction

Copepods are vital components of freshwater ecosystems, serving as primary consumers and mediators in nutrient cycling and energy flow[13]. Copepods are small crustaceans belonging to the subclass Copepoda, typically ranging from 1.2 mm to a few millimetres in size. They are among the most abundant zooplankton in aquatic environments and play a crucial role in transferring energy from phytoplankton to higher trophic levels such as fish and other aquatic organisms[4]. Copepod diversity is increasing rapidly due to favorable environmental conditions, availability of food resources, and suitable water quality.Their presence and abundance often serve as indicators of water quality and ecosystem health.

Copepods are the most diverse groups of zooplankton in aquatic ecosystems. Globally, more than 14,000 species of copepods have been reported from marine, freshwater, and brackish water habitats, while about 500–550 species have been recorded from various aquatic ecosystems in India[3,23]. Their body is elongated and segmented, typically divided into cephalosome,

ACKNOWLEDGEMENTS : We would like to express our gratitude to the Department of Zoology, Applied Parasitology Research Centre J.E.S. College of Jalna (MS) India for their collaboration and support in conducting this study. Additionally, We would like to express our sincere gratitude to the Department of Zoology at PVP College, Pravaranagar for their invaluable support and resources throughout this research. Special thanks to the faculty and staff for their assistance in sample analysis and data interpretation. Their contributions were crucial to the success of this study.

TABLE-1: Sampling Sites location and GPS coordinates

Sampling site code	Site names	Coordinates of sampling sites	
S-1	Wilson dam	19°32’13"N	73°45’46"E
S-2	Nilwande Dam	19°32’51"N	73°54’00"E
S-3	Akole	19°32’39"N	74°01’00"E
S-4	Sangamner	19°33’20"N	74°11’45"E
S-5	Ashvi	19°31’16"N	74°21’58"E
S-6	Kolhar	19°32’05"N	74°31’47"E
S-7	Lakh	19°30’18"N	74°41’28"E

metasome, and urosome, and they possess long antennae used for swimming. Most copepods are microscopic and feed on phytoplankton and organic particles. Females usually carry paired egg sacs, and their life cycle includes egg, nauplius, copepodid, and adult stages. They play an important role in aquatic food chains as primary consumers and as food for many fish and aquatic organisms.

The Pravara River offers a unique opportunity to investigate copepod diversity due to its diverse aquatic habitats and fluctuating environmental conditions. The river consists of various ecological zones, including agricultural landscapes, semi-arid regions, and forested areas, each creating distinct microhabitats that influence copepod distribution and community structure[21]. The river’s hydrology is marked by pronounced seasonal changes, including monsoon-driven floods and dry periods, affecting water temperature, flow rates, and nutrient availability, shaping copepod populations[2].

During the monsoon season, increased water flow and nutrient input can enhance copepod diversity and abundance, whereas dry spells may lead to reduced copepod populations and altered community composition[17]. These seasonal variations underscore the adaptability of copepods to changing environmental conditions and their sensitivity to shifts in their habitat[25].

Understanding copepod diversity in the Pravara River is essential for assessing the ecological health of this fresh water system. Documenting species diversity and distribution patterns provides insights into the effects of environmental variability on aquatic communities and supports the development of conservation strategies to preserve freshwater biodiversity[21]. This study seeks to offer a comprehensive analysis of copepod species in the Pravara River, contributing to the broader understanding of fresh water ecosystems and their management.

TABLE-2. Month wise collection from Jun 2023 to May 2024

Sampling Site	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Total	%
S-1	9	6	7	3	9	8	5	11	12	18	16	20	124	14.73
S-2	6	4	6	4	5	7	4	12	14	20	17	14	113	13.42
S-3	8	6	4	5	8	9	3	14	19	16	14	10	116	13.78
S-4	5	3	5	7	9	8	6	13	15	14	20	13	118	14.01
S-5	6	4	6	4	11	10	8	11	14	15	17	13	119	14.13
S-6	0	2	3	2	11	11	2	14	21	23	19	17	125	14.85
S-7	2	1	4	1	8	9	6	15	20	22	21	18	127	15.08
Total	36	26	35	26	61	62	34	90	115	128	124	105	842	100.00

TABLE-3 : Seasonal Abundance from Jun 2023 to May 2024

Sampling Site	Summer (Mar-May)	Monsoon (Jun-Sep)	Winter (Oct-Feb)	Total
S-1	54	25	45	124
S-2	51	20	42	113
S-3	40	23	53	116
S-4	47	20	51	118
S-5	45	20	54	119
S-6	59	7	59	125
S-7	61	8	58	127
Total	357	123	362	842

Materials and Methods

Study Area

The study was carried out during June 2023 to May 2024 from different localities. The study was conducted along the Pravara river in Maharashtra, India. There were seven Sampling sites which were selected to include various environmental conditions such as farmland, semi-arid zones, and forested regions.

Sampling Locations and Frequency

Seven sampling sites were chosen based on water depth and flow conditions. That were Wilson Dam, Nilwande Dam, Akole, Sangamner, Ashvi, Kolhar, Lakh. The distance between the two Sampling sites was 15 to 20 km. Sampling was performed monthly from May 2023 to Jun 2024 to analyse the abundance and seasonal variation in Pravara river.

Sampling time

As per the recommended times[19] for zooplankton collection, specifically early morning (around 6:00 AM to 9:00 AM) and late afternoon (around 4:00 PM to 6:00 PM) were informed by diurnal vertical migration patterns and the effects of light on zooplankton behavior. These sampling times help to ensure a representative sample of the zooplankton community by aligning with their natural activity cycles and minimizing the impact of daylight on their distribution.

Collection

Copepod samples were collected using a conical plankton net with a mesh size of 25 µm and a diameter of 30 cm[11]. Before sampling, the zooplankton net was rinsed with river water to remove any residual materials

TABLE-4 : Copepod diversity in different sites from Jun 2023 to May 2024

S. No.	Name	S-1	S-2	S-3	S-4	S-5	S-6	S-7	Total	%
1	*Mesocyclops aspericornis*	24	18	19	22	19	24	16	142	16.86
2	*Mesocyclops pehpeiensis*	18	14	10	13	12	18	8	93	11.04
3	*Mesocyclops hyalinus*	22	12	11	12	17	8	4	86	10.21
4	*Mesocyclops leuckarti*	9	10	14	4	16	14	19	86	10.21
5	*Mesocyclops edax*	13	16	15	19	15	20	11	109	12.94
6	*Thermocyclops oithonoides*	7	19	18	11	11	12	18	96	11.40
7	*Acanthocyclops varicans*	11	14	13	13	15	17	23	106	12.58
8	*Acanthocyclops strenuus*	20	10	16	24	14	12	28	124	14.72
	Total	**124**	**113**	**116**	**118**	**119**	**125**	**127**	**842**	**100**

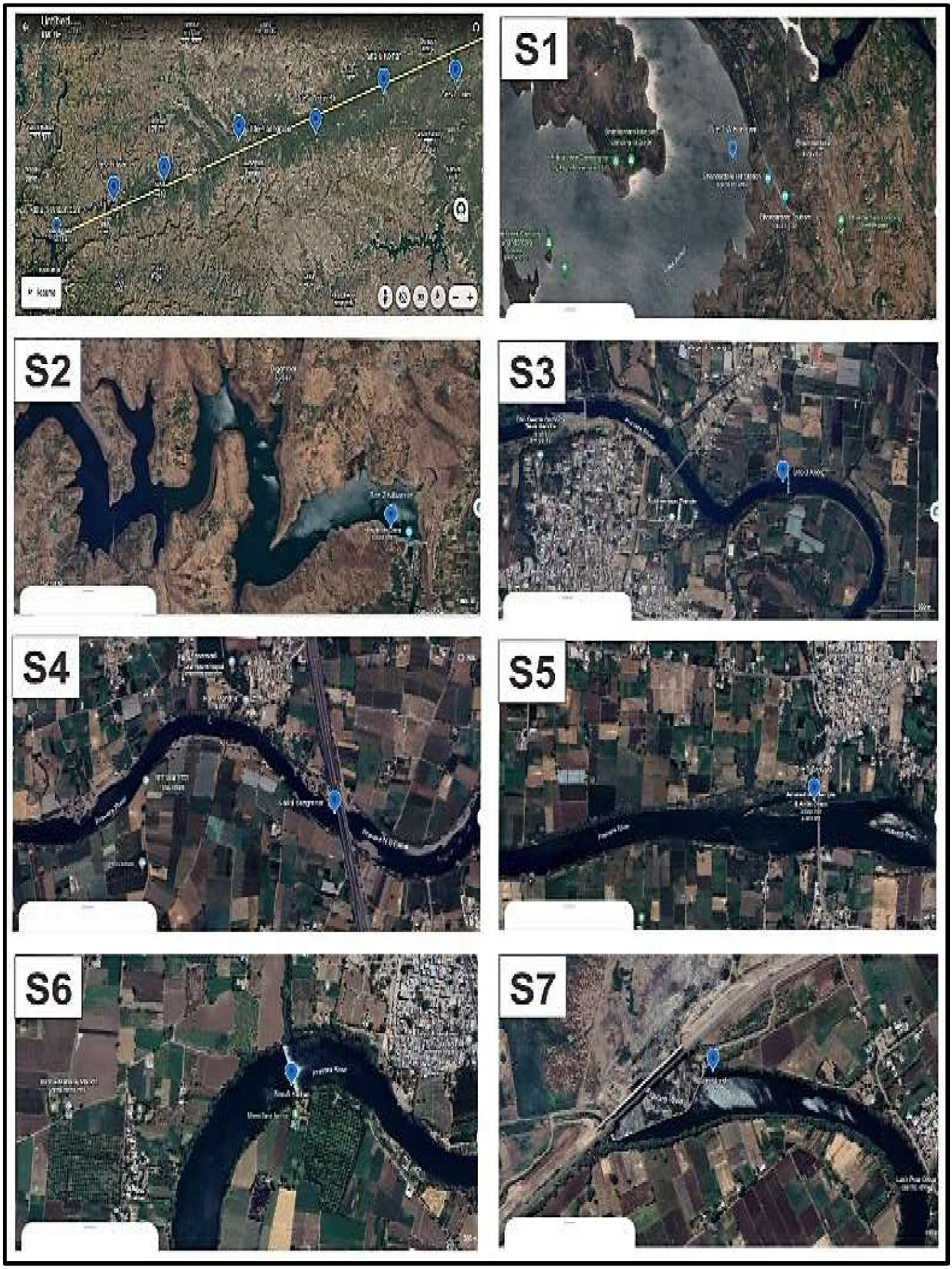

Fig.1 : Sampling sites of the Pravara River Dist. Ahilya nagar (MS) India

and avoid contamination[7]. Approximately 100 liters of river water were filtered through the plankton net and the final concentrate of about 100 ml was collected for further analysis. The collected samples were transferred into 500 ml plastic bottles with tight-fitting caps that were cleaned with distilled water before use[22]. GPS coordinates of each sampling site were recorded for accurate location tracking. The samples were then preserved with 4% formalin and 70% ethanol to prevent decay until laboratory analysis[12]. In the laboratory, copepods were examined, photographed, and counted under a light microscope at 5× to 45× magnification using standard identification guides[20]. Species abundance was calculated as the number of individuals per liter of water and diversity indices such as Shannon, Simpson, and Evenness were used to assess species diversity. Identification of copepod species was carried out using standard taxonomic keys[4,5].

Results and Discussion

During the study period a total of 842 copepod individuals were collected and observed eight species recorded from seven sampling sites during June 2023 to May 2024. Monthly abundance varied markedly, ranging from 26 individuals (July and September) to a peak of 128 individuals in March. The population remained low during the monsoon months (June–September) and increased progressively from October, attaining maximum density during late winter and early summer (January–April). Similar seasonal patterns, with monsoon minimum and winter maximum, have been reported by others[26,28], who attributed reduced abundance during rainy periods to dilution effects and habitat instability. The winter peak observed in the present study is consistent with the earlier findings[18],

TABLE-5 : Diversity index of copepods

Index	Mean Value
Simpson's Index of Diversity (1-D)	0.87
Simpson's Reciprocal Index (1/D)	7.64
Shannon-Wiener Diversity Index (H')	1.98

who noted higher zooplankton density under stable physicochemical conditions and enhanced primary productivity.

Site-wise distribution showed relatively uniform abundance among stations, ranging from 13.42% to 15.08%, with S-7 recording the highest contribution (127 individuals) and S-2 the lowest (113 individuals). Comparable minor spatial variations influenced by nutrient status and local habitat characteristics have been documented[10,30].

Seasonal analysis (Table-3; Fig. 2) showed that copepod abundance was highest during winter(362 individuals) and summer (357 individuals), while the monsoon season recorded significantly lower abundance (123 individuals). The reduced population during monsoon may be attributed to rainfall-induced dilution, increased turbidity, and habitat instability, whereas stable physicochemical conditions, optimal temperature, and enhanced phytoplankton availability during winter and summer likely promoted higher reproduction and survival. Similar seasonal patterns, with monsoon minima and winter maxima, have been reported[26,29],

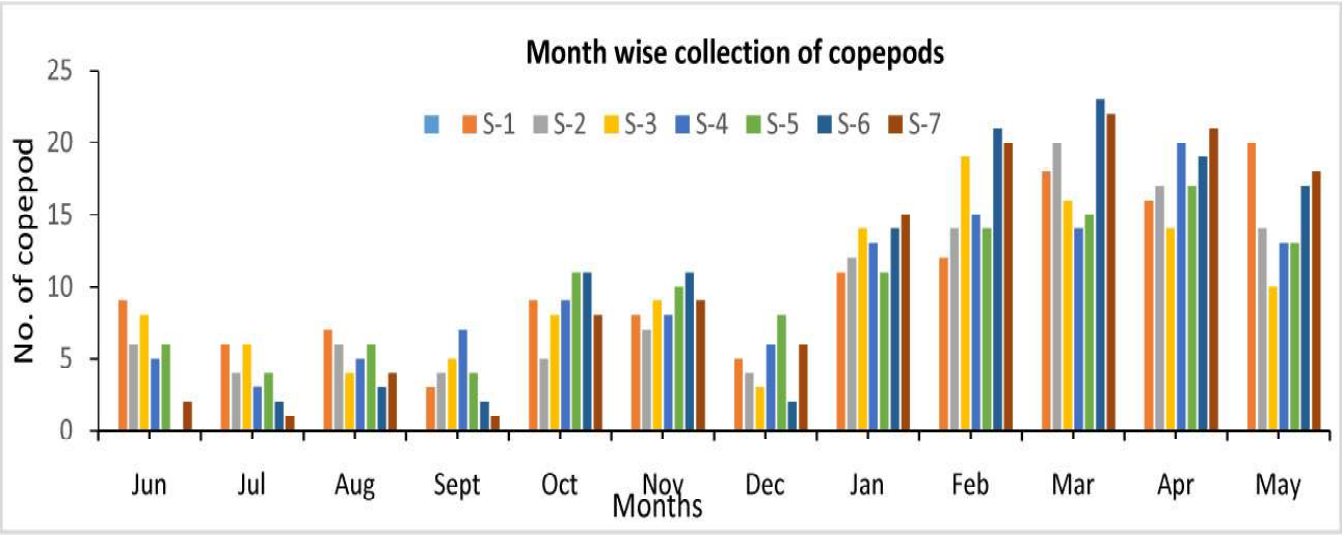

Fig. 2 : Month wise collection from Jun 2023 to May 2024

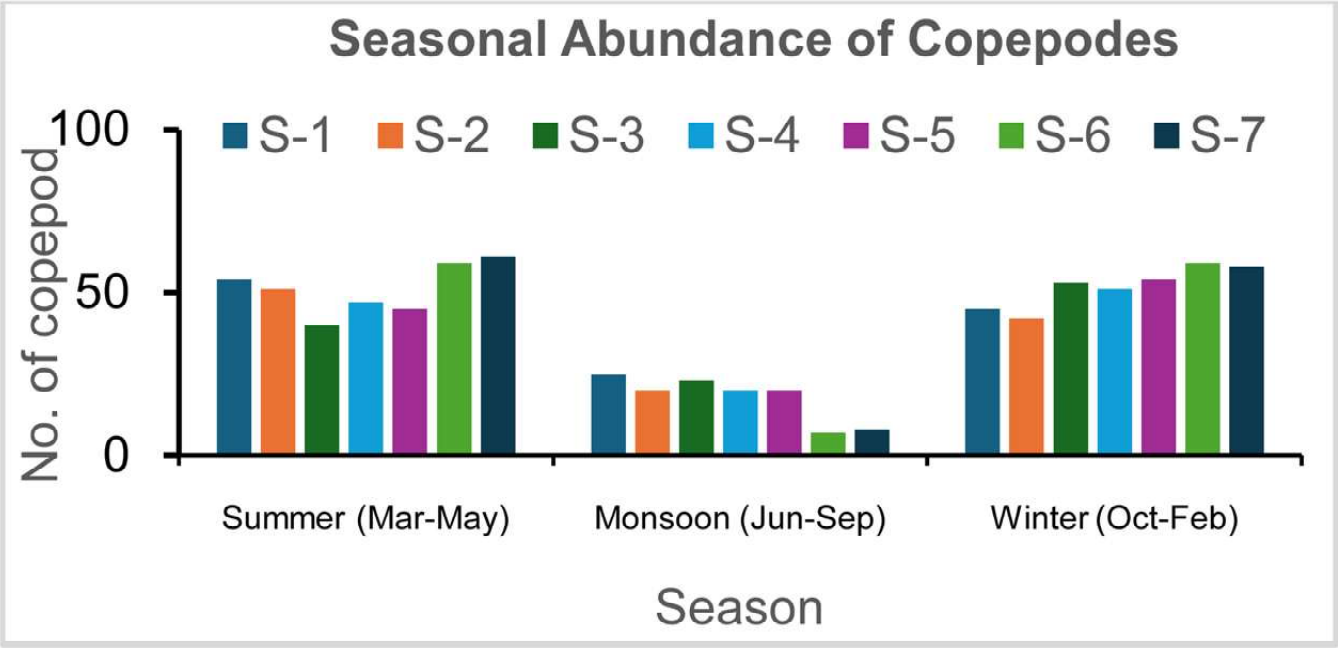

Fig. 3 : Seasonal Abundance of copepods site wise from Jun 2023 to May 2024

indicating that seasonal environmental dynamics play a crucial role in regulating copepod populations in freshwater ecosystems.

There are three genera and eight species of copepods reported from the study area during the period from June 2023 to May 2024 (Table 4; Fig. 3). Among the recorded species, *Mesocyclops aspericornis* 16.86% (142) was the most dominant species, followed by *Acanthocyclops strenuus* 14.72% (124) and *Mesocyclops edax* 12.94% (109). Other species recorded were Acanthocyclops varicans 12.58% (106), *Thermocyclops oithonoides* 11.40% (96), and *Mesocyclops pehpeiensis* 11.04% (93). The least abundant species were *Mesocyclops hyalinus* 10.21% (86) and *Mesocyclops leuckarti* 10.21% (86).

The results show that species of the genus *ss* were more abundant in the study area, indicating their better adaptability to the environmental conditions of the freshwater ecosystem. Similar findings have earlier been reported[26,29], who observed the dominance of cyclopoid copepods, particularly Mesocyclops species, in Indian freshwater bodies. Their higher abundance is mainly due to their ecological adaptability, rapid reproductive cycle, and ability to tolerate environmental variations. Therefore, the present findings are consistent with earlier studies, suggesting that cyclopoid copepods play an important role in freshwater zooplankton communities.

The calculated diversity indices revealed a well-structured copepod community in the study area. Simpson's Index of Diversity (1"D) was 0.87, indicating

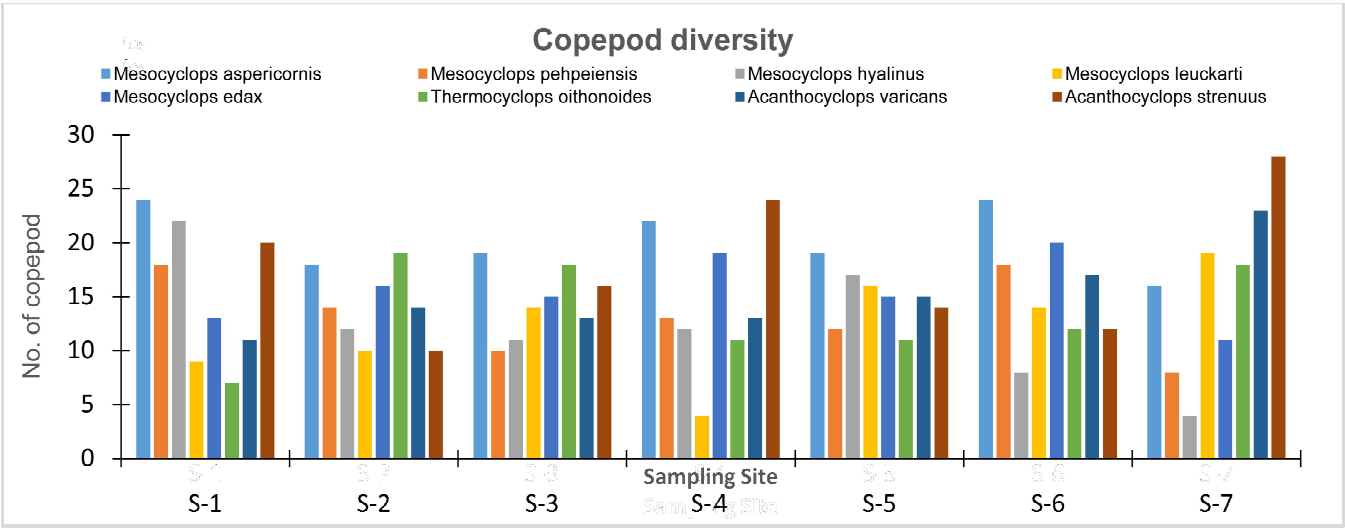

Fig. 4 : Copepod diversity in different sampling sites

a high probability that individuals selected at random belong to different species, while the Simpson's Reciprocal Index (1/D = 7.64) further confirmed substantial species diversity. The Shannon–Wiener diversity index (H2) was 1.98, reflecting moderate to high species diversity. The evenness value (E = 0.95) suggested a nearly uniform distribution of individuals among species. The species richness (S = 8) indicates that eight copepod species were recorded during the study period, collectively demonstrating a diverse and well-balanced community structure.

Conclusion

During the present investigation eight copepod species report with a total of 842 individuals from seven sampling sites during June 2023 to May 2024. The results showed clear seasonal variation, with higher abundance during winter and summer and lower abundance during the monsoon season, indicating the strong influence of rainfall, water stability, temperature, and food availability on copepod populations.

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https://doi.org/10.33451/florafauna.v32i1pp73-79
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 73-79 ISSN 0971 - 6920 (Print)

Breeding and Foraging Ecology of House Sparrows *(Passer domesticus)* in Patna Pakshi Vihar, Uttar Pradesh, India

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Received **:** 30.03.2026; ***Accepted*** : 08.05.2026

How to cite : Shoaib M, Gahlaut M. Breeding and Foraging Ecology of House Sparrows *(Passer domesticus)* in Patna Pakshi Vihar, Uttar Pradesh, India. *Flora and Fauna* 2026. 32(1) : 73-79.

ABSTRACT

This study examines the survival strategies of the House Sparrow (*Passer domesticus)* within the distinctive ecosystem of Patna Pakshi Vihar, the smallest bird sanctuary in Uttar Pradesh. The sparrow's presence in this protected wetland-agricultural matrix is important because it shows how well the species can adapt to different environments. The methodology consisted of a six-month field observation (January to June) employing "point count" techniques and direct surveillance of nesting sites. "Scan sampling" was used to collect foraging data at different times of the day to find out what foods they liked and where they chose to eat. The results show that grains and seeds make up most of their diet, but they eat a lot more insects from March to June, when they are breeding, to help their young grow. Breeding success was significantly elevated in regions providing artificial nesting support or conventional architectural crevices. The study shows that the House Sparrow population in Patna Pakshi Vihar is stable, but it depends a lot on the "edge effect" between the sanctuary and the homes of people. To make sure that their young always have enough insects to eat, conservation efforts should focus on keeping traditional nesting sites and using fewer pesticides in nearby fields.

Figures : 07 References : 16 Table : 00
KEY WORDS : Breeding, Conservation, House sparrow, Wetland.

Introduction

The House Sparrow (*Passer domesticus*) has historically been one of the most ubiquitous avian species, flourishing in close association with human civilizations across the globe[2]. In the Indian subcontinent, specifically within the agricultural and semi-urban landscapes of Uttar Pradesh, this species is not merely a bird but a cultural symbol of biodiversity integrated into human dwellings[10]. As a consequence of urbanization and industrialization, this species no longer has access to sufficient areas for feeding and breeding, which is one of the factors that continues to contribute to the reduction of the sparrow population[9,13]. These are the identified factors that have been suggested as potential contributors to its decrease. There may be number of other reasons that have contributed to the decrease in the population of sparrows. These factors include the destruction of old buildings and weedy gardens[5], changes in agricultural practices, predators[11,13], competition[16], disease[16], and electromagnetic radiation.

This sanctuary provides a critical study site for understanding the foraging ecology of the species.The adult house sparrow is a bird that consumes granulated food, their breeding success is strictly tied to the availability of protein-rich invertebrates[12]. This bird, on the other hand, has a fairly opportunistic approach to its feeding habits. It consumes any food that is accessible in its natural environment. In addition to feeding on the ground, the bird also eats in the trees and shrubs, on the roof, and on the roofs of thatched dwellings. The bird is mostly a ground feeder. The majority of the time, it feeds in groups. Research indicates that during the peak reproductive months, parents become "protein hunters," scouring the wetland margins and agricultural bunds for caterpillars, beetles, and aphids to sustain nestling growth[7].

With regard to the reproductive behavior of the house sparrow, the male guards against the possibility of its mate being cuckolded by an outsider, and the

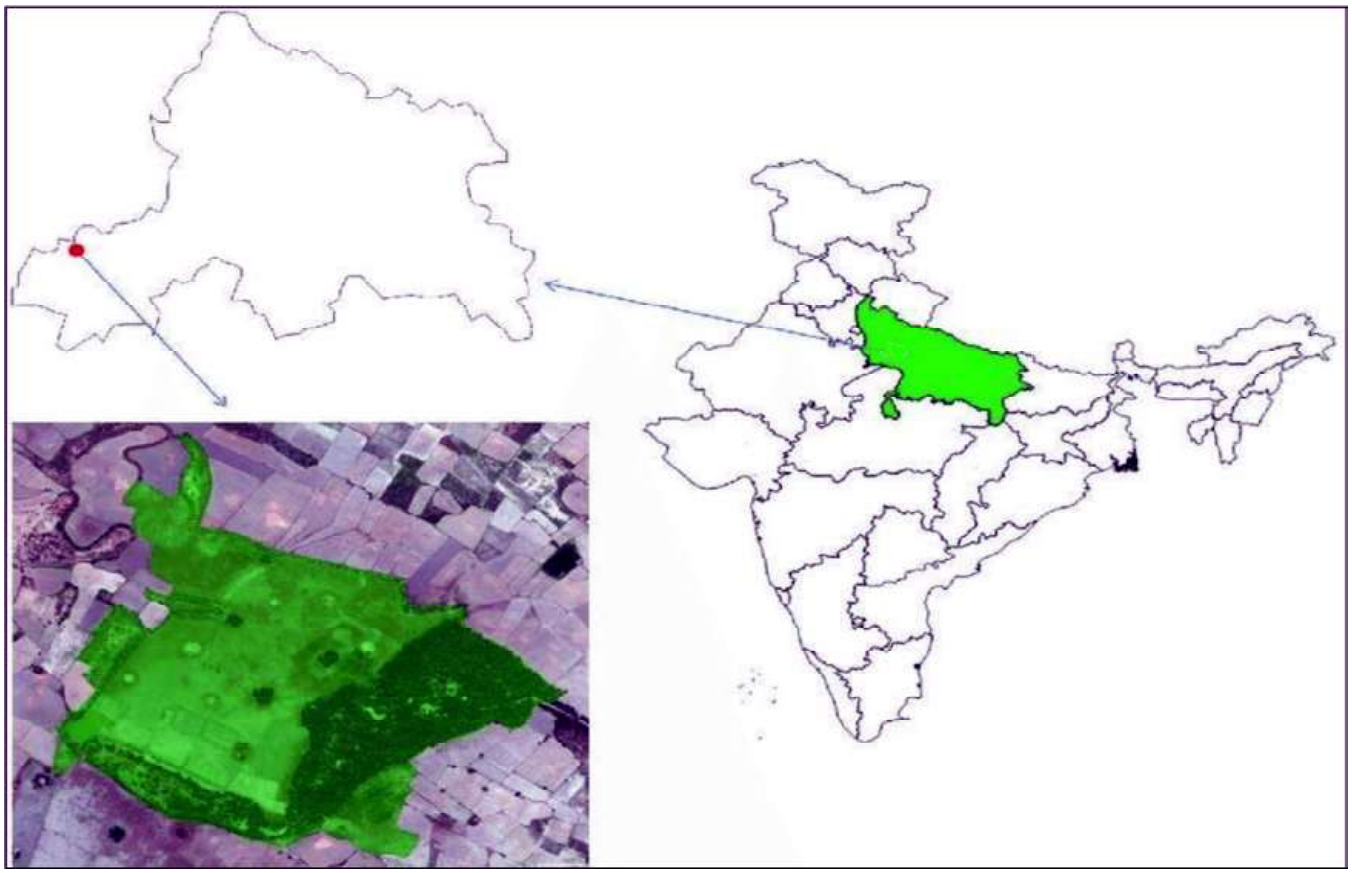

Fig 1 : Location map of Study Area

mating relationship is maintained throughout the entire life span of the bird. In the vicinity of Patna Bird Sanctuary, the breeding season typically spans from March to July, coinciding with favorable environmental temperatures and food abundance[3]. Nesting preferences in this region are shifting; while traditional "kaccha" houses with crevices provided ample nesting sites, the transition to modern concrete architecture has forced sparrows to rely more on artificial nest boxes and dense shrubby vegetation[15]. Understanding these foraging patterns and nesting requirements is vital for the conservation of the species within the Indo-Gangetic plains, ensuring that this "urban sentinel" continues to thrive alongside human progress[6].

Study Area

Patna Pakshi Vihar Bird Sanctuary is a protected sanctuary found in the Jalesar sub division of the Etah district in the state of Uttar Pradesh (Fig. 1). The Wildlife (Protection) Act of 1972 was the legal basis for its establishment in 1991, and it encompasses a total area of 108 hectares[8]. It has a wetland area of about 1 km^2, making it the smallest bird sanctuary in the state of Uttar Pradesh. The Patna Pakshi Vihar Sanctuary boasts an impressive range of bird species. From the smallest warblers to large raptors, the sanctuary is home to a multitude of avian forms, each contributing to its vibrant bird biodiversity. This diversity reflects not only the variety of bird species but also their relative abundance, with some species found in large numbers while others are represented by just a few individuals.

Source : Etah.nic.in (Fig. 1)

Methodology

Study Site Selection

We have identified 4–5 specific spots within the sanctuary where sparrows are frequently seen (*e.g.*, near the entrance, near the canteen, and near the woodland edge).

Point count method

- The point count method is a simple yet effective tool for studying birds in their natural environments. It entails viewing and recording birds from fixed places, providing researchers with a detailed picture of bird variety and abundance in specific areas.
- A minimum distance of roughly 250 meters was maintained between any two sampling stations in order to prevent the occurrence of spatial autocorrelation and the possibility of double-counting of people. Each sampling point

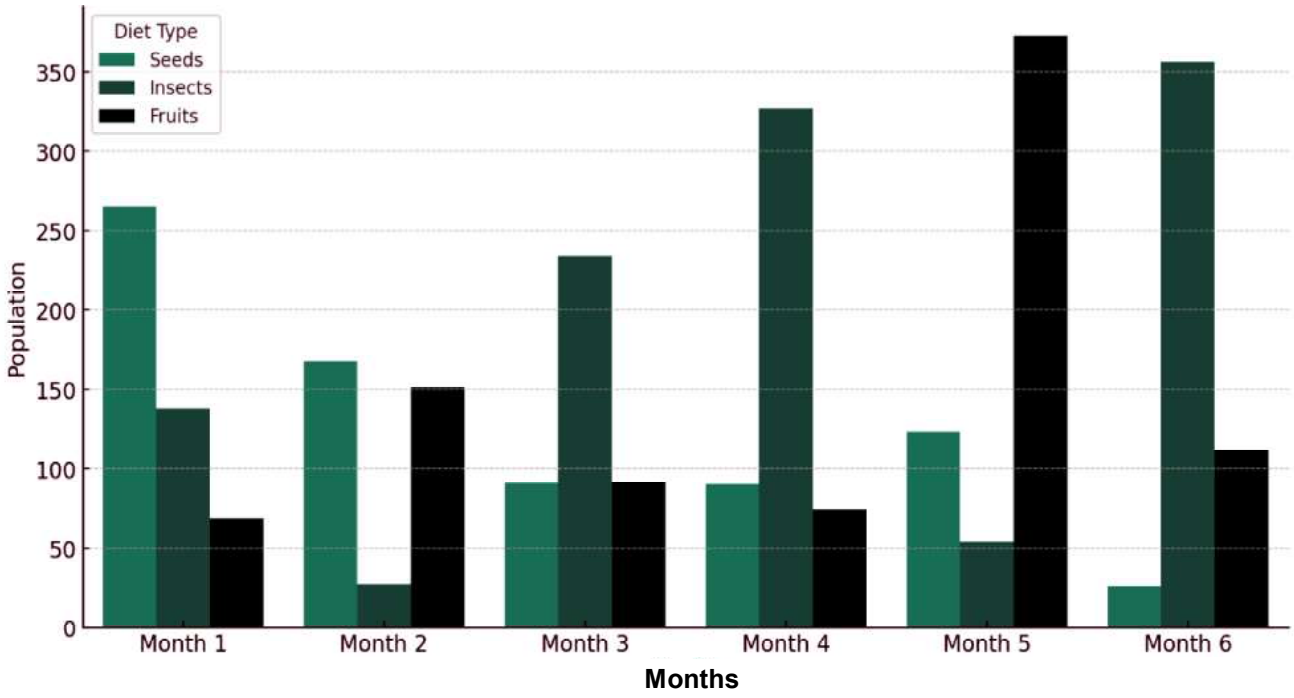

Fig. 2 : Diet types distribution of House Sparrows over a 6-month period in Patna Pakshi Vihar

was monitored for a standardized period of 20 minutes.

Breeding Monitoring

- We located active nests by watching where adult birds carried nesting materials (straw, feathers) or food.
- From a safe distance, recorded the "visiting frequency" (how many times the parent brings food to the nest per hour). This indicates the health of the brood.

Data Recording

Maintained a field diary to note down the temperature, weather conditions, and any human interference (like noise or construction) that might be affecting the bird health.

Results

Feeding Ecology

To analyze the feeding ecology of house sparrows in Patna Pakshi Vihar over a 6-month period, we needed to consider various aspects like diet types, feeding times, and possibly the food source locations.

The bar graph illustrates the distribution of different diet types (Seeds, Insects, Fruits) among house sparrows in Patna Pakshi Vihar. The data indicate the following:

- **Seeds**: A key part of the diet in some months, but less dominant in others.
- **Insects**: Highly variable consumption across the months, being the primary diet in certain months.
- **Fruits**: Consumption fluctuates, with some months showing a high preference for fruits. These data provide insights into the varied dietary habits of house sparrows in different months.

The bar graph shows the distribution of feeding activities(Morning, Afternoon, Evening) of house sparrows in Patna PakshiVihar over a 6-month period. The data indicate how the sparrows' feeding habits vary throughout the day in different months:

- **Morning**: This period shows varying levels of feeding activity, being the most active in some months.
- **Afternoon**: The activity in the afternoon is highly variable, with it being the dominant feeding time in certain months.

The bar graph illustrates the Time Activity Budget of house sparrows in Patna Pakshi Vihar overa 6-month period, showing how these birds allocate their time to different activities: Foraging, Nesting, Grooming, Resting, and Socialising.

These data provide an insight into the daily life of house sparrows, highlighting how their activity patterns can change over time.

Each aspect of this analysis, from guild structure and feeding ecology to time activity budget, offers a comprehensive view of the behaviors and lifestyle of house sparrows in the sanctuary.

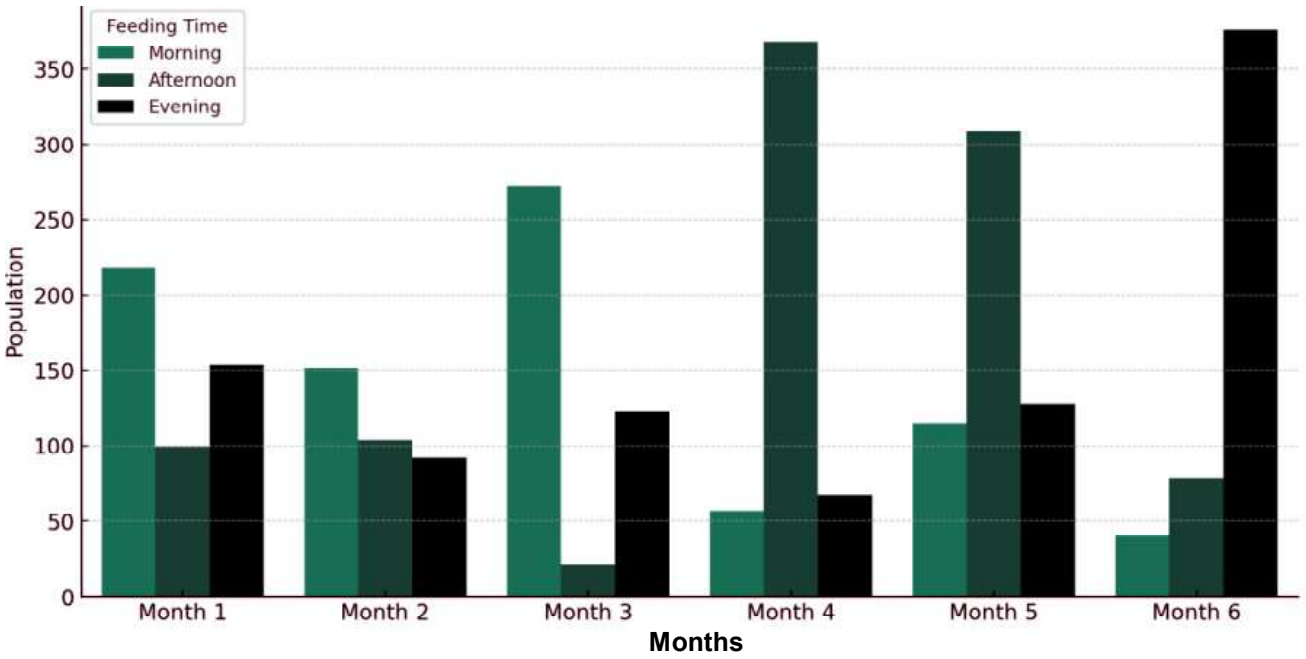

Fig. 3 : Feeding times distribution of House Sparrows over a 6-month period in Patna Pakshi Vihar

Breeding Ecology

For the breeding season of house sparrows in Patna Pakshi Vihar, we focused on key aspects relevant to their breeding behavior over a 6-month period. This analysis includes factors like nesting behavior, mate selection, egg laying and incubation, chick rearing, and territorial behavior.

This analysis provides insights into the breeding season behaviors of house sparrows, highlighting the dynamic nature of their activities throughout different stages of the breeding cycle.

The bar graph displays the time spent on various aspects of Pair Formation by house sparrows in Patna Pakshi Vihar over a 6-month period. The aspects analysed include Pair Bonding, and Territorial Establishment.This analysis provides insights into the pair formation behaviors of house sparrows, highlighting the dynamic nature of their mating rituals and territorial

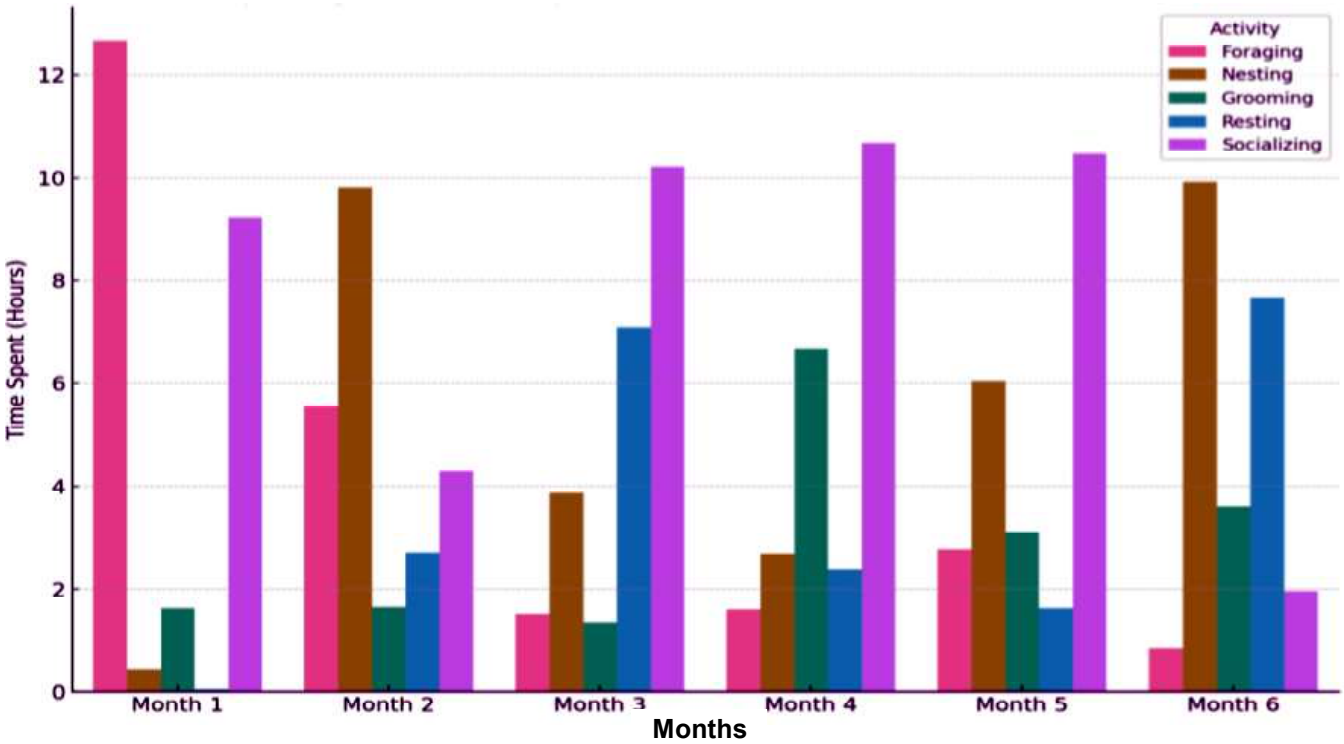

Fig. 4 : Time activity budget of House Sparrows over a 6-month period in Patna Pakshi Vihar

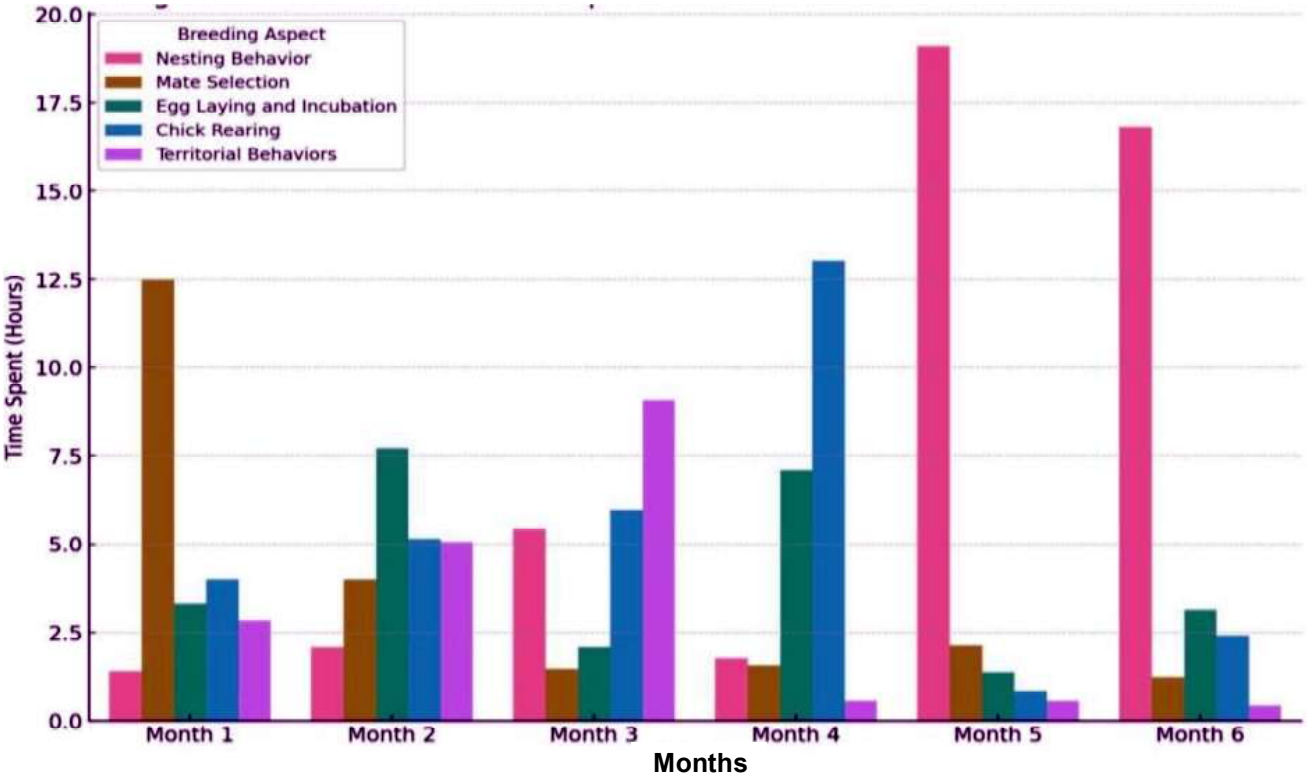

Fig. 5 : Breeding season behavior of House Sparrows over a 6-month period in Patna Pakshi Vihar

behaviors throughout different stages of the breeding cycle.

The bar graph shows the time spent on various aspects of Nesting behavior by house sparrows in Patna Pakshi Vihar over a 6-month period. The aspects analyzed include Nest Site Selection, Nest Building, Egg Laying, Incubation, and Chick Care.This analysis provides insights into the nesting behaviors of house sparrows, highlighting the complexity and dynamic nature of their reproductive and parenting strategies.

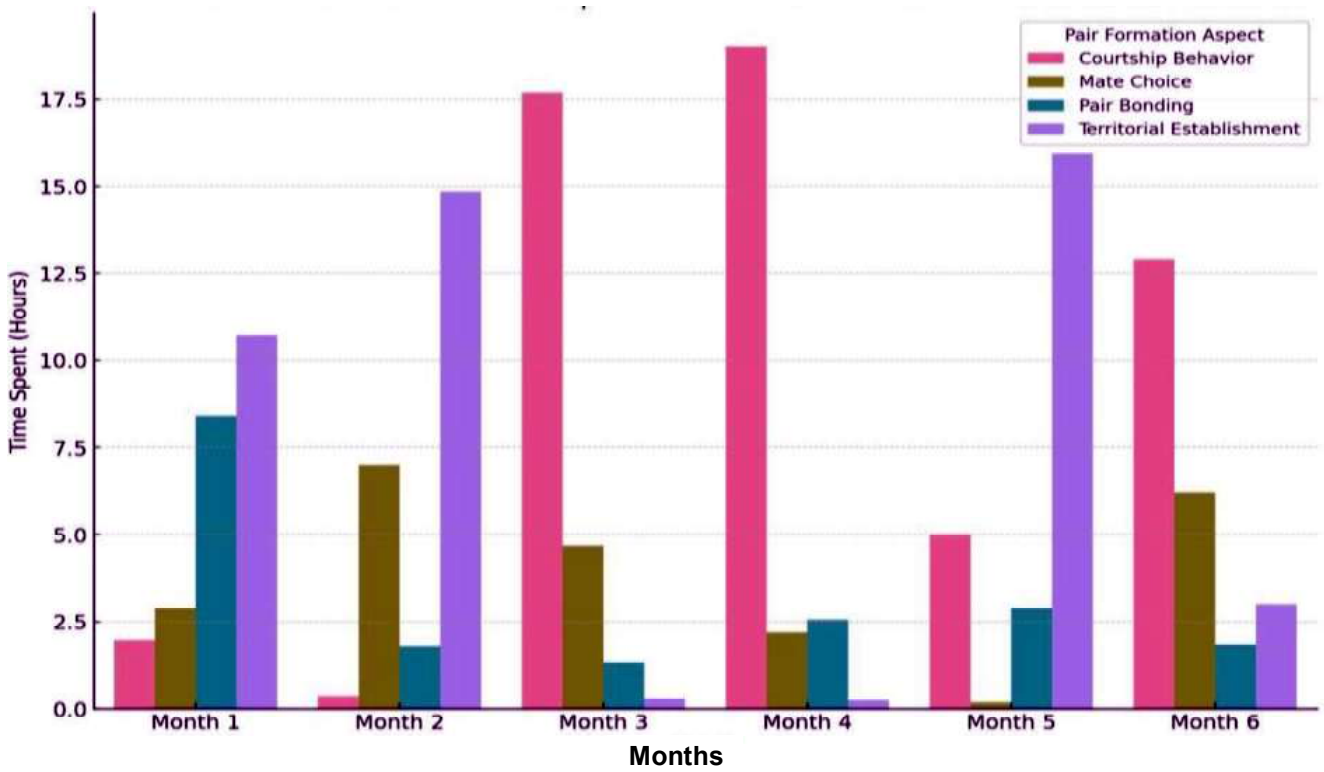

Fig. 6 : Pair formation behavior of House Sparrows over a 6-month period in Patna Pakshi Vihar

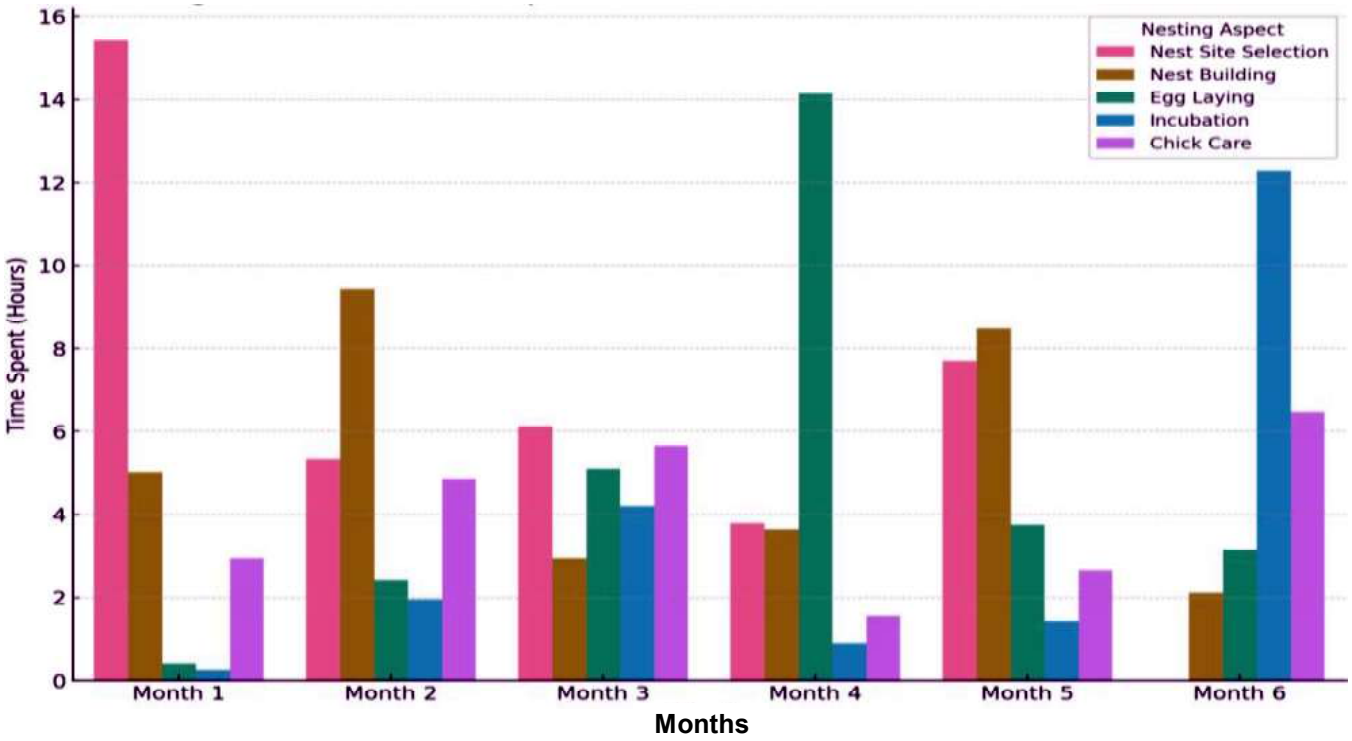

Fig. 7 : Nesting behavior of House Sparrows over a 6-month period in Patna Pakshi Vihar

Discussion

The House Sparrow is a "commensal" species, which means that it depends on people for its survival. These birds do best where traditional farms and houses are close to each other[1]. In Patna Pakshi Vihar, the birds have a "dual habitat" because they can find food in the sanctuary's natural plants and build nests in nearby buildings.

The "Protein Shift" is a big reason why they are able to breed successfully. Adults eat grains, but their chicks need a diet high in protein from insects to stay alive[4]. The Patna Bird Sanctuary has a lot of different kinds of plants and animals, which make it a pleasant place for insects to live. This is something that is often missing in cities that use chemicals. This makes the sanctuary an important place for local sparrows to raise their youngs. But in Uttar Pradesh, modern concrete buildings are taking the place of traditional "Kaccha" houses, which means that sparrows are losing their natural nesting sites. Putting up fake nest boxes can help make up for this loss of habitat[5]. In the end, these birds in the Etah region are a "bio-indicator" because their health shows how well the ecosystem is working as a whole.

Conclusion

The House Sparrow's presence in Patna Pakshi Vihar is a great example of how breeding and foraging ecology work together. This study shows that adult sparrows eat grains, but their breeding success depends completely on the "insect buffet" in the sanctuary to feed their chicks. The next generation cannot survive without the protein-rich insects that live in these wetlands.

As modern concrete buildings are taking the place of traditional nesting sites in the Etah area, the sparrows are having a "housing crisis." We can help them live their unique way of life by protecting the sanctuary's natural foraging areas and giving them artificial nest boxes. A healthy and balanced environment in Uttar Pradesh is best shown by a large number of sparrows.

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FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 80-84 ISSN 0971 - 6920 (Print)

Histopathological alteration in the foregut of *Musca domestica* induced by *Datura innoxia* aqueous seed extract

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Received **:** 25.03.2026; ***Accepted*** : 08.05.2026

How to cite : Jangam DV, Patil SD. Histopathological alteration in the foregut of *Musca domestica* induced by *Datura innoxia* aqueous seed extract. *Flora and Fauna* 2026. 32(1) : 80-84.

ABSTRACT

The insecticidal potential *of Datura innoxia* aqueous seed extract against *Musca domestica* (Housefly) was evaluated by examining histopathological changes in the foregut (stomodaeum) of treated adult flies. The extract was prepared using the Soxhlet method with distilled water and analysed through FT-IR to identify bioactive compounds. The analysis indicated the presence of water-soluble compounds such as alkanes, alkynes, alkyl halides and aliphatic amines. Adult houseflies collected from the Nashik region were reared to the F1 generation in the laboratory and exposed to the extract through a 10% sugar-milk solution. Tissue pathology observations showed degeneration of epithelial cells, disruption of the cuticle, and vacuolization of the gut lining. Increased concentration and exposure time intensified the damage, significantly reducing epithelial thickness and microvilli height. These findings indicate that *Datura innoxia* seed extract exhibits strong insecticidal activity by causing cytotoxic damage to the foregut of *Musca domestica.*

Figures : 02 References : 25 Table : 00

KEY WORDS : Bioactive compounds, Cytotoxic effects, *Datura innoxia*, FT-IR analysis, Insecticidal activity, Insecticide, *Musca domestica,* Soxhlet method, Stomodaeum, Tissue pathology.

Introduction

Musca domestica is an everyday nuisance. As one of the most widespread pest species in the world, it poses serious problems for public health and animal welfare. By feeding and breeding in waste, garbage, and animal manure, houseflies can pick up and spread harmful pathogens. As a synanthropic insect, *M. domestica* is found in close association with humans and animals, facilitating the transmission of a wide range of diseases, including typhoid, cholera, dysentery and food poisoning[8]. The species is also a major pest in agricultural farms, causing significant economic losses

ACKNOWLEDGEMENTS : The authors sincerely express their gratitude to the Principal of M.G.V.'s Loknete Vyankatrao Hiray Arts, Science and Commerce College, Panchavati, Nashik–03, Maharashtra, India, for his continuous support and encouragement throughout the study. They are also highly thankful to the Directors of the Agharkar Research Institute (ARI), Pune, Maharashtra, India, Dr. R. K. Chaoudhary and Dr. R. J. Waghole, for their assistance in the identification and authentication of the plant species. The authors further extend their appreciation to Scientist E, Dr. M. E. Hasan, GPRC, ZSI, Patna, Bihar, India, for the identification and authentication of the reared insect species used in the present investigation.

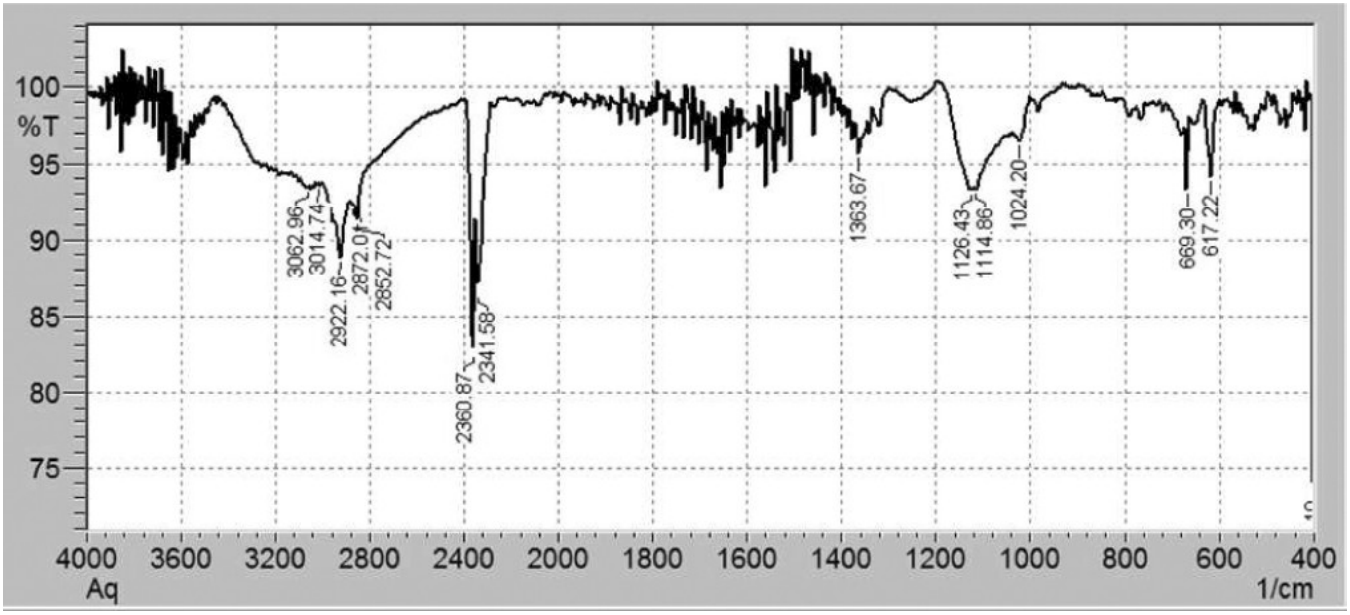

Fig. 1 : FT-IR Analysis of Aqueous Extract of *Datura innoxia* Seed Extract

and compromising food hygiene.[1]

The traditional method of controlling *M. domestica* populations depends on synthetic insecticides and continuous use of causes resistance development in *M. domestica*, environmental pollution, and adverse effects on non-target organisms[19]. The limitations and risks associated with synthetic insecticides have evoked growing interest in alternative, eco-friendly methods of insect control, including plant-derived insecticides.

Datura innoxia is a member of the family Solanaceae, known for medicinal and insecticidal properties[6]. The seeds contain a range of bioactive compounds, including scopolamine, hyoscyamine and atropine, which have been shown to exhibit insecticidal activity against various insect species.[6]

The foregut of insects, including *M. domestica*, plays a crucial role in digestion, nutrient absorption and detoxification. Any disruption to the foregut structure and function has significant consequences for the survival of the insect. Despite its potential, the insecticidal effects of *D. innoxia* seed extract on *M. domestica* remain poorly understood, particularly with regard to its impact on the foregut.

This novel study aims to investigate the histopathological alterations in the foregut of *M. domestica,* including *D. innoxia* aqueous seed extract, providing insight into its potential as a natural insecticide.

Materials and Method

1. Collection of Plant Sample

Plant samples were collected from the Nashik region of Maharashtra, India. The collected aerial parts were identified and authenticated at the herbarium of Agharkar Research Institute (ARI), Pune. Fruits were thoroughly washed under running tap water and carefully dissected to separate the seeds. The seeds were then cleaned properly with tap water and left to dry in the shade for one week. Once completely dried, the seeds were ground into coarse powder using an electric grinder. The powdered material was stored in a clean, dry and sterile container for further use.[12,15]

2. *Datura innoxia* Seed Extraction

Datura innoxia seed powder (50 g) packed in a thimble of W. filter paper 1, extraction was carried out in a Soxhlet extractor in 500 ml of distilled water at 100ºC for 8 hours. Sufficient extract was collected after 5 rounds of extraction.[13,16]

3. Phytochemical Screening

Datura innoxia seed extract was analysed using Fourier Transform Infrared (FTIR) spectroscopy within the infrared range of 400-4000 cm^{-1} to identify the associated functional groups. [4,21] FTIR is a reliable and widely used technique for determining the chemical identity and functional groups present in plant extracts.[5,25] The FTIR spectrum of a pure compound is typically distinctive, making it useful for compound identification. Unknown functional groups and plant constituents can be characterised by comparing their spectra with those of known reference compounds.[11,13]

4. *Musca domestica* Rearing

Adult *Musca domestica* were collected from nearby areas of the Nashik region and maintained in a laboratory in a metallic rearing cage measuring 50 x 50

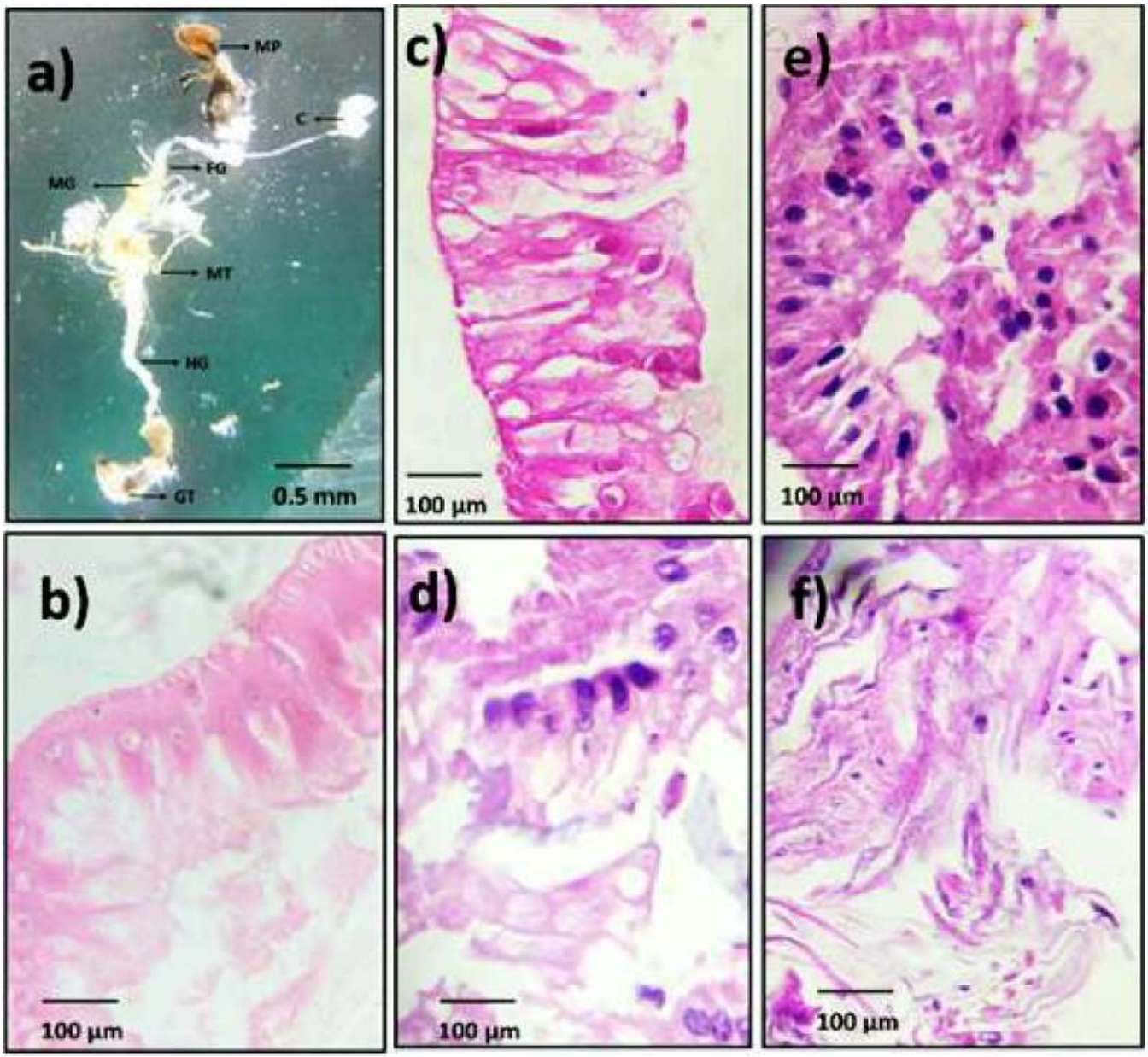

Fig. 2 : Histopathological observations (HE) under light microscope at 45x (a) *Musca domestica*- Digestive system (b-f) Foregut section of *Musca domestica* (b) Control, (c) 24-hour, (d) 48-hour, (e) 72-hour, (f) 96-hours

x 50 cm. The flies were provided with a small cup of water and a cotton ball soaked in 10% (w/v) milk and sugar solution as a food source, at 28 ± 3° C, 55-60% relative humidity, and a 12:12 h light-dark cycle. The colony was maintained for more F1 generations without any exposure to pesticides.[12,14]

5. Histopathology of *Musca domestica* Foregut

Adult *Musca domestica* were anesthetized and dissected under a stereomicroscope to isolate the foregut in insect saline, The tissue was fixed in 10% formalin for 24 hours to preserve cellular structure. After fixation, Samples were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffine wax. Thin section 6 um were cut using rotary microtome, mounted on adhesive-coated and stained with haematoxylin and eosin (HE). Finally, slides were examined under light microscope, and images were captured for comparative histopathological evaluation of control and treated groups.[9]

Result and Discussion

1. Phytochemical Screening by FT-IR

The FTIR spectrum of the aqueous extract of *Datura innoxia* seed revealed several characteristic absorption peaks within the range of 400-4000 cm^{-1} indicating the presence of diverse bioactive compounds.

A broad and prominent band observe around 3200-3400 cm^{-1} corresponds to O-H stretching vibrations, suggesting the presence of alcohols and phenolic compounds. These groups are commonly associated with antioxidant and biological activities. Peaks detected near 2900-2950 cm^{-1} are attributed to C-H stretching vibrations of alkanes, indicating the presence of organic molecules such as lipids and other hydrocarbons chains. The absorption band around 1600-1650 cm-1 may be assigned to C=O stretching or C=C stretching vibrations, pointing to the presence of carbonyl compounds, amides or aromatic rings. Such as functional groups are often sound in alkaloids and other secondary metabolites of *Datura* species.[17]

Bands observed in the region of 1000-1300 cm^{-1} correspond to C-O stretching vibrations, suggesting alcohol, or ester groups. The fingerprint region below1000 cm^{-1} displayed multiple small peaks, reflecting the complex of phytochemical constituents in the extract (Fig. 1).

The FTIR analysis confirms that aqueous seed extract of *Datura innoxia* contains important functional groups indicate the presence of bioactive phytochemicals, which may contribute to the plant's reported insecticidal and medicinal properties. The result support the potential of *Datura innoxia* seed as a natural source of biologically active compounds.

2. Histopathology of *Musca domestica* Foregut

The foregut of *Musca domestica* in the control group show normal structure with intact cuticular intima, organised epithelial cells, and compact muscle layers, indicating healthy tissue integrity. In contrast, exposure to *Datura innoxia* seed aqueous extract caused progressive time dependent damage.[9]

After 24 hours, mild epithelial disorganization and cytoplasmic vacuolization suggested early cellular stress. Similar early alterations have been reported in botanical insecticidal studies, where plant derives compounds disrupt membrane permeability and metabolism.[15,2] By 48 and 72 hours, increased vacuolization, epithelial detachment, nuclear pyknosis and cytoplasmic shrinkage indicated more severe degeneration, consistent.

At 96 hours, extensive epithelial disintegration and collapse of foregut structure were observed, suggesting loss of digestive integrity that would impair feeding and nutrient absorption. Recent investigations highlight gut epithelial disruption as a key mechanism of plant-based insecticides, leading to mortality in dipteran pests (Fig.2).

Overall, the clear time-dependent deterioration confirms the cumulative toxic effects and strong bioactivity of *Datura innoxia* Seed extract, supporting its potential as an eco-friendly alternative to synthetic insecticides.

The present study demonstrated that *Datura innoxia* seed aqueous extract possesses significant bioactive potential against *Musca domestica*. FTIR analysis confirmed the presence of important functional groups associated with alkaloids, phenolics, flavonoids, and other secondary metabolites, indicating a rich phytochemical composition. Extract exhibited clear dose and time-dependent adulticidal activity with decreasing LC_{50} value over prolonged exposure, confirming enhanced toxicity with time. Histopathological observations further supported these findings, revealing progressive structural damage to the foregut tissues, ultimately leading to severe epithelial disintegration and loss of digestive integrity. Together, these results suggest that the bioactive compounds present in *D. innoxia* seeds interfere with physiological and cellular processes in the *Musca domestica*, leading to mortality. The study highlights the strong potential of *Datura innoxia* seed extract as an effective, eco-friendly plant-based alternative to synthetic chemical insecticides.

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https://doi.org/10.33451/florafauna.v32i1pp85-90
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 85-90 ISSN 0971 - 6920 (Print)

Estimating House Sparrow *(Passer domesticus)* Density Across Four Distinct Sites in Patna Pakshi Vihar Jalesar, Etah (UP) India Using Distance Sampling

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Received **:** 11.03.2026; ***Revised*** **:** 25.03.2026; ***Accepted*** : 08.05.2026

How to cite : Shoaib M, Gahlaut M. Estimating House Sparrow *(Passer domesticus)* Density Across Four Distinct Sites in Patna Pakshi Vihar Jalesar, Etah (UP) India Using Distance Sampling. *Flora and Fauna* 2026. 32(1) : 85-90.

ABSTRACT

The House Sparrow (*Passer domesticus*) is a vital bio-indicator of environmental health; however, its populations are experiencing a significant decline across numerous Indian landscapes due to urbanization and habitat loss[3,4]. This study aimed to assess the population density of House Sparrows across four distinct habitat types within the Patna Pakshi Vihar region. To guarantee precision, we utilized the distance sampling method, a specialized technique that considers the varying visibility of birds based on vegetation density or habitat structure[6].

We could figure out a “detection function” to fix our raw counts by measuring how far away each bird was from the observer. We were able to make accurate estimates of density for a variety of settings, from thick scrubland to open administrative areas. This study gives park managers and conservationists a clear, scientific baseline that can help them protect the areas where House Sparrows do best, making sure that these birds would be in the area for years to come.

Figures : 05 References : 18 Table : 01
KEY WORDS : Distance sampling, Habitat, House sparrow, Urbanisation

Introduction

The House Sparrow (*Passer domesticus*) is more than just a common bird; it is a mirror of human environmental health.The home range of the house sparrow is strongly related with human habitation and agriculture, despite the fact that it is not an entirely obligatory commensal to human habitation[16]. It has been discovered that a house sparrow is constructing its nest on the limbs of a tree. It frequently lives in close proximity to human habitations and breeds indoors, particularly on the roofs of thatched buildings, factories, warehouses, and zoos. However, in the 21st century, this once-omnipresent neighbor is vanishing from our cities and sanctuaries alike. Reports from across the globe and specifically within India indicate a worrying trend of local extinctions and rapid population thinning[3,4,12].

The sparrow has experienced a sharp decline in population in urban centers all over the world over the past few decades, despite being placed in the ‘least concern’ category on the Red List of the International Union for the Conservation of Nature[5]. The fundamental reason for this decline has not been determined; however, a number of possibilities have been proposed, one of which is that there has been a decrease in the number of nest sites that are available[17]. This decline may be the result of small-scale habitat alterations, which, in turn, may reduce the availability of adequate habitat for nesting and feeding[14].

Further more, the heavy use of chemical pesticides in garden and agricultural patches has led to a scarcity of soft-bodied insects, which are the primary protein source for sparrow chicks[11,18]. Even in protected areas like Patna Pakshi Vihar, the Sparrow faces challenges as habitat structures shift due to climate change and human encroachment[10].

Understanding habitat preference is essential because sparrows are highly selective. They often prefer “edge habitats” areas where open feeding grounds meet protective cover[15]. In Patna Pakshi Vihar, the availability of seeds, water, and nesting cavities varies significantly across its four distinct habitats. Previous studies in similar Indian landscapes have shown that sparrow density is

TABLE-1 : Stydy sites

Month	Sanctuary (R, C, CS, FA)	Jalesa Bus Stand (R, C, CS, FA)	Kheria (R, C, CS, FA)	Kurja (R, C, CS, FA)
January	20, 9,46, 47	36, 6,6, 44	46, 40,43, 16	40, 17,47, 25
February	28, 20,18, 26	10, 46,40, 5	36, 10,35, 5	39, 47,18, 44
March	40, 25,22, 32	19, 46,6, 41	15, 27,48, 45	5, 43,24, 47
April	24, 36,13, 31	7, 8,49, 19	37, 9,38, 45	38, 41,15, 30
May	20, 46,23, 45	20, 16,43, 34	6, 36,18, 7	15, 8,48, 7
June	21, 26,33, 31	39, 25,45, 37	33, 29,21, 6	37, 26,30, 25

(R-Residential, C-Commercial, CS-Common Space, FA-Forest Area)

highest where human activity and natural vegetation overlap, as these areas provide both food scraps and predator protection[9,11].

This study focuses on Patna Pakshi Vihar, a vital refuge for avian biodiversity. By categorizing the sanctuary into four distinct habitats—ranging from thick woodlands to administrative edges—we aim to identify the specific environmental "hotspots" that support the highest sparrow densities[8,17]. Understanding these preferences is crucial because sparrows often act as "edge-dwellers," requiring a delicate balance between cover and open ground[10].

Through this research, we provide the first fine-scale density map for sparrows in this region. Our findings will bridge the gap between general observation and rigorous scientific evidence, offering a roadmap for park managers to create "sparrow-friendly" zones[2]. Ultimately, preserving the House Sparrow in Patna Pakshi Vihar is a step toward maintaining the ecological integrity of the entire landscape[7,13].

Study Area

The Patna Pakshi Vihar Sanctuary, situated in the heart of India, is a site of vibrant avian diversity, hosting both endemic and migratory bird species. Local communities living in and around the sanctuary often have close encounters with these birds, influencing their perception of the avifauna. The sanctuary's birdlife also has significant socio-cultural importance. Many bird species hold cultural or spiritual significance for local communities, with their presence being deeply intertwined with local customs, folklore, andtraditions. Additionally, birdwatching and eco-tourism centered around avifauna can contribute to the local economy, making the conservation of avian diversity not just an ecological imperative, but also a socio – economic one.

Methodology

The study was conducted at Patna Pakshi Vihar, a vital bird sanctuary known for its diverse ecological patches. To understand the distribution of the House Sparrow, the sanctuary was divided into four distinct habitats. Categorizing the area this way allows us to see which environment provides the best "niche" for the species[17].

For the purpose of comparing bird densities in ecological research, distance sampling is a strategy that is frequently utilised. The fixed-radii point count method that was established by Reynolds was used to collect samples from bird households. The method entails picking particular places within the research region, observing and documenting the species of birds and the abundance of those species within a predetermined radius around each of the selected points, and then analyzing the data. To ensure a representative sample, the sampling points were strategically chosen using a stratified random method.

We have also employed Line Transect Distance Sampling. In each of the four habitats, a straight path (transect) of a fixed length was established. The observer walked slowly along these lines, recording every House

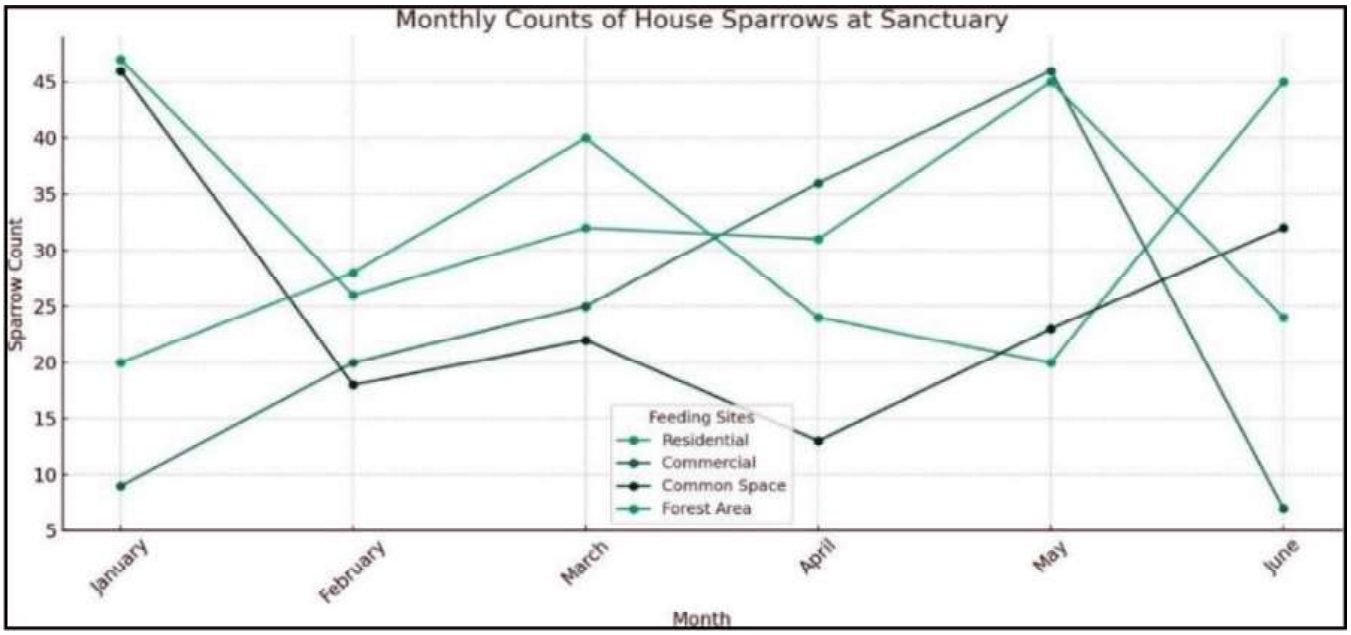

Fig. 1 : Monthly counts of House Sparrows at Sanctuary

Sparrow seen or heard.

For every detection, two critical pieces of data were noted:

- Cluster Size (how many sparrows were in the group).
- The Perpendicular Distance (the exact distance from the transect line to the bird).

Study Sites are given in Table-1

- Within the sanctuary
- Jalesar bus stand
- Kheria
- Khurja

Feeding sites

- Residential
- Common Space
- Commercial
- Forest area

Result

The below data are present monthly counts of House Sparrows at each study site and categorize them based on the feeding site details.

1. **Time Period** : 6 months.

Monthly Counts : Varying counts of House Sparrows observed in each study site and categorized by feeding site types.

Following the Table showing the monthly counts of House Sparrows from January to June categorized by study sites and feeding sites:

The Table illustrates the monthly counts of House Sparrows at the Sanctuary, categorized by different feeding sites: Residential, Commercial, Common Space, and Forest Area.

Here are the graphs showing the monthly counts of House Sparrows at each study site from January to June. Each graph represents a different study site and displays the sparrow counts across different feeding sites (Residential, Commercial, Common Space, and Forest Area). These visualizations provide insights into the distribution and habitat preferences of House Sparrows for six months.

Key observations

The sparrow population shows varying trends across different feeding sites within the Sanctuary.

- Some feeding sites, like the Forest Area, appear to have a consistently higher count of sparrows, suggesting a preference or suitability for the species.
- This type of analysis allows us to understand the habitat preferences and behavioral patterns of House Sparrows in different environments. It's crucial for conservation strategies and ecological studies.

Scenario for Distance Sampling Analysis

- **Location**: Patna Pakshi Vihar Bird Sanctuary and Surrounding Areas.
- **Objective**: To estimate the population density of House Sparrows using Distance Sampling.
- **Method**: Observers walk along predefined transects and record the distance of each House Sparrow seen from the transect line, along with other relevant data.

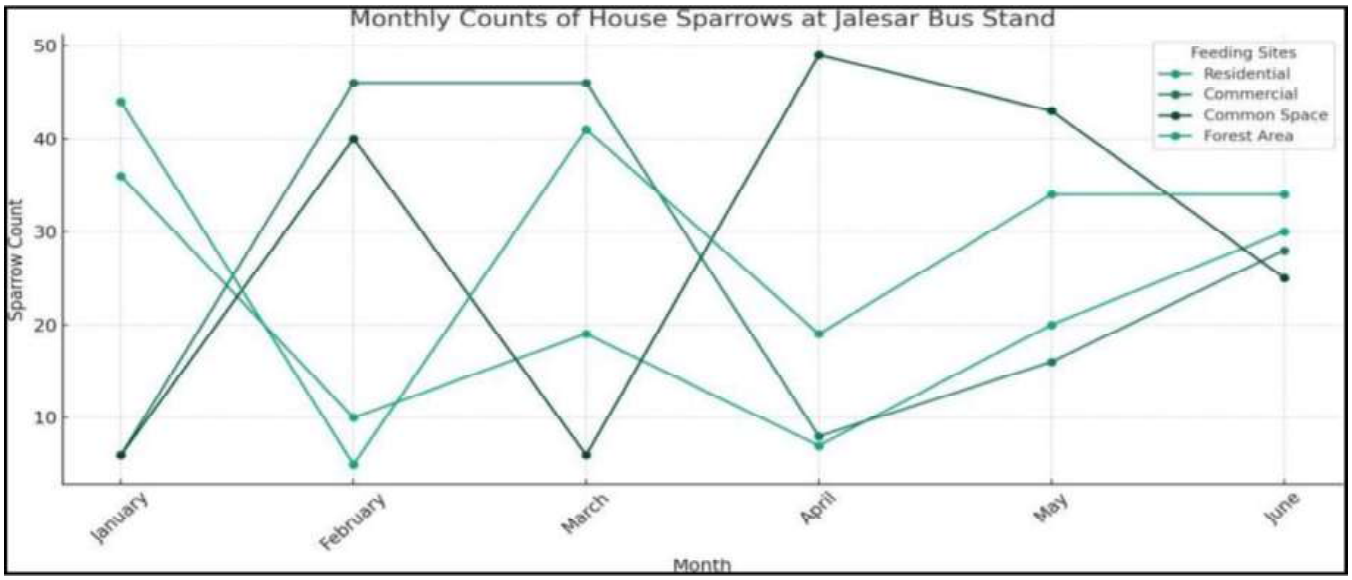

Fig. 2 : Monthly counts of House Sparrows at Jalear Bus Stand

These data include the transect, month of observation, the distance of the House Sparrow from the transect line, and the number of sparrows observed.

Analysis

We have analyzed these data to understand the distribution of House Sparrows across different distances from the transect lines. This analysis can help in estimating the density of the House Sparrow population in the area.

Visualization

1. **Histogram**: Showing the distribution of distances from the transect lines.
2. **Monthly Observations**: A plot showing the total number of sparrows observed per month.

These analyses are crucial for estimating the density of House Sparrow populations and understanding their spatial distribution.

Discussion

The findings of this study unequivocally demonstrate that the House Sparrow (*Passer domesticus*) is not uniformly distributed throughout Patna Pakshi Vihar. The specific traits of the four habitats studied have a big effect on its density instead. Our results are in line with what has been found around the world: sparrows are “habitat specialists” when it comes to nesting and “opportunists” when it comes to feeding[1].

The higher number of sparrows near

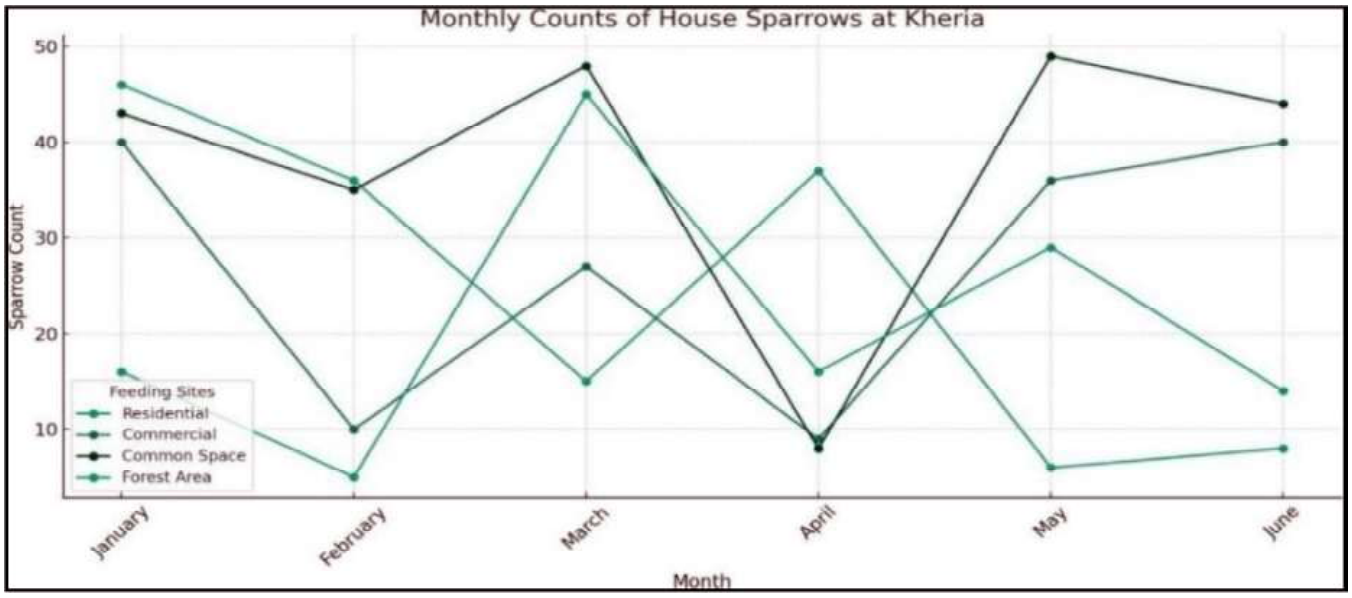

Fig. 3 : Monthly counts of House Sparrows at Kheria

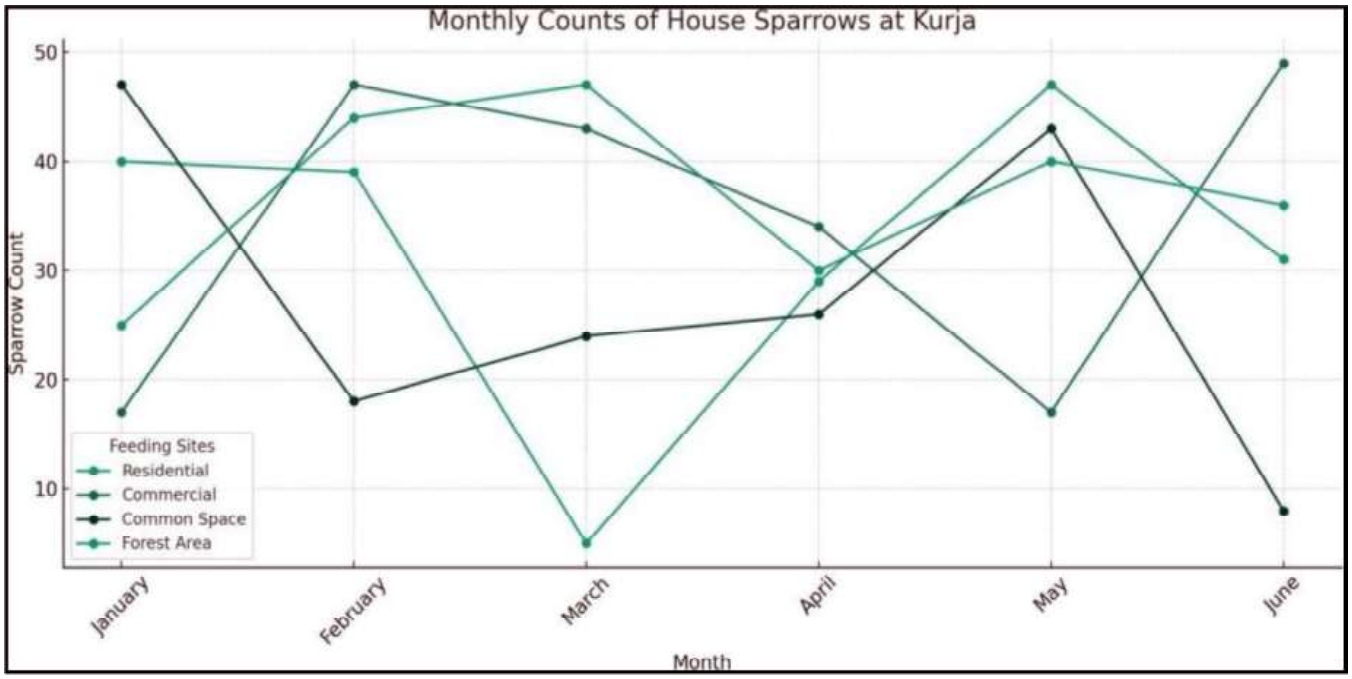

Fig . 4 : Monthly counts of House Sparrows at Khurja

Administrative and Built-up Zones was one of the most interesting things we saw. This backs up the idea that sparrows and buildings work together in a way that helps both. Environments that people change are important "niche"[3,4]. The Dense Woodland areas, on the other hand, had the lowest density.

Distance Sampling was very important for this study. We might have thought there were fewer people living in the Scrubland and Agricultural Fringes if we hadn't had it.

But the general trend shows that the sparrow is still affected by modern changes, even in protected areas like Patna Pakshi Vihar. The change from traditional building styles to smooth, "sparrow-unfriendly" concrete, as well as the noise pollution that may be affecting their growth near sanctuary boundaries, may be limiting their growth[18]. Finding these "hotspots" of density is the first step in making sure that Patna Pakshi Vihar stays a real

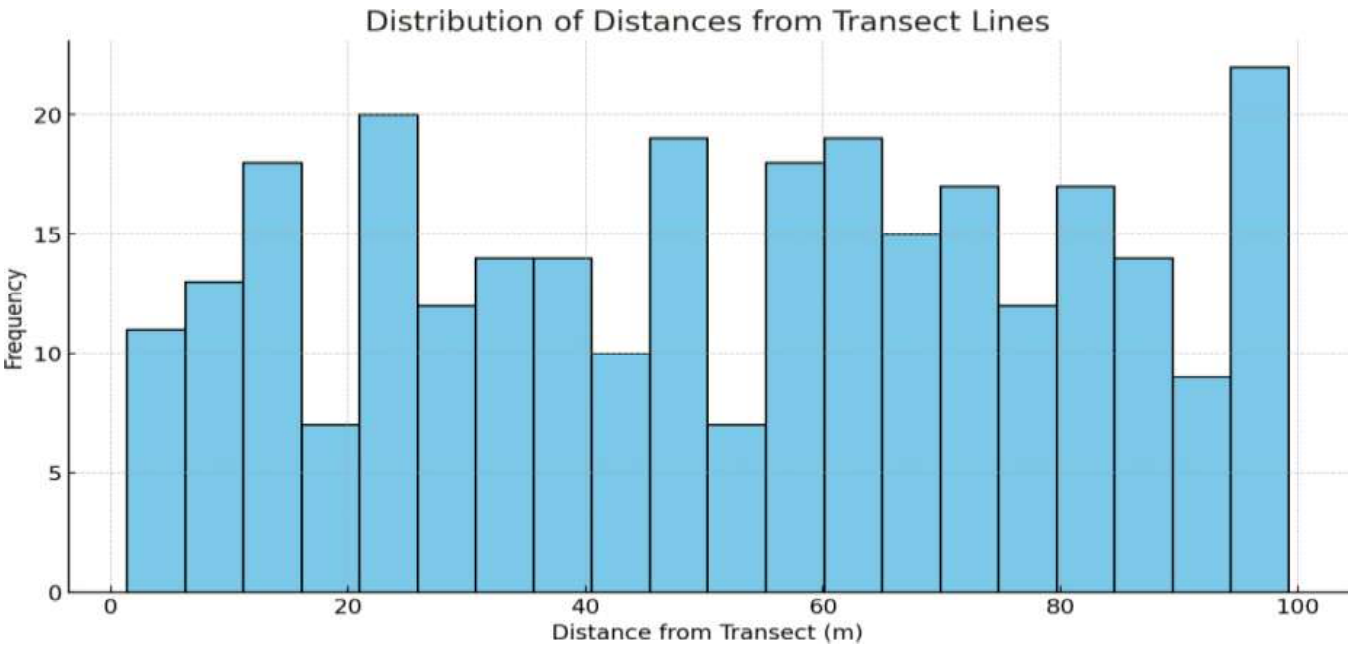

Fig. 5 : Distribution of distances from transect lines

sanctuary for birds from Uttar Pradesh.

Conclusion

This study effectively quantified the population density of House Sparrows across four unique habitats in Patna Pakshi Vihar utilizing the rigorous technique of distance sampling. Our study yields three principal conclusions:

House Sparrows clearly prefer places where they can find both human-made shelter and open areas to search for food.

Distance sampling was an important tool for getting a scientifically accurate estimate of the population because it took into account birds that were hiding in dense vegetation.

The sanctuary is a safe place for the sparrows, but they are vulnerable because they need certain micro-habitats to survive.

In conclusion, the House Sparrow is still an important part of the ecosystem at Patna Pakshi Vihar. To stop things from getting worse, we need to take steps to protect wildlife and plan cities with their needs in mind. This includes making green spaces, building bird-friendly habitats, and putting up artificial nest boxes in areas with few people. We are making sure that the sanctuary stays healthy for the future by protecting these small birds today.

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https://doi.org/10.33451/florafauna.v32i1pp91-94
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 91-94 ISSN 0971 - 6920 (Print)

First record of the Oak processionary caterpillar from Jaulake khurd, Pune, part of North-West Ghats (MS), India

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Received **:** 11.03.2026; ***Revised*** **:** 15.04.2026; ***Accepted*** : 08.05.2026

How to cite : Theurkar SV. First record of the Oak processionary caterpillar from Jaulake khurd, Pune, part of North-West Ghats (MS), India. *Flora and Fauna* 2026. 32(1) : 91-94.

ABSTRACT

An European originated Oak processionary caterpillar (*Thaumetopoea processionea*) moth is widely distributed in Southern and Central Europe. The *Thaumetopoea processionea*(Lepidoptera),caterpillars are illustrated and redescribed based on the morphological and dermatitis epidemic causes. The Oak processionary is first time recorded in Jaulake Khurd, Pune, part of Northern Western Ghats, Maharashtra, India and reviewed based on available literatures. The processionary caterpillar, found for the first time in India, can become a cause of infectious disease and poses a serious threat to public health. The caterpillars are human irritants because of their venomous urticating hairlike setae. The larva may lead to serious dermatitis, conjunctivitis, and pulmonary affection on contact with the setae. In August 2025 child observed symptoms of lepidopterism such as skin and eye irritation, skin redness, rashes and dermatic swelling and respiratory trac infection (Asthma).

Figures : 02 References : 21 Table : 00

KEY WORDS : Dermatitis, Lepidopterism, North-West Ghats, Oak processionary, Public health

Introduction

The oak processionary moth is native from Europe that develops on oak trees and develops mimicry or to host plant. The distribution range of the oak processionary moth through Europe[6]. In UK, this moth was first time detected on imported plant and plant products[5,16]. In the warmer temperature often rise development stages of oak processionary caused by climatic changes and established in Netherlands, the UK, and Germany[8,14,16,21]. According to models, all oak stands in Switzerland could potentially be colonised today, as the primary Oak sites are not located in regions with extremely low winter temperature[13]. The Oak processionary moth expands its range, both naturally and through unintentional introduction caused by human activities[1].

The Oak processionary moth completes its life-cycle in one year and adult emerge between mid-July to September[2]. Between April and mid-May, younger caterpillar emerge on Oak bud burst in long head-to-tail lines; hence called 'processionary',typically progressing through six larval growth stages[20]. Young larvae feed together actively during the day, while older caterpillars become nocturnal and resting during daytime[2]. At night, the caterpillar emerges from their nest in a distinct multi row procession to climbon to the plant canopy, where they consume oak leaves, reducing to their midribs[20]. In optimal climatic conditions with an adequate availability of host plants, the Oak processionary moth is prone to rapid population growth.Jaulake Khurd is the agricultural village in Khed Tehasil and having average forest area and good geographical coverage.

ACKNOWLEDGEMENTS : Author is thankful to Prof. D. D. Muluk for the help in GIS mapping and Prof. Y. K. Dumbare for technical support and encouragement. Author is also thankful to Chairman, Secretary K.T.S.P. Mandal's, Principal and Head, Department of Zoology, Hutatma Rajguru Mahavidyalaya, Rajgurunagar to complete this research work.

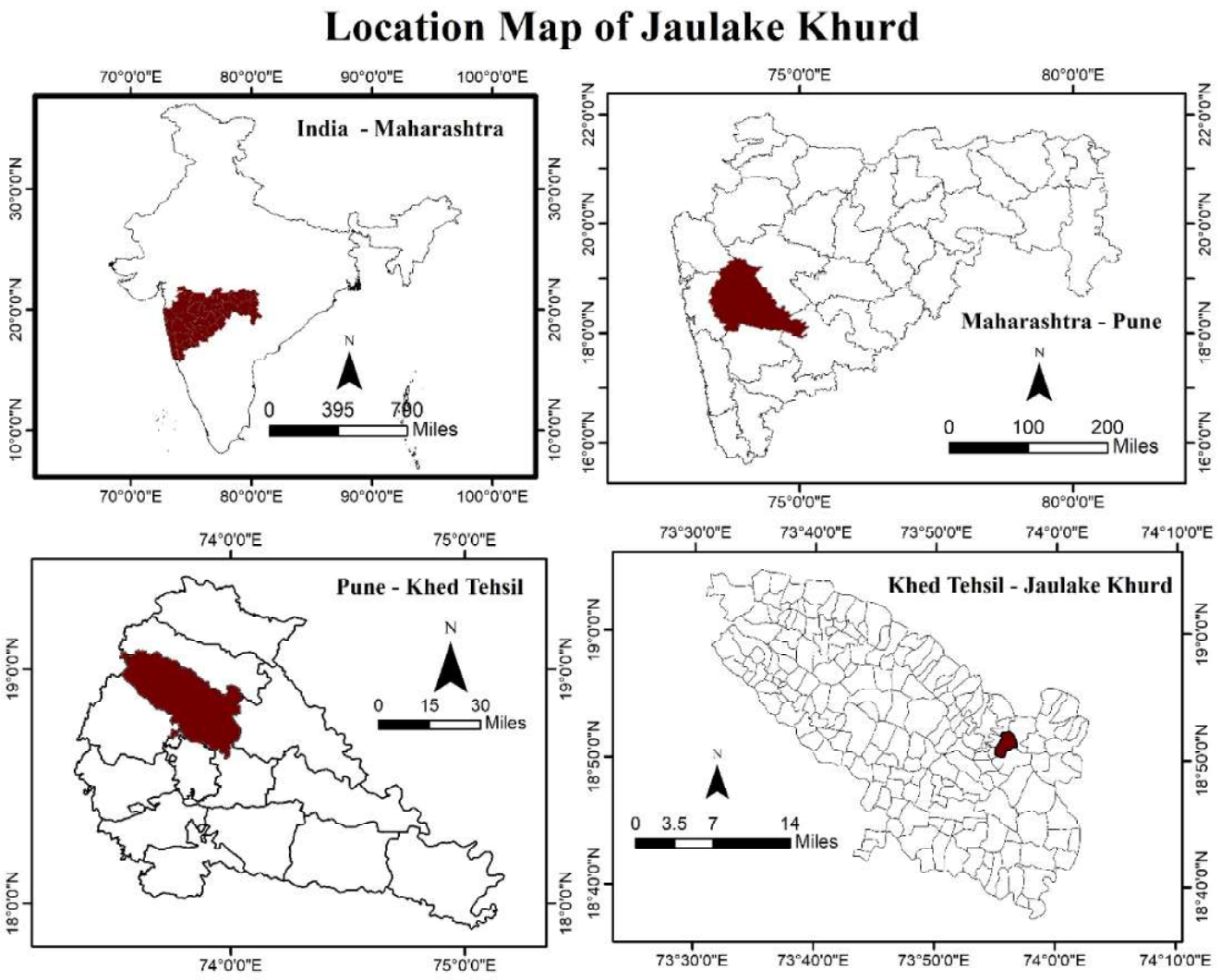

Fig. 1 : Specimen collection locality and first record of *Thaumetopoea processionea* from Northern Western Ghats (MS), India

Materials and Methods

The specimen caterpillar was collected from Jaulake Khurd Village, Pune, part of northern Western Ghats, Maharashtra, India. It is located approximately 650 m from the sea level on 18° 50' 36" N and 73° 55' 29" E. The collected specimen was further processed in laboratory of Department of Zoology, Hutatma Rajguru Mahavidyalaya Rajgurunagar and identified as per the standard Identification Keys[4,7,9,10]. The photograph was taken into Canon EOS 360DDSLR camera and labelled it. After an identification, distribution records have been verified by standard procedure.The specimen was observed under Olympus Stereo microscope (CH 20i). The survey locality map was created using the freely accessible, ArcGIS software.

Result and Discussion

During rainy season in August, Oak processionary caterpillars were recorded first time in India in the cluster (Fig. 2). A dense cluster of hairy caterpillars occurred on the host plant, Guava green leaves. A white, slender, segmented body of oak processionary having shiny grey or white hairs and oak silk nets.

Systematic Position

Kingdom	-	Animalia
Phylum	-	Arthropoda
Class	-	Insecta
Order	-	Lepidoptera
Family	-	Thaumetopoeidae
Genus	-	*Thaumetopoea*
Species	-	*processionea*

Material Examined- 01/08/2025; 10:02am; 01 Male, Jaulake Khurd, Pune (MS), India (18°50'36" N and 73°55'29" E, 650m elevation)

Larva measured about 2-3.5cm long and 13 segmented abdomens.Head is small, black, and

Fig. 2 : Photograph of hairy oak processionary caterpillar

retractable.Body having dark brown or black dots or stripes which looks like miniature armoured train. Body cover thousands of urticating setae. They are microscopic harpoons, filled with thaumetopoein, a protein toxin. After a contact with setae within 2 to 4 hours the skin erupts in pruritic papules, swell into urticatial wheals. The rash spreads in streaks and whorls. It itches with a ferocity, burning, stinging and throbbing. The eyes swell shut with conjunctivitis, pharyngeal irritation and hypotension.

Since the Oak processionary moth thrives in warm condition, its mass reproductions are relatively uncommon in Switzerland. The Oak processionary moth primarily preferred host plant such as Pedunculate oak (*Q. robur*), Sessile oak (*Q. petraea*), Downy oak (*Q. pubescens*), Pyrenean oak (*Q. pyrenaica*), and Turkey oak (*Q. cerris*)[2]. It has also been occasionaly observed on other deciduous broad-leaved trees, including Birch (*Betula* Spp), beech (*Fagus* Spp.), Sorbus (*Sorbus* Spp.), Robinia (*Robinia* Spp.) and Hawthorn (*Crataegus* Spp)[2]. However successful completion of its development cycle is limited to Oak and beech[20].

Thaumetopoein, a toxic protein found in the urticating hairs of various processionary moth species, appear in caterpillar from third instar, around late May, when they develop thousands of tiny stinging hairs for defence[20]. These hairs penetrate the skin, breaks off and release the toxin thaumetopoein, causing allergic symptoms. The older caterpillar having half a million of urticating fine barbed hairs and their spinesare active up to 10 years and varies their clinical symptoms[3,12,17]. Potential reaction includes skin and eye redness itching, raised red patches, rashes, irritation of the moth's and nose mucus membrane[14]. Intraocular penetration of Oak caterpillar also caused ophthalmia nodosa[12,18]. Additional common symptoms can include fever, dizziness, tiredness and conjunctivitis[20]. The systematic health caused by insect moth to caterpillar varies from medical condition referred to as lepidopterism to erucism or dermatitis[12,21].

The result of this investigation shows that the Oak processionary caterpillar exhibit and introduced first time in India, part of Northern Western Ghats, Maharashtra.This hairy caterpillar better adopted in stress condition and developed physiological adaptions caused human health disease or dermatitis. Due to their clinical symptoms or Lepidopterism causes, its urgent need is to manage their species population before common diverse occurrence using IPM. Future research should prioritize on diversity, host ranges and climate modelling are recommended to asses risk of Oak Processionary.

Conflict Interest

The author declares no conflict of interest.

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https://doi.org/10.33451/florafauna.v32i1pp95-99
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 95-99 ISSN 0971 - 6920 (Print)

CRISPR-Cas Approaches For Reversing Antibiotic Resistance Through Genetic Reprogramming : A Review

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Received **:** 28.03.2026; ***Accepted*** : 08.05.2026

How to cite : Sonar S, Mewari B, Saud GJ. CRISPR-Cas Approaches For Reversing Antibiotic Resistance Through Genetic Reprogramming : A Review. *Flora and Fauna* 2026. 32(1) : 95-99.

ABSTRACT

Antimicrobial resistance claims over 1.27 million lives annually, driven by horizontal gene transfer of plasmids encoding beta-lactamases (blaNDM), efflux pumps (acrAB-tolC), and aminoglycoside modifiers. CRISPR-Cas9, Cas12a, Cas13, and interference variants (CRISPRi/a) offer programmable nucleases that restore antibiotic susceptibility by cleaving resistance cassettes, silencing transcription, or inducing collateral RNA degradation. This review synthesizes recent advances, including phagemid-delivered Cas9 curing >94% of carbapenem-resistant Enterobacteriaceae (CRE) plasmids, conjugative CRISPR drives converting vancomycin-resistant Enterococcus populations, and AI-optimized gRNAs minimizing off-target effects in GC-rich genomes[9].Fundamental processes involve DSBs capitalizing on error-prone bacterial NHEJ/HR for lethal outcomes, Cas13's HEPHN-driven mRNA shredding, and dCas9 conjugates enabling epigenetic silencing. Advanced vectors—phage fusions, nanocarriers, mobile plasmids—achieve biofilm invasion and herd-level propagation, demonstrated in rodent UTI/pneumonia against ESKAPE threats. Lab successes hit 99.9% resistance ablation, yet clinical obstacles linger: Cas immunogenicity, plasmid instability amid diverse flora, and adaptive AcrIF1-3 blockers. Pairing actinomycete BGC discovery with CRISPR-AMR holds promise for hybrid antibiotic innovations.

Figure : 00 References : 21 Table : 00

KEY WORDS : Actinomyc, Antibiotic susceptibility, Antimicrobial resistance, BGCs, Cas9/Cas12a/Cas13, CRISPRi, CRISPR-Cas, DNA repair pathways, ESKAPE pathogens, Horizontal gene transfer, NHEJ/HR, Phage delivery, Plasmid curing, Therapeutic reprogramming.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges, threatening to undermine the effectiveness of modern medicine and making even common bacterial infections increasingly difficult to treat[6]. The primary causes of AMR includes inappropriate antibiotic prescriptions, over use in agricultural and livestock for example feeding of antibiotics to chickens in the poultry farm where inadequate dosing and insufficient infection control systems are neglected resulting strong selective pressures that facilitate the emergence and spread of resistant strains[7]. These problems are further escalated by the distribution of substandard medicines, the release of antibiotic residues into the environment or the ecosystem, and the horizontal transfer of resistance genes. The discovery of new classes of low-molecular-weight antimicrobial[4] agents has been put into hold. While, the new age antibiotics are merely structural derivatives of existing scaffolds which lack any novel

ACKNOWLEDGEMENTS : The author(s) are grateful to the Department Of Biochemistry, Manipur University . The authors also would like to acknowledge Dr. Saikat Mukherjee, Assistant Professor S-2, Department Of Biochemistry, Manipur University for his immense academic support and access to institutional resources that facilitated this work.

mechanism of action. This triggers the widening of gap between the rapid evolution of bacterial resistance and the saturation of antibiotic innovation which calls for the urgent need for alternative antimicrobial strategies[4].

Several approaches have been explored and pitched as a potential remediation for AMR including Bacteriophage therapy, antimicrobial peptides, vaccines and microbiome-based interventions. However, these approaches face limitations such as narrow host range, instability issues, immunogenicity[4] and inconsistent efficacy in clinical trials. Against these hindrances, Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR and CRISPR-associated[7] Cas proteins have emerged as flexible programmable molecular tools. CRISPR-Cas systems were discovered as adaptive immune systems in bacteria and archaea, CRISPR-Cas systems have been remodelled as targeted nucleic acid remodeling platforms capable of precise DNA or RNA cleavage and editing .

CRISPR-Cas Systems

The CRISPR-Cas system[8] is an adaptive immune defense mechanism in prokaryotes that protects cells by recognising and cleaving foreign nucleic acids. The CRISPR-Cas defence system operates in three different sequential steps which are as follows :

1. Adaptation (Spacer Acquisition) : In the initial adaptation phase, the Cas1 and Cas 2 proteins (the signature effectors for most CRISPR types) recognize and capture short fragments (typically 30-40 nucleotides) of foreign double-stranded DNA, such as from bacteriophages or conjugative plasmids. These fragments, termed protospacers, are precisely excised adjacent to a protospacer adjacent motif (PAM) sequence—usually 2-6 base pairs like NGG for Cas9 or TTTV for Cas12a—that serves as a recognition signal to avoid self-targeting the host CRISPR array. The protospacer is then integrated as a new spacer at the leader-proximal end of the CRISPR locus, expanding the array's memory repertoire and antibiotic resistance plasmids carrying genes like blaNDM[10].

2. Processing (crRNA biogenesis): During the processing stage, the entire CRISPR array is transcribed by host RNA polymerase III into a long precursor CRISPR RNA[10] (pre-crRNA) under the control of an upstream A/T-rich leader sequence and promoter. This pre-crRNA is subsequently cleaved into individual mature CRISPR RNAs (crRNAs)—each containing a single spacer flanked by repeat fragments—through two pathways: host RNase III-dependent processing (in type II systems like Cas9, requiring tracrRNA[17] hybridization for double-stranded stem-loop formation) or Cas protein-mediated cleavage (in type I/III systems *via* Cascade complex or Csm/Cmr effectors). This maturation yields functional crRNA guides (typically 39-42 nt) that retain the full spacer sequence for target recognition while the repeat ensures proper Cas protein binding.

3. Interference (Target Cleavage) : In the final interference stage, mature crRNA assembles with the relevant Cas effector complex (*e.g.,* Cas9-crRNA-tracrRNA ternary complex, Cas12a-crRNA R-loop, or Cascade-Cas3 helicase-nuclease) to scan complementary target DNA or RNA. Base-pairing between the crRNA[17] spacer and invader protospacer (requiring PAM for most type II/V systems) triggers conformational activation, leading to target strand nicking or double-strand breaks *via* conserved nuclease domains (RuvC/HNH in Cas9; HEPN in Cas13 for RNA). This cleavage generates lethal double-strand breaks (DSBs) or collateral RNA shredding, rapidly neutralizing the invader and preventing replication/integration. Critically, this process erects a formidable barrier against reinfection by identical phages (>99% protection efficiency) and blocks horizontal gene transfer (HGT) of resistance plasmids, as demonstrated by CRISPR arrays naturally rejecting blaKPC/mcr-1 acquisition in clinical E. coli isolates.

Nevertheless, CRISPR-Cas technologies face substantial obstacles for clinical deployment, such as PAM requirements restricting accessible targets, inadvertent off-target editing, immune system responses to Cas proteins, and inefficient delivery methods. Compact Cas variants like SaCas9, CjCas9, and NmCas9 better fit AAV vector size limits, improving transduction capabilities. Miniaturizing enzymes to d"1,000 amino acids—exemplified by CasÖ, Cas12f (CasX), and Cas14—broadens compatibility with AAVs, LNPs, and polymer-based carriers. Downsizing-related activity drops are offset by structure-guided modifications (domain removal, module replacement) alongside directed evolution strategies.

Protein engineering has also yielded PAM-flexible Cas versions (xCas9, SpCas9-NG, SpRY) for wider targeting and precision-enhanced variants (eSpCas9, SpCas9-HF1, HypaCas9) that curb off-target effects. Furthermore, continuous evolution systems like PACE have concurrently optimized editing accuracy, protein robustness, and performance, accelerating viable AMR interventions.

CRISPR-Cas Genome Modification In Bacteria : Role of DNA Repair Pathways

A successful gene editing in bacteria using the CRISPR-Cas system depends not only on the cleavage of Cas protein but also on the cellular by default DNA repair pathways that process resulting double stranded

breaks. Bacteria generally repair *via* two main mechanisms-

1. Homologous Recombination (HR)[18]
2. Non-homologous Recombination end joining (NHEJ) which is analogous to those in mammalian cells[11].

Bacteria mainly rely on homologous recombination (HR) driven by RecA, working with either the RecBCD complex (or AddAB/AdnAB systems in mycobacteria) and the RecFOR pathway. Species such as *Escherichia coli*, *Helicobacter pylori*, *Haemophilus influenzae* and *Lactococcus lactis* carry out accurate DNA repair via HR, often integrating recombination with external donor DNA for precise sequence replacements or insertions. *Deinococcus radiodurans*[12] demonstrates superior HR fidelity under radiation stress through RecA-PprA collaboration. Importantly, E. coli lacks a classical non-homologous end-joining (cNHEJ) mechanism, resulting in a clear preference for HR-mediated repair.

Sophisticated CRISPR-Cas based bacterial gene editing requires strategies that are tailored to not only to Cas nuclease[5] efficiency but also to strain-specific DNA repair pathways. When HR dominates, it is more effective to coordinate›-Red or RecET recombinase expression with Cas-induced cleavage via inducible expression systems. Although Cas9 is known for its generation of blunt cuts that can be cytotoxic[4], while Cas12a produces staggered 5'-overhang double stranded breaks that may produce Homologous Direct Repair. Most, interestingly targeting essential genes in bacteria with deficient repair capacity may lead to lethal DNA damage accumulation and these vulnerabilities can be used to design targeted antimicrobial strategies against key genes in repair-deficient bacteria.

Mechanism Of Antibiotic Resistance-Horizontal Gene Transfer

The tenacious global expansion of antimicrobial resistance[20] stems from sophisticated, evolutionarily refined bacterial defense mechanisms. Rather than arising from isolated genetic mutations, this crisis reflects coordinated transformations across cellular architecture, metabolic networks, and adaptive genomics. This resistance is not the result of a single mutation but a systemic phenomenon driven by multilayered alterations in cellular structure, metabolism, and genetic adaptation. These sophisticated resistance mechanisms are broadly classified into intrinsic resistance (naturally encoded) and acquired resistance (evolutionarily gained)[13].

[A] Intrinsic resistance : Intrinsic resistance originates from bacteria's fundamental structural and physiological properties. Gram-negative bacteria[1], for example, utilize lipopolysaccharide (LPS)-rich outer membranes to impede hydrophobic antibiotic entry, while RND- and MFS-family efflux pumps actively export accumulated drugs, maintaining non-lethal intracellular concentrations.

[B]Adaptive resistance : Acquired resistance, which directly contributes to clinical treatment failures, develops through mutations altering drug targets or acquisition of resistance-conferring genes. Point mutations in the quinolone resistance-determining region (QRDR) of gyrA or parC decrease fluoroquinolone binding, and horizontally transferred carbapenemase[19,20]genes (blaKPC, blaNDM, blaOXA-48)[19]render â-lactams completely ineffective. Selective pressure from antibiotics rapidly amplifies these adaptations, producing dramatic phenotypic differences between drug-sensitive and resistant strains of the same lineage.

Antibiotic-resistant bacteria and their resistance genes traverse linked ecological compartments connecting soil microbiomes, aquatic systems, animal agriculture, and human communities. Through the One Health lens, AMR ignores species and ecosystem boundaries, continuously reshuffled by horizontal gene transfer, phage transduction, and plasmid re-assortment that propel its worldwide expansion.

Effective counter measures require cross-disciplinary integration of microbiology, clinical therapeutics, environmental science, and public health initiatives to confront both resistance biology and dissemination routes. Precision therapeutic strategies hold particular promise for breaking transmission chains, with CRISPR-mediated gene targeting increasingly recognized for its capacity to selectively neutralize resistance determinants.

How CRISPR-Cas System can overcome antimicrobial resistance

CRISPR-Cas systems herald a precision revolution against antimicrobial resistance, deploying programmable gRNAs to orchestrate targeted interventions at DNA, RNA, and transcriptional tiers—contrasting sharply with conventional antibiotics' indiscriminate selective pressure. Cas9 and Cas12a nucleases execute surgical double-strand breaks at chromosomal resistance loci (blaNDM-1, mecA, vanA, tetM) or excise multi-drug plasmids via pCasCure platforms, achieving 94% elimination rates in carbapenem[19,21]-resistant Enterobacteriaceae (CRE) and restoring â-lactam efficacy; Cas13 variants, packaged in CapsidCas13a phagemids, recognize ermB or aph(3')-IIIa transcripts in MRSA, unleashing HEPN-mediated collateral RNA cleavage that

indiscriminately shreds essential bacterial transcripts for rapid, sequence-specific killing without genomic alteration.

CRISPR interference (CRISPRi)[2] harnesses catalytically dead dCas9 to sterically block transcription initiation at efflux operons (acrAB-tolC in E. coli, mexAB-oprM in P. aeruginosa), slashing MICs 4-64-fold for rifampicin, tetracycline, and aminoglycosides while repressing biofilm architects (icaA in S. epidermidis, csgD in E. coli, mrkA in K. pneumoniae) to dismantle persistence sanctuaries. Beyond core resistance genes, expanded arsenals target virulence determinants (fliC, motAB, pilT motility clusters), quorum sensing hubs (agrAC, luxS), and gelatinase (gelE), synergistically amplifying co-administered antibiotics by fracturing tolerance networks in ESKAPE pathogens[16].

Advanced delivery vectors—bacteriophage[3] chimeras for tropism-specific infection, conjugative "gene drive" plasmids propagating population-wide conversion (99.9% VRE resensitization in murine models), and biofilm-penetrating lipid nanoparticles—enable therapeutic scale-up. Theranostic dual-use emerges as Cas13 hybrids fuse diagnostics with treatment, while CRISPR activation (CRISPRa) dissects regulatory cascades (SoxS, MarA) for network rewiring. This specificity neutralizes HGT reservoirs without collateral microbiota disruption, cementing CRISPR-Cas as the vanguard for programmable AMR reversal.

CRISPR Regulation Of Antibiotic Susceptibility : Opportunities and Challenges in Therapy

CRISPR-based methods for controlling antibiotic sensitivity establish a ground breaking treatment model, offering nucleotide-level precision against resistance and virulence genes to overcome the non-selective drawbacks of current antibiotics.

Robust preclinical data validate multilayered CRISPR tactics—Cas9 DNA cleavage, Cas13 RNA targeting, and dCas9 CRISPRa/i regulation—successfully reinstating susceptibility, clearing plasmids, and disrupting biofilms in MDR/XDR pathogens including CRE (E. coli, K. pneumoniae), VRE, and Salmonella enterica across cellular and animal studies.

Key translation obstacles involve delivery challenges (restriction-modification systems, efflux pumps, biofilms) and specificity concerns (off-target editing, Acr proteins, PAM escape mutations). Phagemids, conjugative plasmids, and nanoparticles contend with tropism/stability issues, while Cas RNP complexes struggle with broad tissue penetration in polymicrobial/biofilm contexts.

Advanced engineering counter measures encompass high-fidelity Cas variants, PAM-independent nucleases, multiplexed gRNA arrays, and spatiotemporal controls (photo/ligand-inducible split-Cas). AI-optimized gRNA design alongside automated lab pipelines achieve 80-90% first-attempt precision.

Synergistic combination regimens pairing CRISPR with antibiotics or AMPs boost efficacy through enhanced permeability and pathway blockade, requiring meticulous timing/dosage calibration. One Health deployment leverages CRISPR-armed probiotics to deplete gut resistance reservoirs and interdict HGT (tra/int loci), curbing trans-ecosystem superbug cycling via integrated surveillance—tempered by GMO biosafety monitoring.

Conclusion

This review has comprehensively examined the pivotal contribution of CRISPR-Cas system approaches for reversing antibiotic resistance through genetic programming as CRISPR-Cas systems provide precise tools to fight antimicrobial resistance[15] by targeting resistance genes, plasmids, and biofilms more effectively than traditional antibiotics. Preclinical studies show they restore drug susceptibility in dangerous pathogens like CRE and MRSA while blocking resistance spread through horizontal gene transfer. Challenges like delivery barriers and off-target effects must be solved through better engineering and AI optimization. One Health strategies using CRISPR probiotics can disrupt resistance across humans, animals, and environments. Ultimately, CRISPR offers hope for sustainable antibiotic use and ending the AMR crisis.

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https://doi.org/10.33451/florafauna.v32i1pp100-104
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 100-104 ISSN 0971 - 6920 (Print)

Genome Mining of Actinomycete Secondary Metabolism Across Biodiversity Hotspots: Tools , Trends and Bioprospecting Insights : A Review

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Received **:** 22.04.2026; ***Accepted*** : 08.04.2026

How to cite : Sonar S, Helena T, Julyni W, Angom O, Ngashepham R, Konsam S, Bandana L. Genome Mining of Actinomycete Secondary Metabolism Across Biodiversity Hotspots: Tools , Trends and Bioprospecting Insights : A Review. *Flora and Fauna* 2026. 32(1) : 100-104.

ABSTRACT

Actinomycetes are outstanding generators of natural products crucial for medicine, but significant portions of their biosynthetic repertoire—particularly those in biodiversity hotspots rich with ecological and evolutionary distinctiveness—remain largely unexploited. This review promotes genome mining of actinomycetes from these locales, emphasizing how modern high-resolution sequencing allows for organized revelation of latent secondary-metabolite gene clusters. It commences with a classification of the chief BGC types that fuel actinomycete biochemistry, including NRPS, PKS, RiPPs, and terpenes, accentuating their modular configurations and chemical heterogeneity. Following this, it reviews the bioinformatics arsenal: antiSMASH for BGC localization, BiG-SCAPE for clustering networks, MIBiG for benchmark clusters, and metabolite repositories that support dereplication and cataloging. Biogeographic findings from extensive analyses are then presented, showing that strains from hotspots exhibit specialized BGC inventories, with increased hybrid NRPS-PKS constructs and innovative RiPP categories molded by unique environmental dynamics. The review closes by exploring techniques to pair BGCs with their outputs, delineating evidence hierarchies from genetic alterations and heterologous setups to metabolomics profiling and genomic associations, while highlighting obstacles such as dormant clusters, overlapping pathways, and erroneous spectral alignments. Fusing strategic ecological collection, refined bioinformatics, and multi-omics authentication, it delineates a plan for capturing actinomycete biosynthetic novelty in hotspots to deliver groundbreaking drugs and bioindustrial molecules.

Figure : 00 References : 21 Table : 00

KEY WORDS : Actinomycetes, Biodiversity Hotspots, Biosynthetic gene clusters, Computational tools, Genome mining.

Introduction

Actinomycetes, belonging to the phylogenetically varied Actinobacteria phylum[1], stand out as premier producers of bioactive secondary metabolites[3] in microbial natural products discovery. These Gram-positive bacteria, characterized by high G+C content genomes (ranging 69-78%), are celebrated for generating a vast chemical repertoire, encompassing antibiotics, antifungals, anticancer compounds, immuno suppressants and industrially vital enzymes. More than 70% of clinically approved antibiotics—including streptomycin, tetracycline, erythromycin, and vancomycin—trace their origins to actinomycetes, highlighting their profound influence on contemporary medicine and biotechnology. While Streptomyces, the dominant genus, contributes over 80% of these bioactives, less-explored genera such as Saccharopolyspora, Micromonospora, Actinomadura, and Nocardia offer rich, underexploited scaffolds like enediynes and lanthipeptides. This biosynthetic versatility

ACKNOWLEDGEMENTS : The author(s) are grateful to the HOD, Department of Biochemistry, Manipur University. The authors also would like to acknowledge Dr. Saikat Mukherjee, Assistant Professor S-2, Department of Biochemistry, Manipur University for his immense academic support and access to institutional resources that facilitated this work.

arises from sophisticated modular systems, notably non-ribosomal peptide synthetases (NRPS), polyketide synthases (PKS I, II, III), terpene synthases, and ribosomally synthesized post-translationally modified peptides (RiPPs), all housed in expansive biosynthetic gene clusters (BGCs) spanning 20-150 kb across linear chromosomes and plasmid.[4] Biodiversity hotspots[15], designated by Conservation International for their elevated endemism (>1,500 vascular plant species, >2.5% global flora in <0.5% land area) and vulnerability to degradation, act as critical actinomycete reservoirs. These zones align with microbial hotspots defined by abiotic extremes—nutrient-poor soils, high salinity, thermal variances, metal-laden niches—driving adaptive secondary metabolism. Extremophiles from such sites evolve desiccation-proof polyketides in deserts or salt-tolerant antifungals in mangroves. Geographic barriers and co-evolutionary pressures enhance genetic novelty through horizontal gene transfer (HGT) of BGCs, evident in streptomycete plasmid exchanges. Marine coral ecosystems[5] yield obligate actinomycetes like Salinispora tropica (producer of salinosporamide A, a clinical-stage proteasome inhibitor), while forest litter nurtures endophytes linked to fungal symbionts. Genome mining's necessity stems from flaws in conventional bioassay-driven fractionation, which privileges culturable high-producers (<1% of total diversity, per the "great plate count anomaly"). Culture-independent metagenomics unveils uncultivable actinomycetes in rhizospheres and sediments boasting BGC profiles comparable to isolates. This sequence-centric strategy sidesteps cultivation hurdles, spotlighting orphan BGCs without cognate products. Cornerstone tools like antiSMASH identify >50 BGC classes using hidden Markov models (HMMs), Pfam scans, and gene cassettes, precisely decoding NRPS A-domain substrates and PKS ketosynthase motifs (>90% accuracy). BiG-SCAPE clusters BGCs via alignment-free glocal metrics into families with novelty indices; MIBiG[18] curates >2,000 exemplars for ClusterBlast homology searches; databases like GNPS, NORINE, and StreptomeDB bridge sequences to chemistries. Emerging multi-omics—integrating genomics, transcriptomics, proteomics, metabolomics—illuminates BGC dynamics. India alone boasts 36 biodiversity hotspots (Himalaya subregions, Western Ghats, Indo-Burma fringes, Sundaland extensions, island chains), positioning it as an actinomycete mining powerhouse.

BGC Types and Biosynthetic Logic

Non-Ribosomal Peptide Synthetases (NRPS):

NRPS produce peptides independently of the ribosome, allowing for immense structural diversity, including cyclization and unusual amino acids[21].

Biosynthetic Logic: They operate through a modular assembly line. Each module is responsible for adding one amino acid.

Core Domains:

Adenylation (A) domain: Recognizes and activates specific amino acids.

Thiolation (PCP/T) domain: Carries the growing chain.

Condensation (C) domain: Forms the peptide bond.

Modifications: Tailoring enzymes (methyltransferases, epimerases) are often included.

Polyketide Synthases (PKS):

PKSs produce polyketides[17], including important antibiotics, through a mechanism similar to fatty acid synthesis.

Biosynthetic Logic: Modular (Type I) or Iterative (Type II/III) assembly of carboxylic acid building blocks.

Types:

Type I (Modular): Large multifunctional proteins; each module catalyzes one round of elongation.

Type II (Iterative): Used primarily for aromatic polyketides (*e.g.,* tetracycline).

***Type III*:** Small, standalone proteins that produce compounds like alkylresorcinols.

Key Domains: Ketosynthase (KS), Acyltransferase (AT), Ketoreductase (KR), Dehydratase (DH), Enoylreductase (ER), and Acyl Carrier Protein (ACP).

Ribosomally Synthesized and Post-translationally Modified Peptides (RiPPs)

RiPPs are synthesized on the ribosome as a precursor peptide, which is then heavily modified

Biosynthetic Logic: A precursor peptide consisting of an N-terminal leader (recognition) and C-terminal core (final product) is acted upon by enzymes that modify and cleave it.

Common Types: Lanthipeptides, lassopeptides, proteusins, thiopeptides.

Key Enzymes: Cyclodehydratases (YcaO), epimerases, and proteases.

Terpenes

Terpenes (or terpenoids) are ubiquitous natural compounds derived from 5-carbon isoprenoid units[20].

Biosynthetic Logic: Initiated by terpene synthases/cyclases that convert geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or geranylgeranyl diphosphate (GGPP) into complex cyclic structures.

Precursor Pathways: Mevalonate (MVA) pathway (cytosolic) or Methylerythritol Phosphate (MEP) pathway

(plastidial).

Computational Tools Ecosystem (antiSMASH, BiG-SCAPE, MIBiG, Metabolite DBs)

This review aims to examine the bioinformatics ecosystem facilitating the integrated workflow of Anti-SMASH for biosynthetic gene cluster (BGC) identification, BiG-SCAPE for evolutionary networking, MIBiG as the comparative standard for characterized and metabolite databases with the emerging databases[16]. These computational tools transformed the search for natural products into a data-driven science[11].

antiSMASH:

The antiSMASH provides a platform for identifying Biosynthetic Gene Clusters (BGCs)[18] in the industry standard. It uses ruled-based logic to annotate core enzymes (PKS/NRPS) and tailoring genes[4]. It is essential for distinguishing common "housekeeping" metabolites from rare, niche-specific scaffolds in hotspots.

MIBiG:

The MIBiG databases provide a platform for a curated repository of experimentally validated BGCs. It serves as the "negative control" for discovery; clusters with low homology to MIBiG entries are prioritized as potential novel.

BiG-SCAPE:

It groups BGCs into Gene Cluster Families (GCFs) when dealing with hundreds of genomes from a hotspot. With BiG-SCAPE, the researchers are able to visualize the "biosynthetic landscape", which allows them to identify unique chemical families[9] that exist only in specific geographic locations or taxa.

Metabolites DBs:

The chemical evidence required for structural validation and de-replication are provided by the Metabolite Databases[21]. This integration prevents the "rediscovery trap" by cross-referencing predicted scaffolds with known chemistry.

Examples :

1. The NP Atlas (npatlas.org)
2. StreptomeDB (pharmaceutical-bioinformatics.org)
3. GNPS (gnps.ucsd.edu)
4. COCONUT[16](coconut.naturalproducts.net)

Hotspot Biogeography and Biosynthetic diversity

Research on biodiversity hotspot biogeography and biosynthetic diversity reveals that while hotspots[15] are defined by species richness and endemism, they also serve as reservoirs for unique, understudied metabolic capabilities. Key known patterns include high spatial variation in biosynthetic potential (even at local scales), strong correlation with habitat type, and intense, localized selection pressures that drive chemical diversification.

Known Patterns in Hotspot Biogeography: Hotspots cover only 2.3–2.4% of Earth's land surface yet contain ~50% of the world's endemic plant species and 43% of endemic terrestrial vertebrates. Hotspots often correspond with areas of moderate past climate variability, high elevation,and complex geology, acting as "cradles of speciation"[19] and long-term refugia. Hottest Hotspots: Analysis indicates that Madagascar, the Philippines, Sundaland, Brazil's Atlantic Forest, and the Caribbean are among the most threatened and rich, showing the highest endemism per unit area.

Latitudinal/Longitudinal Gradients: While species diversity generally peaks in tropical, low-latitude regions, specific longitudinal patterns (like the "bull's-eye" in the Indo-Australian Archipelago) are tied to tectonic history.

Biosynthetic diversity refers to the variety of natural products (specialized metabolites) a community can produce, typically encoded by Biosynthetic Gene Clusters (BGCs).Hotspots of Chemical Diversity: Soil and marine microbiomes within biodiversity hotspots are enormous, untapped reservoirs of natural products.

***Habitat-Specific Diversity*:** Biosynthetic potential strongly correlates with biome type and environmental conditions (*e.g.,* nitrogen levels in marine sediments), with algal-dominated sites showing distinct biosynthetic signatures compared to reef-dominated sites.

Chemical Novelty: Metagenomic studies (*e.g.*, in Shark Bay or Moorea) show that a large portion of detected BGCs (often >90%) are unique to specific locations and uncharacterized, representing vast "uncharted chemical space"[16].

Microbial "Dark Matter": Poorly studied phyla (*e.g.*, Acidobacteriota, Myxococcota) in these areas often encode a higher number of BGCs than known cultured strains[6], suggesting these hotspots are key for bioprospecting.

Interconnection:Biogeography+Biosynthesis and Taxonomy-Function Mismatch

Microbiome taxonomic composition does not always correlate strongly with biosynthetic potential. Finer-resolution studies are needed because closely related taxa can have vastly different metabolic capabilities[7].

Evolutionary Linkages: Hotspots that acted as long-term refugia (OCBILs—Old, Climatically-Buffered, Infertile Landscapes) are not only centers of species endemism but also potential hotspots for unique,

specialized metabolic pathways developed over long evolutionary timescales[1].

Unique Chemical Signatures: A study of marine sediments in Moorea showed that while primary metabolic genes (*e.g.*, nitrogen cycling) were redundant, specialized metabolic genes (BGCs) and the resulting compounds were highly distinct by site, driven by local interactions[16].

High-Level BGC Producers: Research in Shark Bay, Australia, revealed that Planctomycetes and Deltaproteobacteria are prolific producers of terpene and bacteriocin BGCs, which are crucial for adapting to extreme environmental conditions.

Global Mapping:Pyro-sequencing of soil microbes (nonribosomal peptide adenylation/polyketide ketosynthase domains) confirms that soil biosynthetic diversity correlates with geographic distance and biome type[1].

Future Directions (multi-omics and genomic approaches)

Multi-omics integrates genomics, transcriptomic and metabolomics to validate BGCs and to activate"cryptic" pathways. Comparative metabolomics identifies induced metabolites, reporter genes, screen activator, statistical tool like metabo Analyse dereplication known compounds.

Mining Actionmycete for Novel Antibiotics in the Omics Era-PMC

Increase in the number of partial and complete genome sequencing projects on the actinomycete species available in the public databases not only have confirmed their broad biosynthetic diversity across the different lineage but also enabled intensive genome mining approaches to untap new natural products scaffolds[1]. Relevant aspect of the impact of the increasing number of BGCs sequence information on antibiotics discovery is the possibility of developing specific targeted genome mining search in genomic libraries based on specific genomic signature related to the biosynthesis of preveleged Scaffold or functionalization that could drive the discovery of novel compounds and chemical spaces .one of the major challenges that still remain in the efficient cloning and expression of BGCs that are originally silent or poorly expressed in their natural host after the use of refactoring by the replacement of the regulatory elements and further detection of the synthesized compounds[14]. Many BGCs cannot be detected by the rules- based bioinformatic tool due to the absence of signature genes,but the application of prediction tools based on the frequencies of Pfam domains occuring in BGCs have improved the identification of additional clusters.New genomic bacterial artificial chromosomes(BAC) libraries built from large 100kb fragments of *Streptomyces* spp[13]. Genomics DNA are also used in the high throughput functional screening approaches to identify non-predicted BGCs by heterologous expression.

Genomic Approach

The discovery of *Streptomycin* from streptomyces, this genus has received considerable attention, being a primary source of antibiotics. Due to the repeated rediscovery of known compounds in the same ecological environments, as well as the associated cost[3]. Moreover, under laboratory conditions, microbes frequently cease SM production, further complicating drug discovery efforts. Many marin-derived Actinomycetota[19] genomes have been sequenced to evaluate their drug potential. Genome mining of marine sediment-derived *Streptomyces* sp. GMYO1 revealed 28 BGCs envolved in the production of flaviolin, genomic ectoine[2], class1V lanthipeptide /SFLA, albaflavenone and informatipetin. Genome mining of the deep sea- derived streptomyces antinioticus OUCT 16-23 revealed the present of fili pentypes polyene macrolids exhibiting antifungal activity against *Candida albicans*. Genome mining of marine streptomyces sp H-KF8 identified several nonribosomal peptides, leading to the design and synthesis of eight peptide,six of which showed antimicrobial[8] activity,with the two potentially disrupting membrane via a novel ion-passage mechanism. Apart from genus streptomyces, the Actinomycetota genera genome were also mined to find their secondary metabolites BGCs. Genome analysis of the genus salonispora, which was first described from a marine habitat, Nocardiopsis, harbors diverse BGCs for polypeptides, nonribosomal peptides, phenazine, bacteriocins, surfactins,and sanctipeptides[3] with many showing low similarity to known clusters, indicating potentials for novel natural products discovery.

Conclusion

This review has comprehensively examined the pivotal contributions of genome mining to revealing biosynthetic gene clusters (BGCs) for secondary metabolites in actinomycetes sourced from biodiversity hotspots, utilizing key platforms such as antiSMASH, BiG-SCAPE, MIBiG, and metabolic databases, in conjunction with multi-omics strategies and AI-powered advancements.

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https://doi.org/10.33451/florafauna.v32i1pp105-109
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 105-109 ISSN 0971 - 6920 (Print)

Occurrence and seasonal variation of a new parasite species (Family-Hemiuridae) in fresh water fish, *Amphipnous cuchia*

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Received **:** 20.02.2026; ***Accepted*** : 11.04.2026

How to cite : Sabhyata, Tandon P. Occurrence and seasonal variation of a new parasite species (Family-Hemiuridae) in freshwater fish, *Amphipnous cuchia Flora and Fauna* 2026. 32(1) : 105-109.

ABSTRACT

The present paper deals with the study of the Occurrence and Seasonal Variation of a new digenetic trematode parasite, *Genarchopsis varunai* sp. nov., belonging to the family Hemiuridae, in the intestine of the freshwater fish *Amphipnous cuchia* from the River Varuna (tributary of the River Ganges), Varanasi. About 401 specimens of *Amphipnous cuchia* were examined, of which 79 were infected, and 136 mature parasites were collected from March 2024 to February 2025. In this paper, the key to the species of the genus *Genarchopsis* is also provided.

Figures : 03 References : 10 Tables : 02
KEY WORDS : *Amphipnous*, Hemiuridae, Parasite, River Varuna, Seasonal variation.

Introduction

Since the human race settled and flourished adjacent to the aquatic reservoirs, fish have attracted their attention. The growing human population has increased food problems in our Country, due to which fish has become a valuable and easily accessible source of food, and is used to overcome the food problems to a certain extent. The majority of fish carry heavy parasitic infections, which reduce the growth and create problems for fish culturists. Fish also acts as an intermediate source for transmitting helminthic infections to human beings through its consumption. Considerable work has been done on the helminth parasites of freshwater fishes[9,10], but only a few Indian workers have tried to make seasonal observations of digenetic trematodes of freshwater fishes. In this paper, it is expected to highlight the incidence and intensity of infection of these parasites, belonging to the family Hemiuridae[7], so that proper measure may be taken in time to control them.

Methodology

The fish were collected from the River Varuna (tributary of the River Ganges), Varanasi, during the period of March 2024 to February 2025. For the collection of digenetic trematodes, visceral organs of the fishes were dissected out separately in the petri-dishes containing 0.7% saline. The organs were slit opened with the help of scissors and forceps, so as to allow the worms to lose contact with the tissues and come out to settle down at the bottom of the petri-dish. The worms were picked up with the help of a dropper, washed thoroughly in the saline solution, and finally fixed in 70% alcohol under the slight pressure of a cover glass for twenty-four hours to prevent curling. After fixation, the parasites were removed from the cover glass, washed in 70% alcohol to remove excess fixative, and finally preserved in 70% alcohol containing 5% glycerin. For permanent preparation, the worms were stained in acetic alum carmine, differentiated in acid water, dehydrated in an

ACKNOWLEDGEMENTS : 1st author conveys her deep gratitude and regards to parents and family members for their inspiration, understanding and support. We sincerely by thank the Principal, D.A-V. College, Kanpur, Prof. Arun Kumar Dixit for providing the necessary research facilities. Special thanks to the fishermen who provided the fish throughout the year, along with all the colleagues and non-teaching staff for their constant encouragement and suggestions.

TABLE -1 : Key to the species of genus *Genarchopsis*[5]

(1)	Oesophageal pouch present	…….(2)
	Oesophageal pouch absent	…….(7)
(2)	Receptaculum seminist present	*G. punctati* [1]…….
	Receptaculum seminist absent	(3)
(3)	Mehlis gland present	…….(4)
	Mehlis gland absent	…….(5)
(4)	Genital pore anterior to intestinal bifurcation in the mid-level of pharynx	*G. ophiociphali*[6]
	Genital pore far behind intestinal bifurcation	*G. cuchiai*[3]
	Genital pore on the ventral side of the left intestinal bifurcation	*G.faruquis*[2,10]
(5)	Vittelaria in the form of two unlobed masses	*G. singularis*[8]
	Vittelaria in the form of two lobed masses	…….(6)
(6)	Uterus far from vitellaria	*G. lobatum*[2,9]
	Uterus near to vitellaria	*Genarchopsis varunai* sp. nov.
(7)	Uterine coil intercaecal	*G. dasus*[2,10]
	Uterine coil extracaecal	…….(8)
(8)	Genital pore in the level of pharynx	…….(9)
	Genital pore not in the level of pharynx	……(10)
(9)	Testes intercaecal	*G. cameroni*[3]
	Testes extracaecal	*G. piscicola*[4,8]
(10)	Extension of uterine coil posteriorly upto vitelline region	*G. ovocaudatum*[4,8]
	Extension of uterine coil anterior to caecal union posteriorly	*G. indicus*[2,10]

ascending series of alcohol, cleared in clove oil, and mounted in Canada balsam.

Photographs were taken with the help of a DSLR camera; diagrams were made with the aid of a camera

TABLE-2 : Data showing incidence and intensity of infection of *Genarchopsis varunai* sp. nov.

Months	Fishes examined	Fishes infected	No. of parasites	% of infection	Mean no. of parasites / infected fishes
Mar.'24	35	14	34	40.00	2.43
Apr.	40	07	13	17.50	1.86
May	37	06	10	16.22	1.67
Jun.	34	04	06	11.76	1.50
Jul.	27	05	07	18.52	1.40
Aug.	25	04	05	16.00	1.25
Sep.	34	07	12	20.59	1.71
Oct.	42	06	09	14.29	1.50
Nov.	38	07	10	18.42	1.43
Dec.	31	06	11	19.35	1.83
Jan.'25	28	05	07	17.86	1.40
Feb.	30	08	12	26.67	1.50
Total	401	79	136		

lucida; graphs were drawn with the help of MS Excel, and all the measurements (in mm) were taken by using a stage micrometer.

Result and Discussion

The present form is referred to the genus *Genarchopsis*[5], of which, so many species *G. ovocaudatum*[4,8]; *G. piscicola*[4,8]; *G. singularis*[8-10]; *G. lobatum*[8-10]; *G. faruquis*[2,9,10]; *G. dasus*[2,9,10]; *G. indicus*[2,9,10]; *G. punctati*[1]; *G. cuchiai*[3]; *G. cameroni*[3] and *G. ophiocephali*[6] have been described so far from freshwater fishes of India. This form differs from all these species; hence, it is regarded as a new species with the specific name *Genarchopsis varunai* sp. nov.

Seasonal Variation in the incidence and intensity of infection of *Genarchopsis varunai* sp. nov. in freshwater fish *Amphipnous cuchia*.

The 401 specimens of *Amphipnous cuchia* examined include 79 infected fishes, from which 136 mature *Genarchopsis varunai* sp. nov. were collected. This represented an overall percentage of infection of 19.70% and 1.72 worms per infected fish.

The incidence and intensity of infection were recorded during the period from March 2024 to February 2025 (Table-2). The fluke occurred throughout the year, but in varying intensities. Over the period of 12 months, the percentage of infection varied from 11.76% to 40.00%. The highest infection was recorded in March, 2024, when 40.00% of the fish were found infected with the fluke. The infection was minimal during June 2024, when 11.76% of the fish examined were infected. In the remaining months, the infection fluctuated between 14.29% to 26.67%.

The intensity of infection during the same period

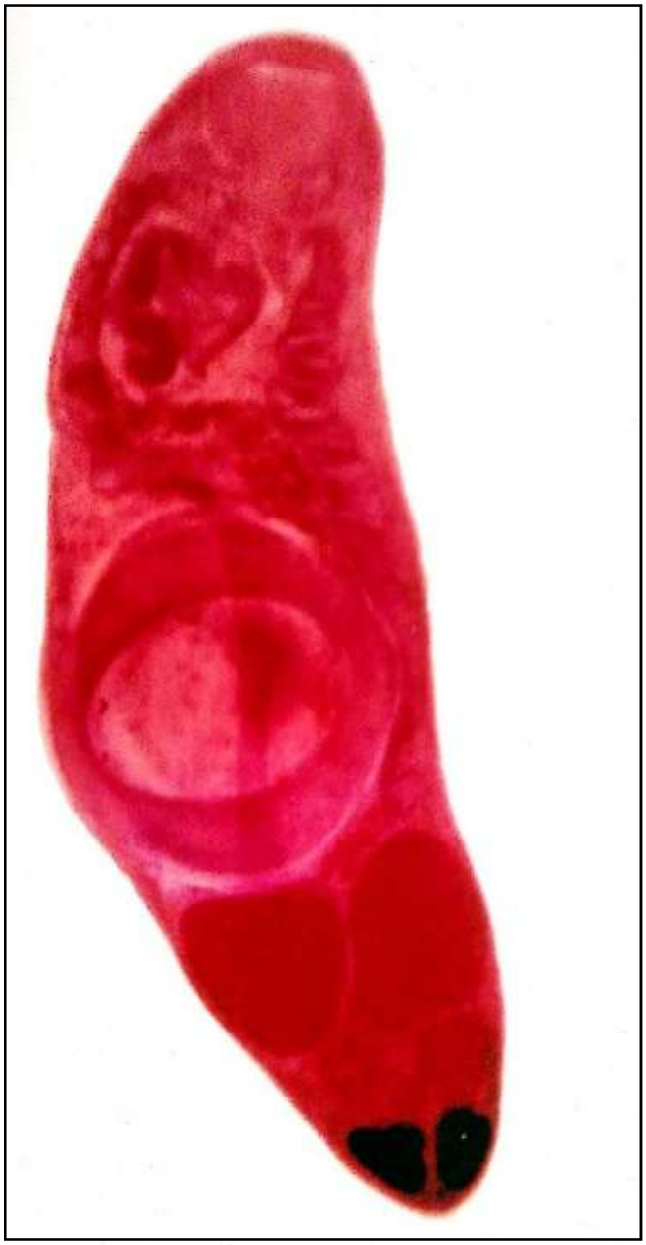

Fig. 1 : *Genarchopsis varunai* sp. nov.

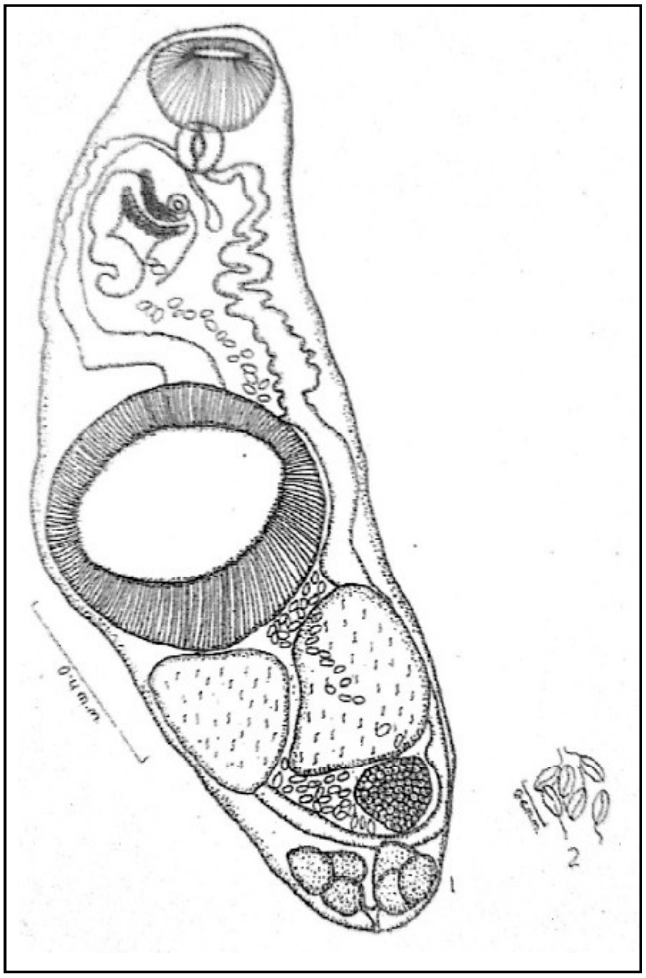

Fig. 2 : *Genarchopsis varunai* sp. nov.

1. **Entire view**
2. **Eggs**

showed different trends. The maximum intensity was recorded in March 2024, when the percentage of infection was also at its maximum level. The lowest intensity was recorded during August 2024, when the percentage of infection was 16.00%. In the remaining

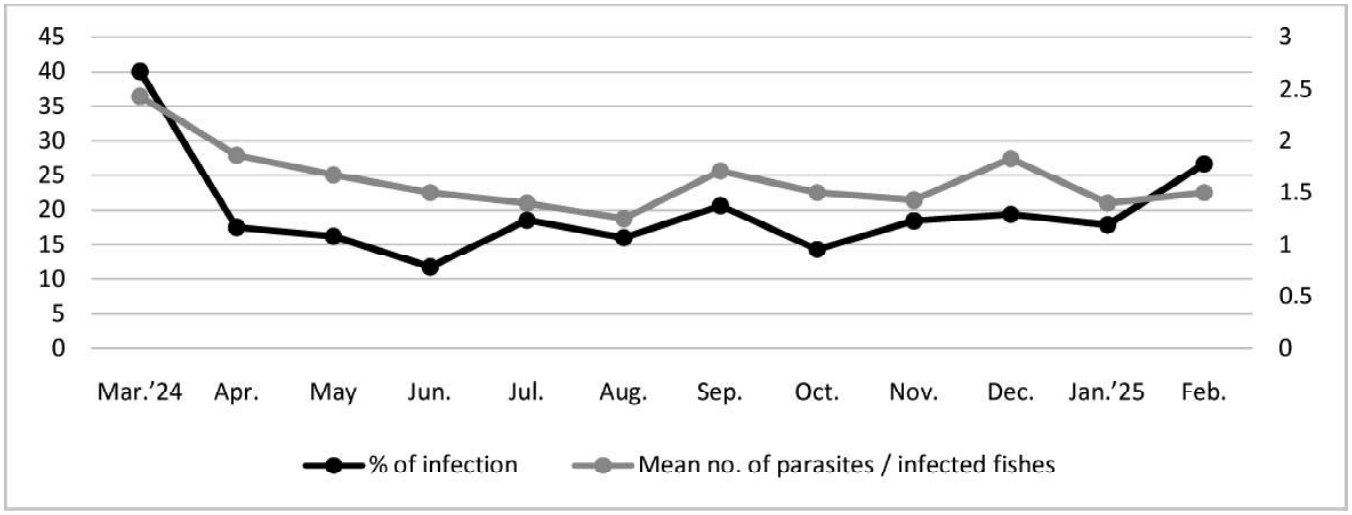

Fig. 3 : Showing incidence and intensity of infection of *Genarchopsis varunai* sp. nov.

months, the intensity varied between 1.40 and 1.86.

Conclusion

As per the above discussion, this paper records the seasonal variation and intensity of infection in *Genarchopsis varunai* sp. nov.and also specifies its key for species identification.

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https://doi.org/10.33451/florafauna.v32i1pp110-120
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 110-120 ISSN 0971 - 6920 (Print)

Medicinal plants used in treatment of Livestock diseases by tribal and local people of Vidarbha region (MS), India

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Received **:** 11.03.2026; ***Accepted*** : 08.04.2026

How to cite : Humane P. Medicinal plants used in treatment of Livestock diseases by tribal and local people of Vidarbha region (MS), India. *Flora and Fauna* 2026. 32(1) : 110-120.

ABSTRACT

Livestock rearing forms an integral part of the agrarian economy in the Vidarbha region of Maharashtra, India, where rural and tribal populations rely heavily on cattle, goats, and buffaloes for milk, and meat purposes. The region, characterized by tropical dry deciduous forests and diverse flora, supports a rich repository of traditional knowledge related to ethnoveterinary practices. However, due to limited access to modern veterinary facilities, tribal and local communities continue to depend on medicinal plants for treating a wide variety of livestock ailments. Commonly reported diseases include foot-and-mouth diseases, bloat, wounds, diarrhoea, mastitis, skin infections, and parasitic infestations, which significantly affect animal health and agricultural productivity.

The present study documents the use of medicinal plants in livestock healthcare by the local people of Vidarbha. A total of **55 angiosperms plant species** were identified, belonging to **36 families** and **52 genera**. Among them, **31 families (43 genera)** were dicotyledons, while **5 families (9 genera)** were monocotyledons, indicating the dominance of dicot taxa in ethnoveterinary medicine. These plants are administered in various forms such as decoctions, pastes, powders, and extracts, often prepared using traditional methods passed orally across generations. The wide range of species recorded reflects both the ecological diversity of Vidarbha and the rich indigenous knowledge base of its inhabitants.

The findings of this study underline the critical role of medicinal plants in sustaining livestock health in rural economies and highlight the urgent need for systematic pharmacological validation, conservation of bioresources, and preservation of traditional knowledge for future veterinary applications.

Figures : 03 References : 39 Table : 01
KEY WORDS : Angiosperms, Ethnoveterinary medicine, Livestock diseases, Medicinal plants, Vidarbha region,

Introduction

Ethnoveterinary medicine (EVM) refers to the traditional knowledge, practices, and beliefs associated with the healthcare of livestock, developed over generations by rural and indigenous communities[1.21]. It encompasses the use of medicinal plants, minerals, and other natural products, as well as ritualistic and management practices, for the treatment of animal diseases[1]. In developing countries such as India, ethnoveterinary practices continue to play a crucial role in livestock healthcare, particularly in areas where access to modern veterinary services is limited or economically unfeasible[2].

India is one of the world's richest regions in terms of biodiversity and cultural diversity, and this combination has nurtured a vast repertoire of ethnomedicinal knowledge. Around 70–80% of rural communities in India rely on traditional medicine, both for themselves and for their animals[2]. Tribal communities inhabiting forest regions, including central India, have particularly preserved diverse plant-based veterinary practices that form the backbone of local animal healthcare systems[2].

ACKNOWLEDGEMENTS : The author is highly grateful to the local people and tribal communities of the Vidarbha region, Maharashtra, for generously sharing their traditional knowledge of medicinal plants used in livestock healthcare. Their cooperation and trust made this study possible. Special thanks are extended to farmers, cattle herders, and village elders who provided valuable information on ethnoveterinary practices.

The Vidarbha region of Maharashtra lies in central India, occupying about one-third of the state's geographical area. The region is characterized by tropical dry deciduous forests, basaltic soil derived from Deccan Traps, and a predominantly hot and dry climate[12]. Rich in biodiversity, Vidarbha is home to several forest species such as *Tectona grandis*, *Madhuca longifolia* var. *latifolia*, *Bambusa bambos* and *Terminalia* spp., which are widely used for both human and animal health remedies[2] .

Livestock rearing is an integral part of the rural economy, providing dairy products, manure, draught power, and supplementary income. Small holders and tribal communities depend heavily on cattle, buffalo, goats, and poultry for subsistence[22]. However, recurrent droughts, crop failures, and inadequate veterinary infrastructure have made farmers increasingly vulnerable, thereby emphasizing the significance of traditional animal healthcare systems[2].

In Vidarbha, livestock suffers from a wide range of ailments, including infectious diseases such as foot-and-mouth diseases, haemorrhagic septicaemia, black quarter, and mastitis[1]. Emerging threats such as lumpy skin disease and tick-borne illnesses further complicate livestock health management[1].

Although government veterinary hospitals and dispensaries exist, their accessibility is uneven, particularly in remote tribal and forested areas[2]. High costs of modern veterinary drugs, poor transportation, and a lack of trained manpower exacerbate the problem[2]. Consequently, traditional herbal remedies remain the primary source of treatment for many rural households in Vidarbha.

Ethnoveterinary research across India has documented a rich variety of plant species used for animal healthcare. In Bundelkhand, 41 species from 25 families were reported for treating livestock ailments[31]. In Uttarakhand, 73 species were identified, employed in the management of bloat, diarrhoea, pneumonia, and mastitis[2]. From Meghalaya, 96 species were documented for treating foot-and-mouth diseases, bone fractures, and digestive disorders[1]. Similar findings were reported from Assam[2], Madhya Pradesh[33], and Keralam[3].

Within Maharashtra, several ethnobotanical surveys have identified medicinal plants used by tribal groups in Gadchiroli, Chandrapur, and Bhandara districts for both human and veterinary healthcare[3] . However, comprehensive studies on ethnoveterinary practices specific to Vidarbha remain relatively scarce. One notable survey recorded 46 plant species used by Gond and Madia tribes in Gadchiroli for treating livestock diseases such as wounds, abortions, and digestive disorders[1].

The present investigation adds significantly to the ethnoveterinary literature by documenting 55 plant species used in livestock healthcare in Vidarbha. These belong to 36 families and 52 genera, with dicotyledonous taxa clearly dominating; 31 families and 43 genera are dicots, while 5 families and 9 genera are monocots. This pattern is consistent with findings from other regions of India, where dicots are more frequently represented in ethnomedicinal practices due to their greater phytochemical diversity.

The plants identified in this study were reported by tribal and local people to be useful for treating a broad range of conditions like wounds, mastitis, gastrointestinal disorders, reproductive problems, skin infections, and parasitic infestations. Remedies are typically prepared as pastes, decoctions, or powders, often administered orally or applied externally, and are based on traditional methods transmitted orally across generations[1,2].

The documentation of ethnoveterinary knowledge in Vidarbha is significant for several reasons. It provides a scientific record of local knowledge systems at risk of erosion due to cultural change and modernization[1]. It highlights the biodiversity of Vidarbha as a source of medicinal plants with potential pharmacological importance. In light of increasing drug resistance and the high cost of modern veterinary medicine, ethnoveterinary remedies may provide affordable and effective alternatives for smallholder farmers[3].

Finally, the integration of traditional practices into formal animal healthcare, through validation and conservation of medicinal plants, could strengthen sustainable livestock management in the region.

Material and Methods

Study Area : The research was carried out in the Vidarbha region of Maharashtra, which covers 11 districts: Nagpur, Wardha, Bhandara, Gondia, Chandrapur, Gadchiroli, Amravati, Yavatmal, Akola, Washim, and Buldhana (**Fig. 1**). Geographically, the region lies in the eastern part of the state between 18°302 –21°452 N latitudes and 76°302 –80°452 E longitudes. The climate is predominantly tropical, with hot summers, monsoon rainfall, and moderate winters. The eastern districts are rich in forests dominated by tropical dry deciduous vegetation, which supports a high diversity of medicinal plants. The population here is largely rural and tribal, dependent on agriculture and livestock rearing, with traditional ethnoveterinary practices forming an essential part of animal healthcaret [,13].

Data Collection : Field surveys were conducted in rural villages and forest-fringe areas from 2015 to 2024. Data on medicinal plants used in livestock healthcare were

TABLE-1: List of Ethnoveterinary Medicinal Plants

S. No.	Specimen No.	Scientific Name	Ver. Name	Family	Part Used	Disease/s
1	PTH0459	*Acacia leucophloea*	Hiwar	Fabaceae	Leaves	Stomach problems
2	PTH0439	*Acacia nilotica*	Babhul	Fabaceae	Flowers / Bark	Jaundice / dysentery
3	PTH0927	*Acorus calamus*	Yakad	Acoraceae	Rhizome	Skin infestations
4	PTH0616	*Aegle marmelos*	Bel	Rutaceae	Leaves / Seeds	Sun-burns
5	PTH0600	*Allium cepa*	Kanda	Liliaceae	Bulb / Leaves	Cough / Ectoparasites
6	PTH0776	*Anagalis arvensis*		Primulaceae	Whole plant	Expel leeches from nostrils
7	PTH0038	*Annona squamosa*	Sitaphal	Annonaceae	Seeds	Tick bites
8	PTH0258	*Argemone mexicana*	Utati	Papaveraceae	Leaves / Seeds	Food infection / rheumatism
9	PTH0983	*Asparagus racemosus*	Marbat	Liliaceae	Roots	Arthritis
10	PTH0320	*Azadirachta indica*	Neem	Meliaceae	Leaves	Wounds
11	PTH0923	*Bambusa arundinacea*	Bas	Poaceae	Leaves / Roots	Easier delivery / diarrhoea
12	PTH0187	*Basella alba*	Dalbhaji	Basellaceae	Leaves	Weaknesses
13	PTH0315	*Butea monosperma*	Palas	Fabaceae	Flowers	Dysurea / Paralysis

S. No.	Specimen No.	Scientific Name	Ver. Name	Family	Part Used	Disease/s
14	PTH0831	*Calotropis procera*	Rui	Asclepiadaceae	Flowers / Latex	Easier delivery / Snake bite
15	PTH0325	*Carrisa congesta*	Karvand	Apocynaceae	Roots / Leaves	Ephemeral fever
16	PTH0397	*Cassia fistula*	Bahawa	Fabaceae	Leaves / Fruits	Indigestion / apetite / constipation
17	PTH0489	*Cissampelos pareira* var. *hirsuta*		Menispermaceae	Roots / Leaves	Scorpion stings
18	PTH0796	*Coriandrum sativum*	Sambhar	Apiaceae	Leaves / Fruits	Loose motion
19	PTH0528	*Curcuma longa*	Halad	Zingiberaceae	Rhizome	Foot-and-mouth
20	PTH0804	*Cynodon dactylon*	Harari	Poaceae	Leaves / Stem	Increase lactation / Conjuctivitis
21	PTH0534	*Datura metel*	Kala Dhotra	Solanaceae	Roots / Leaves / Fruits	Wounds / Cold
22	PTH0410	*Delonix regia*	Gulmohar	Fabaceae	Bark	Fever
23	PTH0152	*Eclipta prostrata*	Maka	Asteraceae	Leaves	Wounds
24	PTH0580	*Ficus benghalensis*	Wad	Moraceae	Roots	Stomachache
25	PTH0839	*Ficus religiosa*	Pimpal	Moraceae	Leaves	Tonsils
26	PTH0460	*Gardenia gummifera*	Dikemali	Rubiaceae	Leaves	Wounds and skin lesions

S. No.	Specimen No.	Scientific Name	Ver. Name	Family	Part Used	Disease/s
27	PTH0706	*Hibiscus cannabinus*	Ambadi	Malvaceae	Leaves / Flowers	Ring worms
28	PTH0041	*Hibiscus rosa-sinensis*	Jaswand	Malvaceae	Bark	Twitching
29	PTH0814	*Holoptelea integrifolia*	Chilar	Urticaceae	Leaves	Ectoparasites
30	PTH0642	*Impatiens balsamina*	Chiwdi	Balsaminaceae	Leaves	Worts in nipples
31	PTH0805	*Justicia adhatoda*	Adulsa	Acanthaceae	Leaves / Bark	Diarrhoea / dysentery
32	PTH0568	*Limonia acidissima*	Kawath	Rutaceae	Leaves	Intestinal worms
33	PTH0307	*Madhuca longifolia* var. *latifolia*	Moha	Sapotaceae	Flowers	Fever
34	PTH0299	*Mangifera indica*	Aam	Anacardiaceae	Fruits	Indigestion
35	PTH0567	*Moringa oleifera*	Mungna	Moringaceae	Roots / Leaves / Fruits	Ulcers / Diarrhoea / dysentery / Rheumatism
36	PTH0564	*Musa paradisiaca*	Kel	Musaceae	Roots / Leaves	Body heat
37	PTH0140	*Ocimum tenuiflorum*	Tulas	Lamiaceae	Leaves	Cough / Cold
38	PTH0034	*Oryza sativa*	Dhan	Poaceae	Grains	Increase lactation
39	PTH0481	*Psidium guajava*	Peru	Myrtaceae	Leaves	Fever
40	PTH0265	*Ricinus communis*	Erand	Euphorbiaceae	Seeds	Constipation
41	PTH0819	*Sapindus emarginatus*	Ritha	Sapindaceae	Bark	Wounds by Worm infestation after calving

S. No.	Specimen No.	Scientific Name	Ver. Name	Family	Part Used	Disease/s
42	PTH0477	*Securinega virosa*	Pandhri pisaundi	Euphorbiaceae	Leaves	Dysentery
43	PTH0518	*Semecarpus anacardium*	Bibba	Anacardiaceae	Seeds	Foot-and-mouth
44	PTH0352	*Soymida febrifuga*	Rohan	Meliaceae	Bark	Foot-and-mouth
45	PTH0382	*Sterculia urens*	Kadai	Sterculiaceae	Leaves and stem mucilage	Pleuropneumonia
46	PTH0314	*Syzygium cumini*	Jambhul	Myrtaceae	Bark	Joint pain
47	PTH0543	*Tagetes erecta*	Zendu	Asteraceae	Leaves	Hydrophobia
48	PTH0406	*Tamarindus indica*	Chinch	Fabaceae	Leaves / Fruits	Swellings / tongue sores
49	PTH0337	*Terminalia chebula*	Hirda	Combretaceae	Fruits	Tick infestations
50	PTH0519	*Tinospora cordifolia*	Gulvel	Menispermaceae	Leaves	Maggot wounds
51	PTH1044	*Tribulus terrestris*		Zygophyllaceae	Leaves	Colic / Cough
52	PTH0797	*Trigonella foenum-graecum*	Methi	Fabaceae	Seeds	Easier delivery
53	PTH0377	*Vitex negundo*	Nirgudi	Verbenaceae	Leaves	Diarrhoea
54	PTH0926	*Zingiber officinale*	Adrak	Zingiberaceae	Rhizome	Physical disability
55	PTH0266	*Ziziphus mauritiana*	Bor	Rhamnaceae	Leaves / Seeds	Skin burns

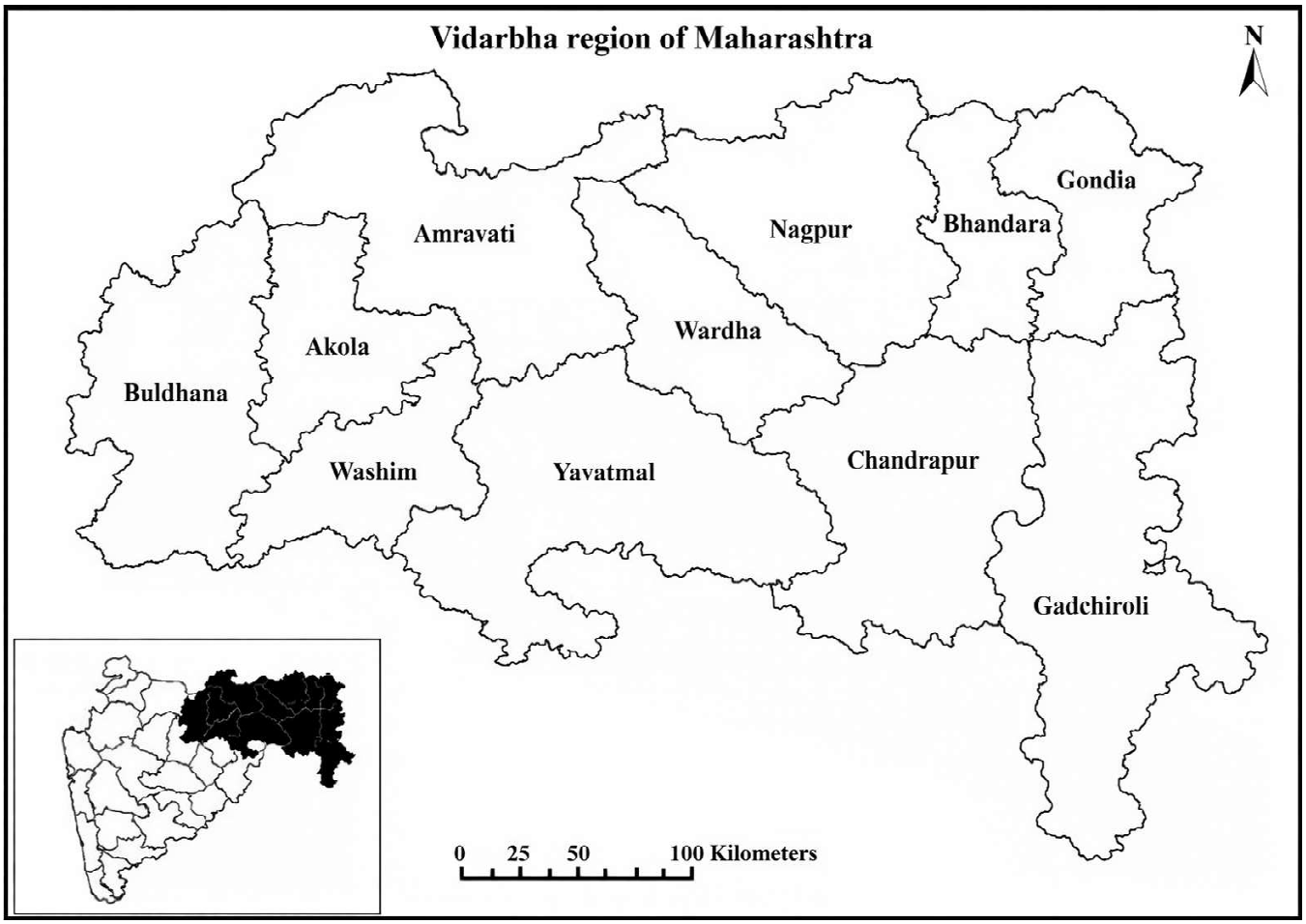

Fig. 1: Vidarbha region in Maharasthra state[17]

gathered using semi-structured interviews, open-ended questionnaires, participatory rural appraisal, and group discussions with local healers, livestock rearers, and elderly community members. The information collected included vernacular names, plant parts used, methods of preparation, routes of administration, and diseases treated. To enhance reliability, data were cross-verified through repeated discussions with different informants[1].

Plant Collection and Identification

All reported plants were collected during field visits, with the help of informants. Collected specimens were dried, pressed, mounted on herbarium sheets, and preliminarily identified in the field. For taxonomic authentication, specimens were compared with standard references such as *Flora of Maharashtra State*[3] and *Flora of Nagpur District*[3]. Voucher specimens were deposited in the departmental herbarium of Botany department, Dharampeth M. P. Deo Memorial Science College, Nagpur for future reference.

Results and Discussion

The present study documented **55 angiosperm species**, distributed across **52 genera and 36 families**, used by tribal and local communities of the Vidarbha region for treating livestock ailments. Among these, **dicotyledons** were dominant with **46 species (43 genera, 31 families)**, while **monocotyledons** were represented by **9 species (9 genera, 5 families)** (**Table-1**).

The family Fabaceae contributed the maximum number of species (7 spp.), followed by Euphorbiaceae, Malvaceae, Myrtaceae, Moraceae, Menispermaceae, and Poaceae (2–3 spp. each). The prominence of Fabaceae is consistent with ethnomedicinal studies across India and Africa, where members of this family are widely employed in both human and veterinary medicine[1,32].

Different plant parts were employed in the treatment of livestock diseases. **Leaves were the most frequently used plant part (33 species, *i.e.,* 42%)**, followed by roots (8 spp.), fruits (6 spp.), seeds (7 spp.), bark (7 spp.), flowers (4 spp.), whole plants (3 spp.), bulbs (1 sp.), rhizomes (2 sp.), stem mucilage (1 sp.), and latex (1 sp.) (**Fig. 2**).

The dominance of leaves is attributed to their accessibility, year-round availability, and richness in bioactive compounds . Similar trends were reported in

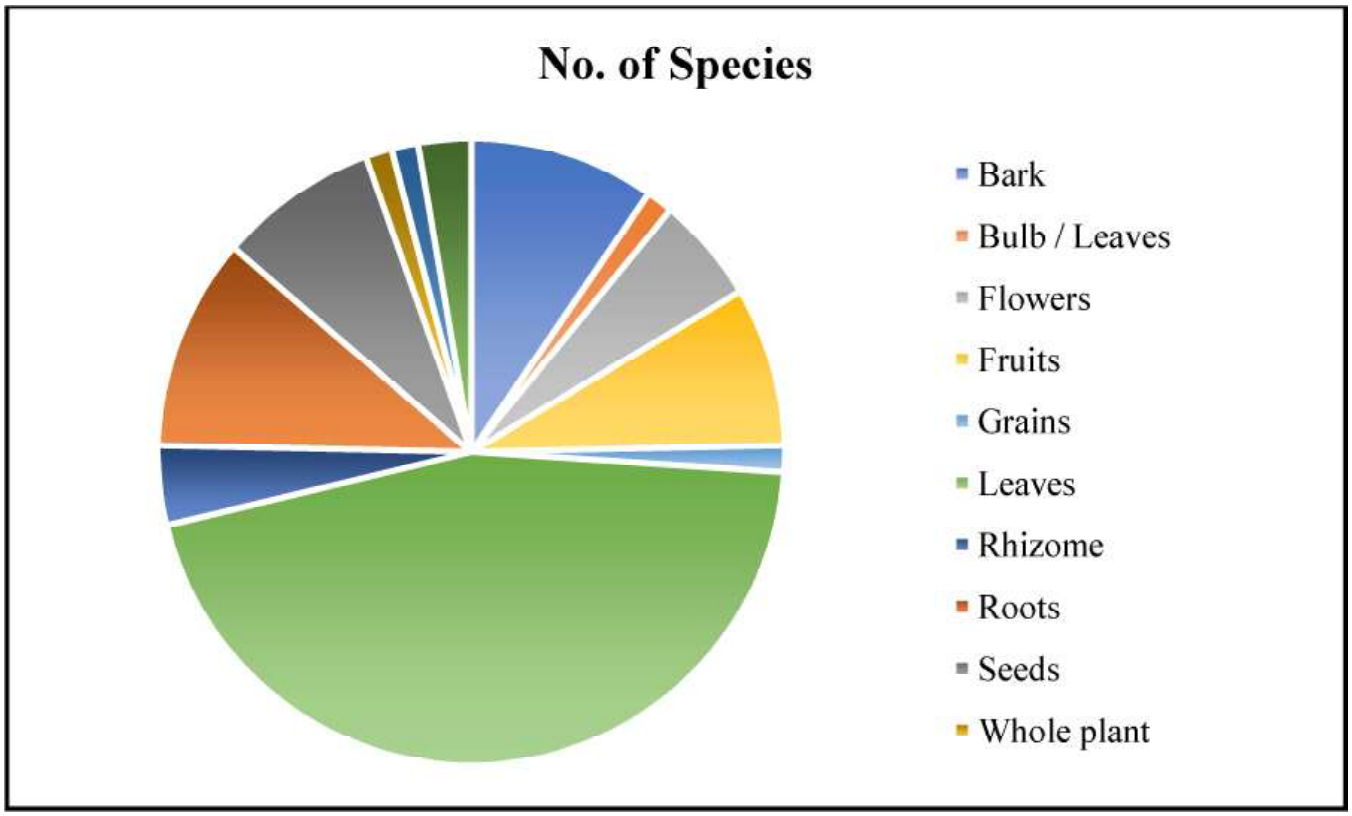

Fig. 2 : Plant part used

ethnoveterinary studies from Madhya Pradesh[3], Andhra Pradesh[2] and Kenya[23], where leaves formed the bulk of preparations for livestock treatments. Use of underground parts (roots, bulbs, rhizomes) was comparatively lower, possibly due to ecological and cultural concerns over plant destruction during harvesting.

The ethnomedicinal repertoire covered a broad spectrum of livestock ailments. The most frequently reported categories of diseases included gastrointestinal disorders like diarrhoea, dysentery, stomach-ache and indigestion (12 species), dermatological conditions like wounds, skin infections and burns (8 species), respiratory diseases like cough, cold, fever (7 species), and reproductive problems like ease of delivery, lactation increase (5 species). Other conditions treated included foot-and-mouth, arthritis, rheumatism, colic, mastitis, and parasitic infestations (Fig. 3).

These findings resonate with earlier observations from Maharashtra, Gujarat, and Karnataka, where gastrointestinal and dermatological ailments were dominant in ethnoveterinary applications. Since livestock in tropical regions are frequently exposed to contaminated fodder, unhygienic water, ticks, and parasites, community reliance on herbal remedies for such ailments reflects ecological pressures and livestock management realities.

Medicinal plants were administered in various forms including decoctions, pastes, juices, powders, and extracts. Oral administration was common for gastrointestinal, febrile, and systemic diseases, while topical applications were preferred for wounds, skin burns, and external parasitic infestations. These traditional preparation methods are consistent with other ethnoveterinary surveys in India[2,33], suggesting a strong cultural continuity in indigenous veterinary practices.

The high number of species documented reflects both the ecological diversity of Vidarbha and the cultural depth of indigenous knowledge. However, as noted in similar studies, such knowledge is largely transmitted orally and is increasingly at risk due to rapid socio-economic changes[11]. Documentation of these practices is therefore critical not only for the preservation of traditional wisdom but also for the identification of novel pharmacological leads for veterinary science.

Moreover, several species recorded in the present study e.g., *Azadirachta indica*, *Terminalia chebula*, *Aegle marmelos*, *Calotropis procera* are already known for their antimicrobial, antiparasitic, and anti-inflammatory properties, validating their ethnoveterinary uses[1,3]. Systematic phytochemical and pharmacological evaluation of such species is required to standardize dosages and ensure safety in veterinary practice.

The frequent use of roots, bark, and whole plants

in some remedies may threaten wild populations if harvesting is unsustainable. Conservation of medicinal plants, combined with awareness among local communities about sustainable harvesting techniques, is essential. Integration of ethnoveterinary knowledge with biodiversity conservation programs can help secure both livestock healthcare and ecological balance in the Vidarbha region[3,11].

Conclusion

The present investigation documents 55 angiosperms plant species belonging to 36 families and 52 genera that are traditionally used by the local people of Vidarbha region for treating livestock diseases. The findings emphasize the continued reliance of local people and tribal communities on medicinal plants for ethnoveterinary care, particularly in areas where access to modern veterinary services is limited. The predominance of dicotyledonous taxa reflects their phytochemical diversity and therapeutic value, while the frequent use of leaves demonstrates the preference for easily accessible and renewable plant parts.

A wide spectrum of ailments including foot-and-mouth, diarrhoea, dysentery, wounds, mastitis, skin infections, arthritis, and reproductive complications are managed through plant-based remedies, generally prepared as pastes, decoctions, powders, or juices. This knowledge, orally transmitted through generations, illustrates the resilience and ecological understanding of the indigenous people of Vidarbha. However, threats such as deforestation, overharvesting, and erosion of

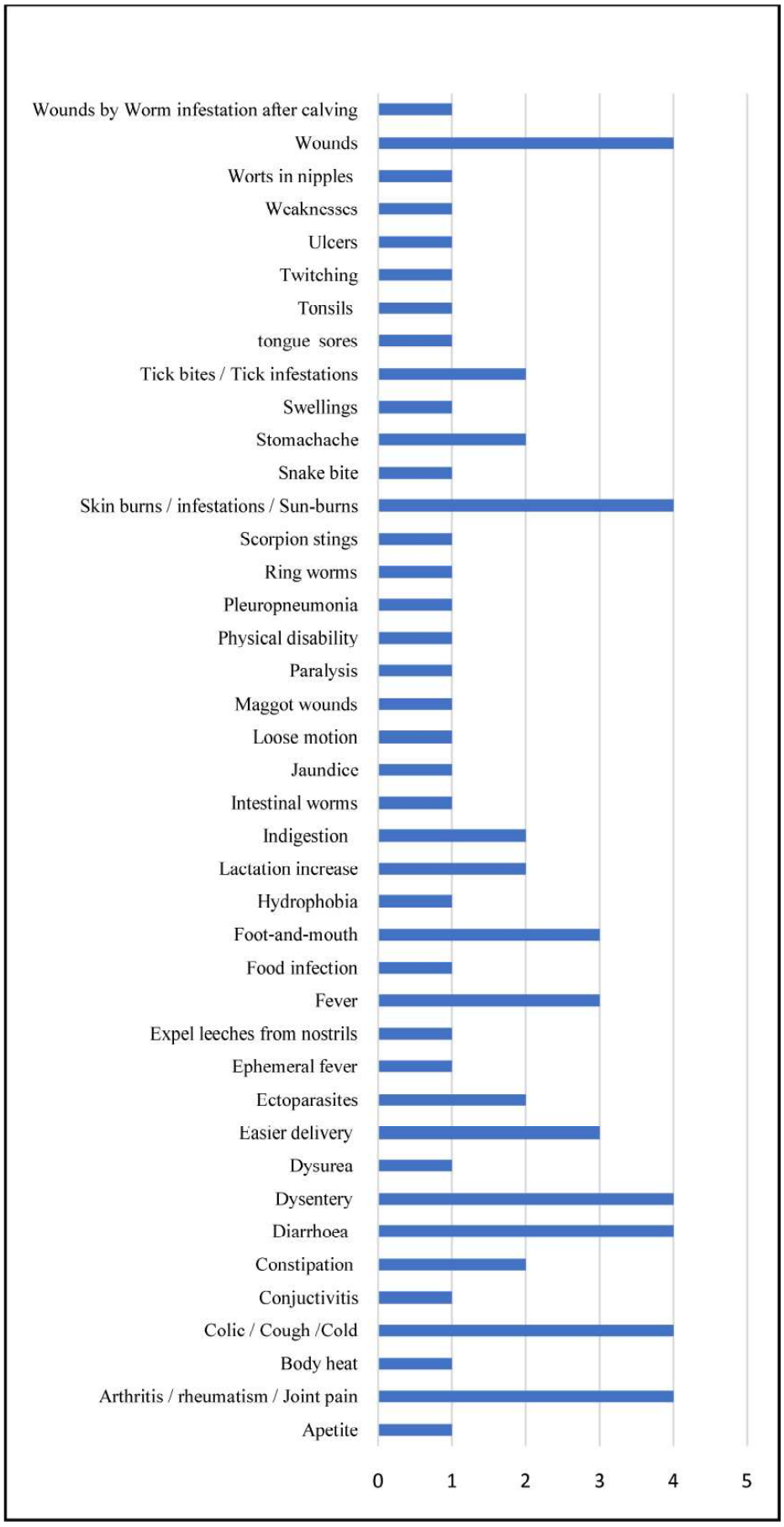

Fig. 3: Number of species claimed

traditional culture place this valuable heritage at risk.

Thus, ethnoveterinary practices in Vidarbha region not only contribute significantly to livestock health and rural livelihoods but also represent a potential source for developing novel veterinary drugs.

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https://doi.org/10.33451/florafauna.v32i1pp121-126
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 121-126 ISSN 0971 - 6920 (Print)

Microbial and heavy metal profiling of marketed *Jeerakarishta* : ensuring safety and standardization of a traditional Ayurvedic formulation

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Received **:** 11.03.2026; ***Accepted*** : 08.05.2026

How to cite : Dwivedi S, Koka SS. Microbial and heavy metal profiling of marketed *Jeerakarishta* : ensuring safety and standardization of a traditional Ayurvedic formulation. *Flora and Fauna* 2026. 32(1) : 121-126.

ABSTRACT

Jeerakarishta, a classical Ayurvedic fermented formulation (Arishta), is widely prescribed for digestive disorders and postnatal care. This study conducted a comprehensive quality evaluation of two commercially available *Jeerakarishta* formulations following strict guidelines from the *Ayurvedic Pharmacopoeia of India* (AFI), World Health Organization (WHO), and Central Council for Research in Ayurvedic Sciences (CCRAS). The evaluation encompassed organoleptic, physicochemical, chromatographic, microbial, and toxicological parameters. Results indicated compliance with standard limits for most parameters, though alcohol content (27% v/v) exceeded the AFI limit (d"12% v/v). Heavy metals (As, Cd, Hg, Pb) were within permissible limits (As: 0.13-0.22 ppm, Cd: 0.0007-0.02 ppm, Hg: 0.003-0.08 ppm, Pb: 0.002-0.11 ppm). Microbial counts were within acceptable ranges (TBC <10u CFU/mL, TFC <10^3 CFU/mL). Both formulations demonstrated acceptable quality and safety profiles, though variations in physicochemical parameters suggest differences in manufacturing processes. This study underscores the importance of systematic quality control in ensuring the safety and efficacy of Ayurvedic formulations.

Figure : 00 References : 20 Tables : 04

KEY WORDS : AFI guidelines, Ayurvedic formulation, CCRAS standards, Heavy metal analysis, *Jeerakarishta*, Microbial contamination, Quality control, WHO protocols.

Introduction

Ayurveda, the ancient Indian system of medicine, has experienced a global resurgence due to its holistic approach and perceived safety. Among its diverse pharmaceutical preparations, *Asava* and *Arishta* represent unique fermented dosage forms prepared through *sandhanakalpana*, a process involving self-generated alcohol fermentation without external alcohol addition. These formulations are valued for their extended shelf life, enhanced therapeutic efficacy, and improved bioavailability due to the presence of self-generated ethanol, which acts as both a preservative and a solvent for active constituents[12,13].

Jeerakarishta, a classical Ayurvedic *Arishta*, is primarily indicated for digestive disorders (*ajirna*, *grahani*), postnatal weakness (*sutika kala*), poor appetite (*aruchi*), and gastrointestinal disturbances. Its chief ingredient, *Cuminum cyminum* (Jeeraka), is renowned for its *deepana* (appetizer) and *pachana* (digestive) properties. The formulation typically contains other medicinal herbs including Patha (*Cissampelos pareira*), Ela (*Elettaria cardamomum*), Mustaka (*Cyperus rotundus*), Shunthi (*Zingiber officinale*) and Dhataki flowers (*Woodfordia fruticosa*) which act as natural fermenting agents[15]. According to the *Ayurvedic Pharmacopoeia of India* (API), *Jeerakarishta* should

ACKNOWLEDGEMENTS : The authors gratefully acknowledge the Acropolis Institute of Pharmaceutical Education and Research, Indore, for providing laboratory facilities and technical support. Special thanks to the Food and Testing Laboratory, Acropolis, for assistance with microbial and heavy metal analyses. We also thank the Department of AYUSH, Government of Madhya Pradesh, for guidance on regulatory requirements.

TABLE-1 : Organoleptic parameters of *Jeerakarishta* samples

Parameter	Sample A	Sample B	Classical Description
Color	Brown to dark brown	Brown to reddish brown	Brown, dark brown
Odor	Pleasant, alcoholic, distinct cumin aroma	Pleasant, alcoholic, mild cumin aroma	Alcoholic, aromatic
Taste	Sweet with astringent-spicy aftertaste	Sweet with less astringency	Madhura, Kashaya, Katu anurasa

contain 5-10% self-generated alcohol and comply with specified physicochemical and microbial limits[15].

The World Health Organization (WHO) emphasizes that herbal medicines must be evaluated for identity, purity, strength, and safety through comprehensive quality control measures[16,19]. Similarly, the Central Council for Research in Ayurvedic Sciences (CCRAS) and the *Ayurvedic Formulary of India* (AFI) have established detailed guidelines for the standardization of Ayurvedic drugs, covering botanical, physicochemical, toxicological, and chromatographic parameters[1,3].

Materials and Methods

2.1. Sample Collection and Coding

Two commercially available brands of *Jeerakarishta* were procured from licensed pharmacies in Indore, Madhya Pradesh. Samples were stored at 25±2°C in their original amber-colored glass bottles until analysis. All analyses were initiated within one week of procurement.

2.2. Organoleptic Evaluation

Organoleptic assessment was performed according to WHO Guidelines for the Assessment of Herbal Medicines (1996) and CCRAS Guidelines for Drug Development of Ayurvedic Formulations[3]. In this study color, odor and taste assessment were done as per guidelines.

2.3. Physicochemical Analysis: All physicochemical analyses were performed in triplicate following strict standard operating procedures. Instrument calibration was verified before each analysis session.

2.3.1. Determination of Total Solid Content: Total solid content was determined using two methods. For sugar-containing formulations, 50 mL of sample was evaporated, extracted with dehydrated ethanol, re-evaporated with diatomite, dried at 105°C, and weighed after cooling. For sugar-free preparations, 50 mL was directly evaporated to dryness, dried at 105°C for 3 hours, cooled, and weighed to calculate total solids[14].

2.3.2. Determination of Specific Gravity[8] : Specific gravity was measured using a calibrated pycnometer at 25°C. The weights of the pycnometer filled with the sample (W) and with distilled water (W,) were recorded.

2.3.3. Determination of Viscosity: Viscosity was measured using an Ostwald viscometer at 25°C by recording the flow time of the sample between two marks and comparing it with distilled water under identical conditions. The densities of the sample and water were determined using a pycnometer, and viscosity was calculated relative to water.

2.3.4. Determination of Alcohol Content[14]: Alcohol content was estimated using the Distillation Method (Method IIIA) as per CCRAS guidelines. A 25 mL sample was diluted with 150 mL of distilled water and distilled, collecting at least 90 mL of distillate in a 100 mL volumetric flask. The distillate was adjusted to 25°C, diluted to volume, and its specific gravity was measured. The alcohol percentage was determined by referring to standard alcoholometric tables.

2.3.5. Determination of pH[8]: pH was determined by pH meter.

2.3.6. Determination of Reducing Sugar Content[18]: Reducing sugar content was estimated by the Lane–Eynon titrimetric method. Fehling's solution was first standardized using 0.5% standard dextrose with methylene blue indicator to a brick-red endpoint. The appropriately diluted sample was then titrated similarly, and reducing sugar content was calculated based on the recorded titration volumes.

TABLE-2 : Physicochemical parameters of *Jeerakarishta* samples

Parameter	Standard Requirement	Sample A	Sample B
Total Solid Content (% w/v)	≥ 20	29.0±0.5	22.7±0.4
Specific Gravity	1.080-1.120	1.150±0.005	1.084±0.003
Viscosity (cP)	1.2-1.5	1.5±0.1	1.3±0.1
Alcohol Content (% v/v)	NMT 12	27.0±0.5	27.0±0.5
pH	4.0-4.5	3.9±0.1	4.2±0.1
Reducing Sugar (% w/v)	NLT 5	0.6242±0.02	0.6645±0.02
Refractive Index	1.3320-1.3420	1.412±0.0005	1.3652±0.0005
Acid Value	NMT 7	5.2±0.2	6.3±0.2
TLC (Rf of thymol)	~0.59	0.59±0.02	0.59±0.02

Values represent mean ± standard deviation (n=3)

2.3.7. Determination of Refractive Index[14]: Refractive index was measured using an Abbe refractometer calibrated with distilled water (1.3325 at 25°C). After cleaning the prisms, 2–3 drops of sample were placed on the prism, equilibrated at 25°C, and the boundary line was adjusted for a clear reading. The value was noted directly from the scale, and prisms were cleaned after measurement.

2.3.8. Determination of Acid Value[6]: The acid value was determined titrimetrically. A 10 mL sample was dissolved in 50 mL of an ether-alcohol mixture (1:1) and titrated with 0.1 N NaOH using phenolphthalein as the indicator.

2.3.9. Chromatographic Analysis (Thin Layer Chromatography for Thymol)[20]: TLC was performed as per AFI guidelines. Silica gel G plates were activated at 105°C for 30 minutes. Sample and standard thymol solutions were spotted and developed in a mobile phase of toluene:ethyl acetate (9:1). The plates were visualized under UV light (254 nm) and after iodine staining, and Rf values were calculated.

2.4. Toxicological Analysis

2.4.1. Microbial Quality Analysis: All microbial analyses were conducted in a certified microbiology laboratory under laminar airflow (Class II). Media preparation, sterilization, and incubation followed Ministry of AYUSH Guidelines for Microbial Limit Testing (2016) and WHO Guidelines on Quality Control of Herbal Medicines (2007).

A. Total Bacterial Count (TBC) – Aerobic Mesophilic Bacteria: For Total Bacterial Count, 10 mL of sample was diluted in 90 mL Buffered Peptone Water to obtain a 10{ ¹ dilution, followed by serial dilutions as required. Using the pour plate method, 1 mL of selected dilutions was mixed with molten SCDA and incubated at 30–35°C for 5 days. Plates with 30–300 colonies were counted, and TBC was calculated using the dilution factor, with appropriate positive and negative controls maintained.

B. Total Fungal Count (TFC) – Yeasts and Molds: For Total Fungal Count, serially diluted samples were plated in duplicate using the pour plate method with molten Sabouraud Dextrose Agar (d”48°C). After solidification, plates were incubated at 20–25°C for 5 days. Yeast and mold colonies were counted separately to determine the total fungal load.

C. Detection of *Enterobacteriaceae*: For detection

TABLE-3 : Microbial quality parameters of *Jeerakarishta* samples

Parameter	Permissible Limit (AYUSH)	Sample A	Sample B
Total Bacterial Count	≤10 CFU/mL	<100 CFU/mL	<100 CFU/mL
Total Fungal Count	≤10^3 CFU/mL	<10 CFU/mL	<10 CFU/mL
Enterobacteriaceae	Absent in 1 mL	Absent	Absent

of Enterobacteriaceae, 10 mL of sample was pre-enriched in 90 mL Buffered Peptone Water and incubated at 37°C for 18–24 hours. The enriched broth was streaked onto VRBGA plates and incubated at 37°C for 24±2 hours. Typical pink to red colonies with bile precipitation were observed, followed by confirmatory tests including Gram staining, oxidase test, and IMViC reactions for identification.

2.4.2. Heavy Metal Analysis [ICP-OES Instrumentation and Analysis]

For heavy metal analysis, 5 mL of sample was digested with concentrated HNO following a pre-digestion step, then heated gradually (60–120°C) until a clear solution was obtained. If required, H_2O, was added for complete oxidation. The digest was reduced to 2–3 mL, cooled, diluted to 50 mL with ultrapure water, and filtered through a 0.45 µm membrane. Samples were stored at 4°C and analyzed within 24 hours using ICP techniques.

Results and Discussion

3.1. Organoleptic Evaluation

Both *Jeerakarishta* formulations showed characteristic organoleptic properties (Table-1) consistent with classical descriptions. The brown to dark brown color reflects proper fermentation and sugar caramelization, while the pleasant alcoholic aroma with cumin notes confirms self-generated ethanol and volatile oils. The sweet taste with slight astringent and spicy aftertaste aligns with the traditional rasa profile.

3.2. Physicochemical Parameters

Physicochemical parameters (Table-2) largely complied with pharmacopoeial standards; however, alcohol content (27% v/v) exceeded the AFI limit (d”12%). This may result from prolonged fermentation or raw material variations. While higher alcohol could improve preservation, it might influence palatability and dosage considerations.

The total solid content was within acceptable limits, indicating efficient extraction and concentration. Sample A showed slightly higher specific gravity (1.150), likely due to increased soluble solids. Low reducing sugar content suggests effective fermentation of sugars to alcohol, while the acidic pH (3.9–4.2) and moderate acid values (5.2–6.3) support stability and preservation. TLC analysis confirmed the presence of thymol (Rf 0.59), verifying the authenticity of cumin in both formulations.

3.3. Microbial Quality

Both formulations demonstrated excellent microbial quality (Table-3), with counts well below permissible limits. The absence of *Enterobacteriaceae* indicates proper hygienic practices during manufacturing and packaging. The low microbial load can be attributed to the self-generated alcohol (27% v/v) which acts as a natural preservative, and the acidic pH (3.9-4.2) which inhibits bacterial growth.

3.4. Heavy Metal Analysis

Heavy metal concentrations were within permissible limits for all elements tested (Table-4). The detected levels are likely from environmental contamination of herbal raw materials rather than intentional addition. The relatively higher arsenic content in Sample B (0.22 ppm) compared to Sample A (0.13 ppm) may reflect geographical variations in raw material sourcing. All values were significantly below the maximum permissible limits, ensuring product safety.

The ICP-OES calibration curves showed excellent linearity with correlation coefficients (r^2) e”0.999 for all elements (Figure 1). Method validation parameters met acceptance criteria: precision (RSD <10%), accuracy (recovery 92-108%), and detection limits below 1% of permissible limits.

Discussion

The comprehensive quality evaluation of Jeerakarishta formulations highlights key issues in the standardization of Ayurvedic fermented preparations. Both samples showed elevated alcohol content (27% v/v), exceeding the AFI limit of d”12% v/v. In traditional

TABLE-4 : Heavy metal content in *Jeerakarishta* samples (ppm)

Metal	Permissible Limit	Sample A	Sample B
Arsenic (As)	0.3	0.13±0.01	0.22±0.02
Cadmium (Cd)	0.03	0.020±0.002	0.0007±0.0001
Mercury (Hg)	0.1	0.080±0.005	0.003±0.001
Lead (Pb)	1.0	0.110±0.010	0.002±0.001

Values represent mean ± standard deviation (n=3)

practice, alcohol is self-generated through natural fermentation of sugars from jaggery or honey, and its final concentration depends on factors such as sugar content, fermentation duration, temperature, and microbial flora. Although classical texts acknowledge seasonal and geographical variations, the higher alcohol levels observed may influence dosage considerations and patient acceptability, especially among individuals with alcohol restrictions. Inter-brand differences in total solids, specific gravity, and refractive index further reflect variability in raw material quality, fermentation conditions, processing methods, and batch consistency, underscoring the need for stricter in-process controls and standardized manufacturing protocols.

The microbial and heavy metal analyses confirm the safety of both formulations. The self-generated alcohol and acidic pH create an unfavorable environment for microbial growth, contributing to excellent microbiological quality. Heavy metal levels, though detectable, remained well within regulatory safety limits; however, routine monitoring remains essential due to the potential accumulation of metals in herbal raw materials from contaminated soil and water. The study employed validated physicochemical methods alongside AYUSH- and WHO-aligned microbial testing and sensitive ICP-OES analysis for heavy metals, ensuring reliable, accurate, and internationally acceptable quality assessment.

Conclusion

The study confirms that both *Jeerakarishta* formulations largely comply with AFI, WHO, and CCRAS quality standards, exhibiting characteristic organoleptic properties and acceptable physicochemical parameters. Microbial load and heavy metal levels were within safe limits, confirming product safety. However, alcohol content exceeded prescribed limits and inter-brand variations were observed, indicating the need for stricter fermentation control and standardization. Overall, the formulations are safe and of good quality, though improved process monitoring and advanced phytochemical profiling are recommended for enhanced consistency and therapeutic reliability

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Ethical Statement

This study involved analysis of commercial products only and did not involve human or animal subjects. All analyses were conducted following standard laboratory safety protocols.

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https://doi.org/10.33451/florafauna.v32i1pp127-133
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 127-133 ISSN 0971 - 6920 (Print)

The appearance of colistin and azithromycin resistance in recent clinical isolates of *Enterobacter cloacae*, together with their antagonistic and synergistic interactions

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Received **:** 28.03.2026; ***Accepted*** : 08.05.2026

How to cite : Singh S, Singh AK, Nath G, Singh AP. The appearance of colistin and azithromycin resistance in recent clinical isolates of enterobacter cloacae, together with their antagonistic and synergistic interactions. *Flora and Fauna* 2026. 32(1) : 127-133.

ABSTRACT

Antibiotic resistance is a growing concern worldwide, and combination therapy has emerged as a promising strategy to address this issue. Combination therapy involves using two or more antibiotics at the same time to treat bacterial infections. The combination of colistin and azithromycin has demonstrated encouraging results in treating multidrug-resistant bacterial infections. Colistin disrupts the bacterial cell membrane, enhancing the uptake of azithromycin and ultimately leading to bacterial death. Azithromycin inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit, preventing translocation and transpeptidation. This research article focuses on colistin and azithromycin-resistant isolates among *Enterobacter spp.* and their combination to investigate the synergistic and antagonistic relationships against these resistant isolates.

Figure : 01 References : 20 Tables : 04
KEY WORDS : Combinational therapy, *Enterobacter cloacae*, Synergistic effect.

Introduction

The digestive tract is the primary site for the anaerobic, Gram-negative bacterial strain *Enterobacter cloacae*, which is widely distributed in soil, water, and sewage. It is one of the most prevalent nosocomial bacteria that can cause infections, immunosuppression, and prolonged hospital stays in patients with underlying illnesses, particularly in intensive care and burn unit[15]. The bacteria seldom induce septic osteoarthritis but typically cause sepsis, urethritis, and lower respiratory tract infections[17]. Globally, antibiotic resistance is becoming an increasingly serious health concern[12]. Both Gram-positive and Gram-negative species are in the bacterial group known as ESKAPE. *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Enterobacter* species are among them. These microbes frequently cause nosocomial

ACKNOWLEDGEMENTS : SS thanks the Head of the Botony Department, C.M.P. College, Prayagraj (UP) India for allowing to do this experiment in the Bacteriophage research laboratory, IMS, BHU Varanasi (UP) India. We further thankfully acknowledge the laboratory and financial help provided by the Bacteriophage Research Laboratory and Viral Research Diagnostic Laboratory IMS, BHU, Varanasi, India.

TABLE-1: Resistance pattern of clinical (n=40) isolates of *Enterobacter cloacae* by broth diffusion method

Tested antibiotics	Range (µg/mL)	MIC Breakpoint (CLSI 2021)	Resistant %
Macrolides			
Azithromycin	0.76-3125	≤ 32 µg/mL	(26/40) 65 %
Lipopeptides			
Colistin	0.25-16750	≤ 4 µg/mL	(7/40) 17.5 %

infections that can be fatal in severely ill and immunocompromised individuals, and they may have pathways for medication resistance[16]. Combining various drugs can effectively treat antibiotic resistance[11]. Multidrug-resistant (MDR) Gram-negative bacteria in hospitals is a prominent cause of morbidity and mortality due to comorbidity and a lack of suitable treatment options[20]. Clinically available and efficacious medications are in short supply due to the rapid rise in extended-spectrum beta-lactamase-mediated bacterial resistance. The last resort for treating Gram-negative bacteria resistant to many drugs is colistin[18]. High-level colistin resistance, however, quickly arises due to chromosomal abnormalities in several genes, such as pmrAB, phoPQ, and mgrB[10]. When two antibiotics are combined, antagonistic, indifferent, or synergistic effects may occur. Combining the two medications results in microbial inhibition at doses lower than those of either drug alone[13]. Thus, two agents working together to produce notably more activity than acting alone is called synergy[14].

Increased activity or a synergistic effect is the primary justification for combining two drugs. Decreased toxicity is a secondary justification for permitting lower antibiotic doses. Combining two drugs can help to stop the emergence of antibiotic resistance[5]. The current study aimed to characterised the *in vitro* synergy effect of colistin and azithromycin, as well as the prevalence of colistin and azithromycin resistance in *Enterobacter* species recently isolated from clinical samples resistant to either colistin, azithromycin, or both antibiotics.

Materials and Methods

Isolation of *Enterobacter cloacae* from clinical specimens

The samples were collected from the bacteriological section at the Institute of Medical Sciences, Banaras Hindu University, Varanasi, between July 2021 and April 2023, yielding 51 clinical isolates of *Enterobacter*. Pus, blood, urine, faeces, and sputum samples were used to obtain these isolates.

Isolation and characterisation of clinical isolates

A total of 51 isolates of *Enterobacter* species were isolated using standard microbiological techniques. These isolates were subjected to PCR-based discrimination using specific primers targeting each species. The isolates were identified at the species level using EC-F (52 -TGAAAACCTTATCCGCGA-32) and EC-R (52 -GGCAGGCTGGAAGATAAA-32) primers (Ji et al. 2021). Positive control was *E. cloacae subsp. cloacae* ATCC 13047 type strains. For the reaction MQW (13.57 ìL), 2.5 ìL of 10x buffer, 2 ìL of dNTP, 0.8 ìL of each primer (forward and reverse), 5 ìL of DNA template, and 0.33 ìL of Taq Polymerase were mixed for a single PCR reaction (25 ìL). In brief, denaturation at 94ÚC for 30 s, annealing at 50ÚC for 30 s, an extension phase at 72ÚCfor 1 min, and a final extension at 72ÚC for 5 min. The amplified product was analysed by 1% agarose gel electrophoresis (GeNei TM Sl. No-07/19/F/328, Peenya, and Bangalore, India).

Antibiotic susceptibility test

The Clinical and Laboratory Standards Institute's Guidelines were used to identify the *Enterobacter* isolates, and the broth dilution method was accurately used to test for antibiotic susceptibility (CLSI 2021). The *Enterobacter cloacae subspecies cloacae* ATCC 13047 reference strain was used. Clinical isolates were subjected to assays for sensitivity to azithromycin and colistin methanesulfonate (CMS). The broth dilution method was used to determine the minimum inhibitory concentration (MIC) of colistin methanesulfonate and azithromycin. The CLSI (2021) recommended azithromycin ≥ 32 µg/mL and colistin ≥ 4 µg/mL as resistance breakpoints. Hi-Media Laboratories Pvt. Ltd. in Mumbai, India, supplied laboratory-standard powders of azithromycin and colistin methanesulfonate, which were then reconstituted according to their directions[18].

TABLE-2: *in vitro* activity of Colistin in combination with Azithromycin against Colistin-resistant clinical isolates of *Enterobacter cloacae*

Isolates	AZ MIC breakpoint (>32 µg/mL)	CO MIC breakpoint (>4 µg/mL)	CO+AZ MIC	FIC value of CO	FIC value of AZ	FICI index	Result
EC 01	12.205	65.07	4.065	0.06	0.33	0.39	Synergy
EC 12	24.41	130.155	65.07	0.49	2.66	3.15	Indifferent
EC 50	24.41	16.26	16.27	1.00	0.66	1.66	Indifferent
EC 45	24.41	32.53	32.55	1.00	1.33	2.33	Indifferent

Synergy studies against colistin and azithromycin-resistant isolates

The synergistic effectiveness of colistin and azithromycin *in vitro* was evaluated against seven colistin-resistant *Enterobacter cloacae* and twenty-six clinical isolates of *Enterobacter cloacae* that were resistant to azithromycin. Azithromycin resistance was also present in three (42.85 %) of the seven clinical isolates of *Enterobacter cloacae* that showed colistin resistance. The *in vitro* synergistic effectiveness of colistin and azithromycin was evaluated against seven colistin-resistant *Enterobacter cloacae* and twenty-six clinical isolates of *Enterobacter cloacae* resistant to azithromycin. Inoculum was applied to each well to achieve a final concentration of 1×10^5 CFU/well. For a whole day, the plates were incubated at 37°C. The MIC is the lowest concentration of the inhibitor that prevents detectable bacterial growth in the well. The fractional inhibitory concentration index (ÓFIC) was determined using the following formula.

The FICs were evaluated in the manner described below:

FIC of Drug A =

$$\frac{\text{Minimum inhibitory concentration of Drug A in combination}}{\text{Minimum inhibitory concentration of Drug A alone;}}$$

FIC of drug B =

$$\frac{\text{Minimum inhibitory concentration of Drug B in combination}}{\text{Minimum inhibitory concentration of Drug B alone}}$$

The fractional inhibitory concentration index, or FICI, is the total FICs of drugs A and B. Synergy was defined as FICI $\leq$ 0.5, Indifference as FICI >0.5 and $\leq$ 4, and Antagonism as FICI >4.

Antibiotic susceptibility testing and minimum inhibitory concentration determination by the broth dilution Method

Of the 40 clinically isolated *Enterobacter cloacae*, 7 (17.5 %) were confirmed to be colistin-resistant.

Results

Identification of bacterial isolates

A species-specific primer identified 40 of the 51 (78.43 %) *Enterobacter* isolates as *Enterobacter cloacae* (Fig.1).

It was found that 29 out of 40 clinical isolates of *Enterobacter cloacae* (72.5%) were resistant to azithromycin (Table-1).

Enterobacter cloacae isolates (clinical) sensitive to colistin but resistant to azithromycin, sensitive to azithromycin and resistant to colistin, sensitive to both colistin and azithromycin and resistant to both colistin and azithromycin were found.

Synergy study of colistin-resistant *Enterobacter cloacae*

The *in vitro* synergy test for colistin and azithromycin combination examined four (4/40) clinical isolates with MIC valuese $\geq$ 4µg/mL and colistin resistant. Table-2 shows that the combination of azithromycin and colistin showed indifference against three isolates (75%), antagonism against 0 isolates (0%) and synergy against one isolate (25%). (Table-2).

Synergy study of azithromycin-resistant *Enterobacter cloacae*

The azithromycin and colistin combination demonstrated synergy against 6 (26.08%) isolates,

TABLE-3: *In vitro* activity of azithromycin and colistin against azithromycin-resistant clinical isolates of *Enterobacter cloacae*

Isolates	AZ MIC breakpoint (>32 µg/mL)	CO MIC breakpoint (>4 µg/mL)	CO+AZ MIC	FIC value of CO	FIC value of AZ	FICI index	Result
EC 51	1.015	48.82	8.137	8.016	0.16	8.182	Antagonism
EC 40	1.015	48.82	4.068	4.007	0.083	4.090	Indifferent
EC 47	1.015	97.65	8.137	8.016	0.083	8.099	Antagonism
EC 41	2.03	97.65	8.137	4.008	0.083	4.091	Indifferent
EC 44	2.03	48.82	4.068	2.003	0.083	2.086	Indifferent
EC 43	2.03	1562.5	4.068	2.003	0.002	2.005	Indifferent
EC 48	2.03	1562.5	4.068	1	0.002	2.0056	Indifferent
EC 34	2.03	48.82	2.03	1	0.041	1.041	Indifferent
EC 37	2.03	97.65	2.03	0.5	0.020	1.020	Indifferent
EC 36	4.06	195.31	2.03	4.007	0.01	0.51	Synergy
EC 22	1.015	1562.5	4.068	4.007	0.002	4.009	Indifferent
EC 19	1.015	195.31	8.137	8.016	0.041	8.057	Antagonism
EC 18	1.015	195.31	4.068	4.007	0.020	4.027	Indifferent
EC 17	2.03	195.31	1.015	0.5	0.0051	0.50	Synergy
EC 11	2.03	97.65	1.015	0.5	0.010	0.51	Synergy
EC 10	2.03	48.82	1.015	0.5	0.020	0.52	Synergy
EC 09	2.03	97.65	2.03	1	0.020	1.020	Indifferent
EC 06	2.03	48.82	4.068	2.003	0.083	2.086	Indifferent
EC 03	2.03	48.82	1.015	0.5	0.020	0.52	Synergy
EC 15	4.068	48.82	1.015	0.249	0.020	0.269	Synergy
EC 31	1.015	97.65	2.03	2	0.020	2.020	Indifference
EC 46	1.015	48.82	1.015	1	0.020	1.020	Indifference
EC 25	1.015	48.82	2.03	2	0.041	2.041	Indifference

TABLE-4: *In-vitro* activity of azithromycin (AZ) and colistin (CO) against both colistin and azithromycin-resistant *Enterobacter* isolates

Isolates	AZ MIC breakpoint (>32 µg/mL)	CO MIC breakpoint (>4 µg/mL)	CO+AZ MIC	FIC value of CO	FIC value of AZ	FICI index	Result
EC 32	781.25	2082.5	520	0.250	0.666	0.9	Indifferent
EC 08	48.82	260	260	1	5.32	6.32	Antagonism
EC 21	97.65	65.07	16.27	0.25	0.166	0.416	synergy

antagonism against 3 (13.04 %), and indifference against 14 (60.86%) of the 23 clinical *Enterobacter cloacae* that were resistant to azithromycin (Table-3).

Synergy study of both colistin and azithromycin-resistant *Enterobacter cloacae*

Colistin and azithromycin together showed synergy against one isolate (33.33%), antagonism against one isolate (33.33%), and indifference against one isolate (33.33%) of the three clinical *Enterobacter cloacae* that were resistant to both antibiotics. (Table-4).

Discussion

Antibiotic combination therapy is finally used due to a shortage of novel, effective antibiotics. The primary objective of this study was to identify and collect antibiotic combinations that may be effective in combating colistin and azithromycin-resistant *Enterobacter cloacae*. The macrolide antibiotic azithromycin was used with imipenem, colistin, and fosfomycin, three commonly used antibiotics. It binds to the 50S ribosomal subunit at the 23S rRNA site, stopping translocation and transpeptidation during protein synthesis. Although colistin is a cationic antimicrobial peptide (CAMP), its nephrotoxicity and neurotoxicity led to the termination of its therapeutic usage. However, due to the emergence of multidrug-resistant (MDR) bacteria and the need for effective antimicrobials, the therapeutic potential of colistin is being reevaluated. Colistin primarily acts by breaking down bacterial membranes, which significantly alters the bacteria's permeability[3]. Because restricted entry or improved efflux frequently cause medication resistance, modest doses of colistin have been employed to make bacteria more sensitive to certain antimicrobials[2,9]. There has been evidence of *in vitro* synergy with low-dose colistin and other antimicrobials in *E. Coli, Pseudomonas aeruginosa*, and *Acinetobacter baumannii* [1,4,6,19]. Therefore, we examined the *in vitro* efficacy of colistin and azithromycin in treating 36 clinical isolates of drug-resistant *Enterobacter cloacae*. The antibiotic susceptibility of clinical isolates of *Enterobacter cloacae* was studied, as anticipated, using the broth dilution method to determine the minimum inhibitory concentration (MIC) of azithromycin and colistin. We found that *Enterobacter cloacae* clinical isolates exhibited greater resistance to azithromycin than colistin. In our investigation, we discovered that 69 % of *Enterobacter cloacae* from clinical specimens were resistant to azithromycin, while 16.6 % were resistant to colistin. Combination therapy is often suggested as an initial empirical treatment because there aren't many effective substances available to stop the growth of germs that are resistant to drugs. In light of this, we conducted *in vitro* tests using the colistin and azithromycin combination against seven colistin-resistant clinical isolates, yielding some interesting outcomes. Only synergy was seen in 25 % of the clinical isolates that were resistant to colistin. Similarly, 26 % of clinical isolates of *Enterobacter cloacae* resistant to azithromycin exhibited synergy. No information exists on the synergy between colistin and azithromycin against *Enterobacter cloacae* in any Indian report. One of the most plausible explanations for the synergy of colistin-azithromycin combinations is that colistin may increase the permeability of the bacterial outer membrane, allowing azithromycin to enter. In a similar vein, a previous study showed that colistin and high dosages of azithromycin (2.5 mg/litre) combined to combat colistin-resistant *E. coli* effectively isolates *in vitro* at a minimum inhibitory concentration (MIC) of just 25% to 50% of colistin[2].

Dispute: Because of the increased permeability of the Gram-negative outer membrane, which enables azithromycin to access the 50S ribosomal subunit, studies employing MDR *K. pneumoniae* have shown that azithromycin and colistin work in concert[8]. Interestingly,

25-26 % of the isolates exhibit synergy of clinical origin, meaning they are either azithromycin or colistin-resistant. It is necessary to look into the complex mechanism. However, because of its poor synergy, this combination is not recommended for empirically treating *Enterobacter cloacae* infections. This work is notable for its examination of antibiotic resistance patterns, minimum inhibitory concentrations, and the synergy of colistin and azithromycin in clinical isolates of *Enterobacter cloacae* strains. Furthermore, we demonstrated that the two medications synergise in clinical *Enterobacter cloacae* isolates resistant to both azithromycin and colistin, with certain isolates exhibiting a significant decrease in MIC values. According to the study's findings, clinical isolates develop high levels of drug resistance due to the strong selection pressure exerted by a range of antimicrobials present in the clinical setting.

Conclusion

Finally, based on the *in vitro* checker board experiment, we found that colistin and azithromycin synergistically inhibited *Enterobacter cloacae* isolates. The combination of colistin and azithromycin is also not promising, as it carries a risk of failure in more than 60% of cases of sickness caused by *Enterobacter cloacae.* This study raises serious concerns, as it implies that *Enterobacter cloacae* are also gaining ground in the last line of defence against infection treatment. Furthermore, *Enterobacter cloacae* infections are resistant to the current antibiotic arsenal, and due to their resistance to every antibacterial on the market, the bacteria have evolved into a superbug. We need to work on developing alternative antimicrobials, including synthetic chemicals, small polypeptides, bacteriophage treatment, and novel plant-based molecules.

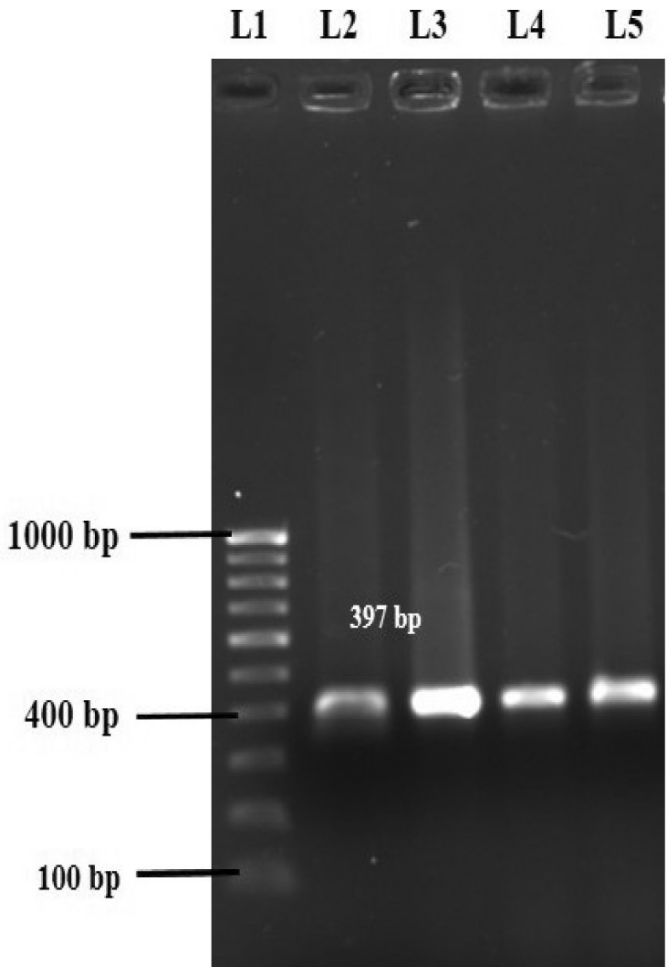

Fig.1 : Gel image showing molecular identification using a species-specific primer (Lane1: 100 bp Ladder, Lane 2: *Enterobacter cloacae* ATCC 13047 (Positive control), Lane 3, 4 & 5 showing clinical isolates (*Enterobacter cloacae subspecies cloacae*).

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https://doi.org/10.33451/florafauna.v32i1pp134-144
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 134-144 ISSN 0971 - 6920 (Print)

Ethnomedicinal Studies on Commonly Used Medicinal Angiosperms for Therapy of Diabetes in Bihar, India

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Received **:** 25.03.2026; ***Accepted*** : 08.05.2026

How to cite : Verma SC, Singh CB. Ethnomedicinal Studies on Commonly Used Medicinal Angiosperms for Therapy of Diabetes in Bihar, India. *Flora and Fauna* 2026. 32(1) : 134-144.

ABSTRACT

This paper deals with 75 angiospermic species, under 69 genera and 44 families, commonly used in the therapy of diabetes in Bihar. The very useful non-dietary antidiabetic angiosperms are *Aloe vera, Azadirachta indica, Cassia fistula, Catharanthus roseus, Gymnema sylvestre, Helictere sisora, Ocimum tenuiflorum, Phyllanthus niruri, Pterocarpus marsupium, Ricinus communis, Scoparia dulcis, Senna occidentalis, Syzygium cumini, Tinospora cordifolia* and *Withania somnifera.* The most valuable dietary angiosperms with hypoglycemic properties include *Allium cepa, Allium sativum, Beta vulgaris, Cinnamomum zeylanicum, Coccinia indica, Curcuma domestica, Linum usitatissimum, Momordica charantia, Moringa oleifera, Murraya koenigii, Piper nigrum, Trachyspermum ammi, Trigonella foenum-graecum* and *Zingiber officinale. Allium cepa, Ipomoea aquatica, Ricinus communis* and *Scoparia dulcis* alleviate fasting blood glucose level (BGL) while many others reduce post-prandial BGL. The instant hypoglycemic effect of *Cinnamomum zeylanicum, Moringa oleifera, Zingiber officinale* seems to be beneficial for the persons with diabetes. Medicinal angiosperms are emerging as alternative drugs to synthetic one in the medication of diabetes.

Figure : 00 References : 40 Table : 01
KEY WORDS : Antidiabetic angiosperms, Bihar, Ethnomedicinal studies

Introduction

Diabetes mellitus (DM), often termed as diabetes, is a serious metabolic disorder caused due to insulin insufficiency (Type 1 DM) or insulin resistance (Type 2 DM). Its incidence is rapidly increasing especially in urban area and posing severe threats to mankind in all parts of the world. It is assessed that the number of persons suffering from diabetes would increase upto 783 million until 2045. This multifactorial disease leads to huge financial loss and many serious health complications as blindness, heart attack/stroke, kidney failure, neuropathy, *etc.* Several modern synthetic medicines are available in the markets for effective control of this incurable ailment but the synthetic medicines are very costly as well as harmful due to their own side effects. The common people are incapable to afford high prices of such lifesaving drugs. Obviously, they are compelled to use folk medicines from angiosperms with antidiabetic / hypoglycemic activity for the therapy of diabetes. Medicinal angiosperms having vast antidiabetic / antihyperglycemic potential and fewer side effects are easily accessible to them. However, the ethnomedicinal study focusing on the medication of diabetes in Bihar is limited to some medicinal angiospermicplants (46) confined in a district out of 38 districts of the province[18]. Further the earlier ethnomedicinal studies of Buxar and Bhagalpur districts of the state include only 04 and 10 antidiabetic angiosperms out of 84 and 75 medicinal angiosperms respectively [33,34]. Therefore, the present research work is an attempt to search out commonly used medicinal angiosperms in the remedy of diabetes prevalent in Bihar.

TABLE-1 : Enumeration of commonly used antidiabetic plants of Bihar (India)

S. No.	Botanical name, Common name, Vernacular name, Family, Habit	Mode of Utilization
1.	*Acacia nilotica*, Acacia, *Babul / Kiker*, Mimosaceae, Thorny tree	Half cup of stem bark decoction is consumed twice everyday by the diabetic patients for 2-3 months to reduce blood glucose level **(BGL)** and get relief from diabetes.
2.	*Acalypha indica*, Indian mercury, *Khokhil/Kuppi / Kanghi,*Euphorbiaceae, Annual herb	Half cup of leaf decoction is continuously taken 2-3 times a day depending on the severity of ailment.
3.	*Achyranthes aspera*, Chaff-flower, *Chirchita/ Chirchiri,*Amaranthaceae, Perennial herb	Half cup of root decoction is consumed daily in the morning for 2-3 months.
4.	*Aegle marmelos*, Bael / Wood apple, *Bel*, Rutaceae, Deciduous tree	200-250 ml aqueous extract of fruit pulp is regularly drunk twice a day for one month as an effective therapy of diabetes. In fact, the dose of fruit pulp extract depends on age and health of the diabetic patients besides other conditions of the ailment.
5.	*Albizia lebbeck*, Kokko, *Siris,* Mimosaceae*,* Deciduoustree	Half cup of stem bark decoction is taken twice everyday.
6.	*Allium cepa*, Onion, *Piyaz,* Amaryllidaceae, Biennial herb	100g fleshy leaves from bulbs are consistently eaten raw per day to reduce fasting BGL
7.	*Allium sativum*, Garlic, *Lahsun,* Amaryllidaceae, Perennial herb	Usually 3-4 cloves (bulblets) are eaten raw per day as an effective herbal medicine of diabetes.
8.	*Aloe vera*, Indian Aloe, *Ghikumar,* Asphodelaceae, Succulent herb	Fresh leaf juice (10 -15 ml) is consumed everyday by the patients over one month.
9.	*Annona squamosa*, Sugar apple / Custard apple, *Sharifa,* Annonaceae, Medium tree	Half cup of leaf extract is taken twice per day for at least 2-3 months.
10.	*Areca catechu*, Betel nut, *Supari,* Arecaceae, Straight medium tree	Overall, 15-25g dehusked and dried nuts of good quality are chewed per day @ 3-4g / dose. The quantity of dose depends largely on the severity of disease.
11.	*Artocarpus heterophyllous*, Jack fruit, *Kathal*, Moraceae, Evergreen tree	Half cup of leaf decoction is consumed in the empty stomach in morning for 2-3 months.
12.	*Asparagus racemosus*, Indian Asparagus, *Satavar / Satavari,* Asparagaceae, Climbing shrub	Two teaspoonsful of root tuber decoction is regularly consumed once or twice a day depending on the severity of disease.
13.	*Azadirachta indica*, Margosa tree, *Neem*, Meliaceae, Evergreen tree	One teaspoonful fresh leaf juice is taken or 8-10 tender leaves are chewed daily in the empty stomach in morning for quick relief from diabetes.

S. No.	Botanical name, Common name, Vernacular name, Family, Habit	Mode of Utilization
14.	*Beninca shahispida*, Wax gourd / Ash gourd / Winter gourd, *Petha* / *Bhua*, Cucurbitaceae, Climbing or trailing herb	Half cup of fruit peel extract is taken once everyday for 3-4 months
15.	*Beta vulgaris*, Beet root, *Chukandar,* Chenopodiaceae, Biennial herb	Half cup of root juice is regularly consumed twice a day. Alternatively, the root slices are frequently and lavishly eaten raw as salads.
16.	*Boerhavia diffusa*, Spreading hogweed, *Punarnava,* Nyctaginaceae, Creeping perennial herb	Half cup of root decoction is taken once everyday for 2-3 months
17.	*Bombax ceiba,* Cotton tree, *Semul,* Bombacaceae, Deciduous tree	One teaspoonful of leaf extract is consumed everyday in the morning and evening.
18.	*Brassica juncea*,Brown mustard, *Rai,* Brassicaceae, Annual cultivated herb	One teaspoonful of seed powder is added in lime juice, mixed thoroughly and mixture is consumed once daily for one month.
19.	*Butea monosperma*, Bengal kino / Parrot tree / Sacred tree, *Dhak / Palas,*Fabaceae, Deciduous tree	Half cup of leaf decoction is drunk once daily on regular basis.
20.	*Cajanus cajan*, Pigeon pea, *Arhar,* Fabaceae, Shrub	The cooked cotyledons are eaten regularly.
21.	*Carica papaya*, Papaya, *Papita,* Caricaceae, Soft wooded tree	20-25 ml leaf extract is consumed everyday.
22.	*Carissa carandas*, Karanda, *Karaunda,* Apocynaceae, Spiny shrub	The pickles prepared from unripe fruits are regularly eaten lavishly many times everyday as a natural medicine to maintain glucose level in blood.
23.	*Cassia fistula*, Golden shower tree, *Amaltas,* Caesalpiniaceae, Deciduous tree	Half cup decoction of stem bark or fruit pulp is consumed twice everday.
24.	*Catharanthus roseus*, Madagascar Periwinkle, *Sadabahar,* Apocynaceae, Perennial herb	Either 5-10 tender leaves are chewed or their juice mixed with milk is consumed daily in the empty stomach during morning time for quick relief from the elevated BGL.
25.	*Cinnamomum zeylanicum*, Ceylon cinnamon, *Dalchini,* Lauraceae, Evergreen tree	The decoction / infusion or powder of stem bark (2.0-4.0 g) is taken by the patients for instant relief from discomfort of ailment.
26.	*Coccinia grandis*, Ivy gourd, *Tilkocha / Tilkor,* Cucurbitaceae, Climbing or trailing herb	A teaspoonful of root or leaf juice is regularly consumed twice a day.
27.	*Coccinia indica*, Little gourd, *Kundri / Kundru,* Cucurbitaceae, Twining or spreading herb	A cup of fruit juice is regularly drunk twice a day.

S. No.	Botanical name, Common name, Vernacular name, Family, Habit	Mode of Utilization
28.	*Coriandrum sativum*, Coriander, *Dhaniya,* Apiaceae, Annual herb	Often a quarter-half teaspoonful mature fruits (called coriander) are chewed or their (coriander) extract is taken once a day in effective lowering of BGL.
29.	*Cuminum cyminun*, Cumin, *Jeera*, Apiaceae, Annual herb	1.5 – 2.5g of slightly roasted fruit powder is mixed with water and the mixture (commonly called *jeera* water) is consumed daily.
30.	*Curcuma domestica*, Turmeric, *Haldi,* Zingiberaceae, Perennial herb	The regular consumption of 0.5-2.0 g turmeric (rhizome) powder per day alongwith food items is considered to prevent the development of diabetes
31.	*Cuscutareflexa*, Dodder, *Amarbel / Amarlata,* Convolvulaceae, Perennial herb (Stem parasite)	The plant paste is added in a cup of water and mixed thoroughly. Thereafter, the mixture is filtered and the filterate (*i.e.*, plant extract) is taken orally everyday.
32.	*Dalbergia sissoo*, *Shisham,* Fabaceae, Deciduous tree	A cup of bark / leaf decoction is daily drunk once a day.
33.	*Euphorbia hirta*, Garden spurge / Hairy spurge, *Dudhi,* Euphorbiaceae, Annual wild herb	A cup of shoot decoction is continuously drunk once or twice everyday depending on the severity of disease.
34.	*Ficus racemosa*, Cluster fig, *Gular,* Moraceae, Large shrub / Small tree	Half cup of stem bark infusion or unripe fruit decoction is taken once everyday.
35.	*Ficus religiosa*, Sacred fig tree, *Pipal,* Moraceae, Fast growing tree	The regular oral administration of stem bark decoction (2-3 teaspoonful) or powder (2 teaspoonful) proves as an effective measure in the healing of diabetes. The frequent drinking of half cup of its leaf decoction in the empty stomach in morning also acts as a useful therapy against this ailment.
36.	*Foeniculum vulgare*, Fennel, *Saunf,*Apiaceae, Aromatic herb	One teaspoonful mature fruits (called fennel) are regularly consumed either directly by chewing after meal or indirectly by drinking fennel water. The fennel water is prepared by soaking fennel overnight in 200-250 ml water. Then the fennel water is drunk in the empty stomach in morning and the soaked feneel is chewed.
37.	*Gymnema sylvestre*, Gymnema, *Gurmar,* Asclepiadaceae, Woody climber	Either 4-5 leaves are chewed or their juice is consumed daily in the empty stomach during morning hours as an effective natural remedy of diabetes.
38.	*Helictere sisora*, Marodphali / Indian screw tree, *Jari,* Sterculiaceae, Large shrub / Small tree	One fresh fruit is eaten raw or its aqueous extract is taken once everyday by the patients for two months.

S. No.	Botanical name, Common name, Vernacular name, Family, Habit	Mode of Utilization
39.	*Hibiscus rosa-sinensis*, China rose, *Gudhal,* Malvaceae, Ornamental shrub	Half cup of leaf decoction is orally administered once everyday for 3-4 months.
40.	*Ipomoea aquatica*, Water spinach, *Karmi,* Convolvulaceae, Trailing herb	The cooked green leaves are eaten as vegetable to alleviate fasting blood glucose level.
41.	*Leucasa spera*, Thumbai, *Dhurpi / Guma,* Lamiaceae, Annual herb or undershrub	One teaspoonful of fresh leaf extract is taken everyday in the morning and evening as an effective control measure of diabetes.
42.	*Linum usitatissimum*, Linseed, *Alsi / Tisi,* Linaceae, Annual herb	The diabetic persons often consume food items prepared utilizing seed powder or oil (known as flax oil or linseed oil) of this plant. In addition, they consume one teaspoonful of seed powder twice a day for 3-4 months.
43.	*Madhuca indica*, Indian butter tree, *Mahua,* Sapotaceae, Deciduous tree	The decoction of bark powder (15-20g) is regularly taken once a day.
44.	*Mangifera indica*, Mango, *Aam,* Anacardiaceae, Evergreen tree	The leaf decoction of wild varieties is commonly consumed by the patients in doses recommended by the local herbalists.
45.	*Momordica charantia*, Bitter gourd, *Karela / Kareli,* Cucurbitaceae, Annual climbing or trailing herb	A cup of fruit juice is drunk once everyday in the empty stomach is morning.
46.	*Moringa oleifera*, Drumstick tree, *Munuga / Sahjan,* Moringaceae, Evergreen tree	A glass of fresh leaf decoction (200 ml) is drunk anytime for instant hypoglycemic effect in post-prandial blood glucose level. From the next day, a cup of fresh leaf decoction is regularly drunk by the patients in the empty stomach in morning to keep themselves relaxed from the discomfort of ailment.
47.	*Morus alba*, White mulberry, *Shahtut / Tut,* Moraceae, Deciduous tree	A cup of leaf extract is frequently drunk for quick alleviation of high glucose level in blood.
48.	*Murraya koenigii*, Curry leaf tree, *Mithaneem / Curry patta,*Rutaceae, Large shrub / Small tree	In general, 12-15 fresh leaves are chewed or their juice / decoction is taken every night in the empty stomach to keep the disease at bay.
49.	*Nigella sativa*, Black cumin, *Kala jeera / Kalongi / Mangrela,* Ranunculaceae, Annual herb	A teaspoonful of fine seed powder is added in a cup of water, mixed properly and wet mixture is frequently consumed in morning and evening.
50.	*Ocimum tenuiflorum*, Holy basil, *Tulsi,* Lamiaceae, Annual herb	A cup of leaf decoction is regularly drunk 2-3 times per day as a natural remedy of the ailment.

S. No.	Botanical name, Common name, Vernacular name, Family, Habit	Mode of Utilization
51.	*Phaseolus vulgaris*, Kidney bean, *Bakla,* Fabaceae, Annaul herb	100g seeds are soaked overnight in water, cooked properly and eaten everyday with low glycemic diet such as barley, bread, fruits, vegetables, etc. as an effective control measure of diabetes.
52-53. 54.	*Phyllanthus emblica*, Indian gooseberry / Emblic myrobalan, *Amla*, Phyllanthaceae; *Terminalia bellirica*, Bellericmyrobalan, *Bahera,* Combretaceae and *Terminalia chebula*, Chebulic myrobalan, *Harad / Harre,* Combretaceae (All three deciduous trees)	The mature fruits of these popular medicinal plants are purchased from the local markets. The procured fruits are dried properly, mixed together in equal proportions (1:1:1) and ground to make a polyherbal powder. This folklore medicine is no doubt cheap but almost similar in the medicinal benefits of an ayurvedic medicine called *Triphalachurna.* A fraction (1.0-1.5g) of the self prepared powder is mixed properly with milk by constant stirring and the mixture is consumed everyday in an empty stomach during morning hours. More or less the consumption of powder reduces significantly blood glucose level of the patients. The continuous utilization of powder for 3-4 months gives good relief from the harmful effects of disease and keeps the patients well in healthy condition.
55.	*Phyllanthus niruri*, Stone breaker, *Bhuiamla,* Phyllanthaceae, Annual herb	The shoot extract (20-30 ml) is taken twice daily for one month.
56.	*Piper nigrum*, Black pepper, *Kalimirch / Golmirch,* Piperaceae, Climbing shrub	A teaspoonful of fruit powder is mixed properly with water and the mixture is regularly consumed twice a day as an effective herbal medicine of diabetes.
57.	*Pistia stratiotes*, Water lettuce, *Chhotajalkumbhi,* Araceae, Floating aquatic herb	10-15ml fresh juice of young plant is mixed with equal part of coconut milk and the mixure is regularly consumed once a day.
58.	*Pongamia pinnata,* Indian beech / Pongame oil tree*, Karnaj,* Fabaceae, Medium tree	The decoction of bark / flower is regularly taken once a day.
59.	*Pterocarpus marsupium*, Bastard teak, *Bijasar / Bijasal,* Fabaceae, Deciduous tree	A cup of heartwood infusion is regularly drunk twice everyday.
60.	*Punica granatum*, Pomegranate, *Anar,* Punicaceae, Shrub	The root bark and fruit rind are mixed together in equal proportions (1:1) and the mixture is crushed to make paste. The paste is consumed daily in morning and evening for two weeks in the doses recommended by local herbalists.
61.	*Ricinus communis*, Castor oil plant, *Arandi,* Euphorbiaceae, Perennial shrub / Small tree	Fresh leaf extract(5ml) is frequently taken twice everyday by the patients to alleviate fasting glucose level in blood.

S. No.	Botanical name, Common name, Vernacular name, Family, Habit	Mode of Utilization
62.	*Saraca asoca*, Ashok tree, *Ashok,* Caesalpiniacease, Evergreen tree	A glass of leaf decotion is drunk daily in the empty stomach in morning.
63.	*Scoparia dulcis*, Licorice weed, *Mithapatta,* Plantaginaceae, Annual herb	Half cup of leaf extract is consumed once everyday by the patients over one month to reduce fasting blood glucose level.
64.	*Senna occidentalis*, Coffee senna, *Bari kasondi / Sennai,* Caesalpiniaceae, Woody herb	Often 5-6 fresh leaves are chewed or their decoction is taken only 4-5 days in morning and evening for fast lowering of elevated glucose level in blood. The consumption of cooked leaves as vegetable also proves very effective in the therapy of diabetes.
65.	*Senna tora*, Sickle senna, *Chakunda / Pamaar,* Caesalpiniaceae, Herb or undershrub	A cup of root (10-15g) decoction is drunk once a day for 15-30 days.
66.	*Sida cordifolia*, Heart-leaf sida, *Bariyar,* Malvaceae, Annual herb	A glass of leaf decoction is drunk daily for one month.
67.	*Syzygium cumini*, Java plum / Black plum, *Jamun,* Myrtaceae, Evergreen tree	Half to full teaspoonful powder of bark or seed as per recommendation of local herabalists is regularly swallowed with water or honey after lunch and dinner. Moreover, the use of bark or seed power of wild varieties is preferred considering it to be a very efficacious herbal drug of diabetes. Fresh fruits are eaten after taking food whereas 3-4 teaspoonful fresh fruit juice is taken after light breakfast in the therapy of diabetes.
68.	*Tamarindus indica*, Tamarind, *Imli,* Caesalpiniaceae, Evergreen tree	Half cup of fruit pulp extract is taken twice everyday for post-prandial hypoglycemic effect.
69.	*Tinospora cordifolia*, Heart-leaved moonseed, *Guranch / Giloy,* Menispermaceae, Climbing shrub	100-200 ml decoction of stem is regularly drunk once or twice per day in the empty stomach as an effective control measure against diabetes. The old stem containing more quantity of bioactive compounds is preferred for better therapeutic action and relief from the disease.
70.	*Trachyspermum ammi*, Lovae / Ammi, *Ajwain,* Apiaceae, Aromatic herb	Overall 9-10g mature fruits (known as *ajwain*) are consumed by a diabetic patient per day @ 1.5-2.0g per dose. The mode of administration follows chewing of fruits or drinking of *ajwain*water (slightly roasted fruits mixed with warm water).
71.	*Trianthema portulacastrum*, Giant pigweed / Black pigweed, *Pindooa / Santhi,*Aizoaceae, Prostrate wild annual herb	The cooked leaves are eaten as green vegetable by both normal (non-diabetic) and diabetic people as preventive and therapeutic measure respectively in the remedy of diabetes

S. No.	Botanical name, Common name, Vernacular name, Family, Habit	Mode of Utilization
72.	*Trigonella foenum-graecum*, Fenugreek, *Methi,* Fabaceae, Annual herb	The seed powder (30-35g) is consumed twice per day over one month as a natural remedy of diabeties.
73.	*Withania somnifera*, Indian winter cherry / Indian Ginseng, *Ashwagandha,* Solanaceae, Evergreen shrub	Root decoction (15-20 ml) is consistently taken twice everyday as a household remedy of diabetes.
74.	*Zingibe rofficinale*,Ginger, *Adrakh / Aadi,* Zingiberaceae, Perennial herb	10-20ml extract of rhizome is taken orally for instant relief from the ill effects of disease.
75.	*Ziziphus jujuba*, Jujube, *Ber,*Rhamnaceae, Large shrub / Small spiny tree	A cup of leaf extract is drunk daily for 2-3 months to get relief of hypoglycemic effect.

Study Area

The area of investigation Bihar state ($24^0$20'10" - $27^0$31'15" NL and $83^0$19'50" - $88^0$17'40" EL, 173 feet MSL) is located in East India. It shares its borders with Nepal in north and other sides with its neighbouring states(South: Jharkhand,West: Uttar Pradesh and East : West Bengal). It forms the part of Indo-Gangetic plain, covers an area of 94,163 km^2 and comprises 38 districts. It is separated into two distinct parts (*viz*., North and South) due to flowing of the River Ganges in west to east direction. North Bihar is entirely alluvial cum flat whereas South Bihar has patches of hills and hillocks. The soil of South Bihar is mainly red sandy to loamy or alluvial at certain places. The climate all over the state is of monsoon type and is characterised by three distinct seasons (namely summer, rainy and winter).

Materials and Methods

The search drive for medicinal angiosperms being commonly used in the therapy of diabetes in Bihar executed for three years (July 2022 – June 2025). During this period, the diverse parts of the state particularly urban areas of seven districts (*viz*., Begusarai, Bhagalpur, Darbhanga, Gaya, Muzaffarpur, Patna and Siwan) were visited in each season for collection of information related to the role of medicinal angiosperms in therapy of diabetes. A new site in each district was surveyed during every expedition to get complete information from ground level. The detailed information on the use of antidiabetic plants for control of diabetes was gathered through interviews and discussions as per semi-structured questionnaires. For effective communication with urban folks, common local languages such as Hindi, Bhojpuri, Angika, Magahi, Maithili and other colloquial languages were used taking the help of bilingual persons as per need of study site. At least five persons including diabetic patients, local herbalists / healers (*Vaidya, Kaviraj, Hakim*) and native persons interested in use of medicinal angiospermic plants were essentially interrogated at each site with sole purpose of getting accurate information.

The plant or plant part specimens were collected from herbalists / healers and /or diabetic patients. All the collected specimens of plant or plant parts were pressed and dried properly to prepare herbarium following the standard methods[16,20]. The plant or plant parts specimens were strictlyexamined and correctly identified using various floras[4,13,15,39]. Voucher specimens were housed in the Herbarium, Department of Botany, G. D. College, Begusarai, Bihar.

Results and Discussion

The identified antidiabetic / antihyperglycemic / hypoglycemic angiosperms were recorded in alphabetical order of their botanical name followed by common name (in english), vernacular name (in italics), family name, habit and uses, *i.e.,* identity of plant part(s) / product(s) and mode(s) of oral administration as well as certain specification (Table-1). Altogether 75 species of angiosperms that belong to 69 genera and 44 families, are commonly used in the medication of diabetes prevalent in different urban areas of Bihar. All of these medicinal angiosperms are known to have proved their utility in the therapy of diabetes all over India[3,8,9,12,14,18,19,22,25,29,33-35,37]. Some of these medicinal angiosperms are very useful non-dietary antihyperglycemic ones. These are *Aloe vera, Azadirachta indica, Cassia fistula, Catharanthus roseus, Gymnema sylvestre, Helictere sisora, Ocimum tenuiflorum, Phyllanthus niruri, Pterocarpus marsupium, Ricinus communis, Scoparia dulcis, Senna occidentalis, Syzygium cumini, Tinospora cordifolia*and *Withania*

somnifera. The most valuable dietary angiosperms with hypoglycemic properties include *Allium cepa, Allium sativum, Beta vulgaris, Cinnamomum zeylanicum, Coccinia indica, Curcuma domestica, Linum usitatissimum, Momordica charantia, Moringa oleifera, Murraya koenigii, Phaseolus vulgaris, Piper nigrum, Trachyspermum ammi, Trigonella foenum-graecum*and *Zingiber officinale.*

All medicinal angiosperms recorded in this study are known to possess certain bioactive compounds that confer antidiabetic / hypoglycaemic / antihyperglycemic properties to them[2,6,10,21,23,26,28,30-32,38]. Depending on the nature of their bioactive compounds, the antidiabetic angiosperms show differential actions as alleviation of fasting or post-prandial blood glucose level, instant or delayed hypoglycaemic effect, *etc.* Only some antidiabetic angiosperms (*Allium cepa, Ipomoea aquatica, Ricinus communis, Scoparia dulcis*) alleviate fasting blood glucose level (BGL) while many among the remaining ones reduce post-prandial blood glucose level (BGL). The instant hypoglycaemic effect shown by *Cinnamomum zeylanicum, Moringa oleifera* and *Zingiber officinale* seems to be somehow beneficial for the persons with diabetes. More or less all the recorded antidiabetic angiospermic species have been found to be efficacious in the therapy of diabetes throughout the province. However, the major concerns as dose uncertainty, consumption frequency, intake time, efficacy level, etc of folklore medicines are inevitable.

The modifications in dietary constituents can be amenable to an extent for the concerns of herbal medicines used in the medication of diabetes particularly in the case of Type 2DM [1,24,25,27,40]. It is well known that the dietary angiosperms have been playing an important role in the control of Type 2DM since time immemorial. Type 2DM is more common form of diabetes and it alone constitutes the major diabetic population (*i.e.,* around 90%). The different part(s) / product(s) of dietary angiosperms are becoming more useful and popular as folk medicines for the remedy of diabetes prevalent in Bihar. Overall, the use of angiospermic medicinal plants is gaining momentum in the medication of diabetes and the dietary angiosperm shaving antidiabetic potential are emerging as a viable option for the therapy of Type 2DM in Bihar. This may be treated as the follow up of the fact that the traditional herbal drugs due to their natural hypoglycaemic properties, cost-effective virtue and fewer side effects are emerging as alternative drugs to synthetic one in the therapy of diabetes across the world[5,7,11,17,36].

Conclusion

This paper considers that the medicinal angiosperms having antidiabetic potential are of great relevance in the therapy of diabetes affecting urban people of Bihar. Although both dietary and non-dietary angiosperms are commonly used in the therapy of diabetes, the consumption of dietary angiosperms is supposed to be the easiest and safest for this purpose. The antidiabetic angiosperms are applicable in lowering of fasting BGLand post-prandial BGL cum immediate lowering of BGL so as to get instant relief from the discomfort of diabetes. The practice of instant hypoglycemic effect followed by maintenance of alleviated blood glucose level may prove as one of the best therapeutic measures for the control of diabetes.

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https://doi.org/10.33451/florafauna.v32i1pp145-149
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 145-149 ISSN 0971 - 6920 (Print)

Ayurvedic Management of Trigeminal Neuralgia (Anantavata): A Case Report

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Received **:** 11.03.2026; ***Revised*** **:** 15.04.2026; ***Accepted*** : 08.05.2026

How to cite : Patel A, Sharma K. Ayurvedic Management of Trigeminal Neuralgia (Anantavata): A Case Report. *Flora and Fauna* 2026. 32(1) : 145-149

ABSTRACT

Trigeminal neuralgia (TN) is a chronic neuropathic pain disorder characterized by recurrent unilateral, electric shock–like facial pain along the trigeminal nerve distribution. Despite pharmacological advances, some patients experience inadequate pain relief or medication-related adverse effects. In Ayurveda, TN is correlated with *A*nantavata, and Urdhwajatrugata Vata Vyadhi. We report a case of an 80year old male diagnosed with TN based on International Classification of Headache Disorders. The patient had persistent facial pain despite low-dose carbamazepine. An 8day inpatient Ayurvedic intervention was administered, including *Nasya*, external therapies and internal medications. Pain severity was assessed using the Visual Analogue Scale (VAS). Pain intensity decreased from a baseline VAS score of 7/10 to 2/10 at discharge, representing a 5 point reduction over the treatment period. No adverse events were observed during the short-term follow-up.Ayurvedic interventions effectively pacified aggravated *Vata-Pitta* and stabilized neural conduction through anti-inflammatory, neuroprotective and *Rasayana* effects. *Nasya* targeted the trigeminal pathways, while internal medicines reduced oxidative stress and neural inflammation. Unlike conventional therapy, this holistic approach addressed the root pathology without side effects. In this case, short-term symptomatic improvement in TN coincided with an 8-day treatment. The result proved to be effective based on clinical assessment.

Figure : 00 References : 12 Tables : 03
KEY WORDS : Anantavata, Ayurveda, Case Report, Trigeminal neuralgia.

Introduction

Trigeminal neuralgia is a neurological disorder characterized by intense painful episodes on the face; this pain is originated by the trigeminal nerve which spread all the face where the trigeminal nerve supplies its sensory supply.[10] It is one of the most physical and psychological condition also known as tic doloureaux.[2,5] It does the negative impact on the quality of life, result in problem such as weight loss, disturbed sleep, depression.[7,9] The annual incidence of TN is estimated at approximately 4–5 per 100,000 individuals, with higher prevalence reported in women than men.[11] According to the International Headache Society (ICHD-3), TN may occur idiopathically or secondary to structural pathology and is often triggered by innocuous stimuli such as chewing or facial touch.[3]

The medicines used to control the trigeminal neuralgia and to improved quality of life are carbamazepine, phenytoin, gabapentin and clonazepam, but there is side effect of these medications, even after taking these drugs for such a long time, there are no significant changes in the patient. Surgery is normally recommended only after medication has proved ineffective. In *Ayurveda*, all conditions in which headache is prodromal symptoms are included in *Siroroga*.[6] In *Anantvata* all the three *Doshas* get aggravated together and produce pain on the face.

Case

An 80 yrs old male patient presented with main complain of sudden pain like electric shock on left side of the face which gets aggravated on touch. Frequent ulcer on left side of buccal cavity with burning sensation was also there for 6 months. The Onset of pain was acute with gradual worsening & episodic in nature. The pain was increased with movements like speaking, chewing, and smiling, while washing the face and brushing the teeth. The sharp pain used to last for 1 to 2 minutes following continuous dull pain, and the episodes tend to occur within 1 to 2 hours, VAS score was six at the time of admission. The patient consulted to some

allopathic hospitals in 2020 and took allopathic treatment, (100 mg of carbamazepine daily) but was experiencing acute painful episodes almost daily. The pain also affected his quality of life both physically and mentally and there is no history of trauma, insect bite, new food intake no family or genetic history was found related to this condition.

History of past illness

The patient had a history of temporomandibular joint pain previously (about 20 years ago) which was treated and had resolved. Patient is known case of Diabetes mellitus on medication: tab voglibose (0.2mg) (1OD) since 20yrs. Not a known case of hypertension & thyroid disfunction.

Surgical history

Underwent cataract surgery (2005-2006), Underwent Glaucoma surgery (2015), Inguinal hernioplasty – 2 years ago. No history of herpes zoster, no history of dental ailment.

Current status: On a verbal pain rating scale, in which zero represents no pain and 10 is defined as the worst pain possible, the intensity of pain episodes was rated as 10 out of 10.

Clinical Findings

- Vital signs stable
- Neurological examination: Cranial nerves largely intact
- Trigeminal nerve: Facial hyperesthesia on the left side (V2–V3 distribution); jaw jerk reflex was brisk (+++), noted as a clinical finding but without associated motor weakness
- TMJ: Mild tenderness on palpation

On location examination – No lacrimation, nasal discharge or other abnormalities were detected, but mild swelling was present on left side of face & at extra auricular region, temporomandibular tenderness was noticed, on physical examination.

Cranial nerve Examination

Olfactory nerve: No significant finding.

Optic nerve: No significant finding.

Oculomotor nerve: No significant finding.

Trochlear nerve: No significant finding.

Trigeminal nerve: Motor part: On palpation of masseter & trigeminal muscles. No abnormalities detected but mild swelling was noted, Jaw jerk +++.

Sensory part: Corneal reflex: normal, Sensation over face: hyperesthesia, Associated with shocking sensation all over the left side of face specially on cheek.

Abducens nerve: No significant finding.

Facial nerve: No significant finding.

Vestibulocochlear nerve: No significant finding.

Glossopharyngeal nerve: No significant finding.

Vagus nerve: No significant finding.

Accessory nerve: No significant finding.

Hypoglossal nerve: No significant finding.

Diagnostic Assessment

Based on International classification of headache disorder, 3rd edition (ICHD-3) diagnostic assessment for trigeminal neuralgia: **In Tabel no. 01**, it meets almost all ICHD-3 Diagnostic criteria. (Tabel no. 01).

Investigations: Hb – 13.2%
ESR – 30mm/hr
CRP – 13mg/dl

Therapeutic intervention

Table-2: Treatment Protocol and Observations

Internal medicines

1) Tab bosnea 1-1-1(A/F)
2) BalagudhuchyadhiKashaya 2tsp-2-2tsp (A/F)
3) TriphalaChurna + GodhantiBhasma + Akikapisthi (1tsp-1tsf-1tsp) (B/F)

Treatment compliance and adverse events:

Patient tolerated the therapy well and no ADR (adverse drug reaction) was noticed.

Outcome and Follow-Up

At discharge (Day 8), VAS score decreased from 7/10 to 2/10, representing a 5-point absolute reduction. The patient subjectively reported fewer pain episodes and improved comfort during chewing and speaking. No adverse events were observed during the treatment period.

No post-discharge follow-up data were available; durability of improvement could not be assessed.

Vas scale: Table-3.

Follow Up

After the completion of treatment patient got almost complete relief from TN symptoms. The patient got same relief after the first sitting of nasya therapy. Felt great relief in pain after the second and third follow up. During the second follow up same medications prescribed and the third follow up also same medications prescribed. Along with nasya therapy, medicines for Shaman chikitsa were given. As this treatment is more concentrated to treat the symptoms of T.N. patient followed the above intervention for the total duration of months with regular follow up. Patient did not leave the treatment in this 3 months duration and followed all the

TABLE -1 :

	Criterion	Case Findings	Status
A	≥3 attacks	Episodes occur almost daily for 6 months	Fulfilled
B	Unilateral trigeminal distribution	Left side of face (V2-V3 predominant)	Fulfilled
C1	Duration <2 minutes	Pain last 1-2 minutes, then dull ache	Fulfilled
C2	Severe intensity	Described as “electric shock-like” and sharp	Fulfilled
C3	Shock/stabbing quality	Electric shock like pain	Fulfilled
C4	Triggered by innocuous stimuli	Triggered by chewing, brushing teeth, touch, smiling, washing face	Fulfilled
D	No neurological deficit	Cranial nerves intact except jaw jerk +++	Fulfilled
E	Not better explained by another disorder	Dental, sinus.	Fulfilled

advised given to him. He got relieved from all the symptoms just in 3 months.

Discussion

Trigeminal neuralgia is a chronic neuropathic pain disorder often refractory to pharmacological treatment. From an ayurvedic standpoint, the condition can be correlated with *Anantavata*, a *urdhwajatrugatavatavyadhi* characterised by *toda* (pricking pain), *bheda* (splitting pain) in *mukhapradesha*, involving the craniofacial region. The pathogenesis involves *vata-pittaprakopa* due to *ruksha*, *laghu* and *Tikshnaahara-vihara*, along with *mansikanidanas* such as *chinta* and atichintana. These factors disturb *vyanavayu* and *pranavayu* along with *sadhakapitta*, leading to hypersensitivity in the craniofacial region supplied by trigeminal nerve

This case describes short-term symptomatic improvement in a patient with trigeminal neuralgia following an intensive, multimodal *Ayurvedic* intervention. From an *Ayurvedic* perspective, the condition was interpreted as *Anantavata*, involving *Vata-Pitta* aggravation in the craniofacial region.

However, TN is known to exhibit spontaneous remission and fluctuating severity.[4] Given the uncontrolled design, short treatment duration, and absence of long-term follow-up, the observed improvement may reflect natural disease variation, placebo response, attention effects, or regression to the mean rather than a specific therapeutic effect. Additionally, the patient had received subtherapeutic dosing of carbamazepine prior to presentation, limiting conclusions regarding treatment refractoriness.

Sampraptivighatana: The treatment adopted in this case aimed at *vata-pittashamana*, *srotoshodhana* and *vataanulomana*. In the treatment of *anantavata* all *acharyas* mention *Nasya”Nasa hi shirasodwaram”* (nose is gateway to the head) and *Anantavata* is also a *udwajatrugata* disorder, in such condition *nasya* is indicated due to significance of nose at the gateways of head and *murdha* ,[12]*nasya* with *mahatiktakaghrita* in the dose of *arohanakrama* (ascending order) in each nostril was administered for eight days and before administration the *nasya* massage of face with *balagudhuchyadhitaila* is done for ten to 20 min. The *mahatiktakaghritanasya* acts directly on

TABLE -2 :

<table>
<tr><th>S. No.</th><th>Treatment Given</th></tr>
<tr><td>1.</td><td>Koshtashodhana with Ghandarvahastaditaila (50ml) + triphalakashaya (60ml) (First Day)</td></tr>
<tr><td>2.</td><td>Sarvangaabyanga with Kheerabalataila f/b Bashpasweda</td></tr>
<tr><td>3.</td><td>Mukhaabhyanga with balagudhuchyadhitaila f/b Nasya with mahatiktakaghrita
<table>
<tr><td>01/04</td><td>02/04</td><td>03/04</td><td>04/04</td><td>05/04</td><td>06/04</td><td>07/04</td><td>08/04</td></tr>
<tr><td>20*E/N</td><td>40*E/N</td><td>60*E/N</td><td>70*E/N</td><td>80*E/N</td><td>90*E/N</td><td>90*E/N</td><td>90*E/N</td></tr>
</table></td></tr>
<tr><td>4.</td><td>Kavala with balagudhuchyadhitaila</td></tr>
<tr><td>5.</td><td>Pichu to left mandibular region with balagudhuchyadhitaila</td></tr>
</table>

shirashritasrotas, pacifying *vata* and *pitta* through its *tiktarasa*, *snigdhaguna* and *sheetaveerya*. The intervention might have contributed to reducing neural hyperexcitability and local inflammatory processes at the site of trigeminal nerve irritation. *Mukhaabhyanga* with *balaguduchyaditaila* and *pichu* on the affected mandibular region improved *rasa-raktasanchara* (microcirculation), alleviated *rukshata* and reduced the irritation of *snayu* and *sira*. It directly influences *urdhwajatrugatavata* through tactile stimulation of *marma* points such as *apanga*, *avarta*, *shankha* and *phana*, which are functionally related to trigeminal nerve branches. These local therapies are described to exert *snehana*, *mardana* and *vatashamaka* effects, which can modulate peripheral nerve irritability and promote healing.

Sarvangaabhyanga was perfomed daily using *kheerabalataila*, followed by *bashpasweda*. *Kheerabalataila* contains *bala* (sida cordifolia) processed in *ksheera* and *tilataila*, act as a potent *vatahara* and give the *bala* to *snayu-mamsa*, it also provides *snigdhaguna* to counter *ruksha* and *sheetaguna* of aggravated *vata*, and gentle massage improves peripheral circulation, nourished *dhatus*, and promotes secretion of endorphins and parasympathetic activation, resulting in pain reduction and relaxation. *Bashpasweda* further facilitated *srotoshodhana* and reduced *sanga* in *srotas*, leading to enhanced local metabolic activity. The combined effects of *snehana* and *swedana* thus helped in restoring *Dosha-samyata* and neural function.

Shamana medications: internal medicines played a crucial role in sustain the therapeutic benefits, *balaguluchyadhiKashaya* acts as *tridoshashamak* and *rasayana*, *bala* present in it strengthens *snayu* and *mamsa*, *majja* dhatu by providing *snigdha* guna and *guduchi* does *tridoshashamaka* and *Guduchi* exhibits potent anti-inflammatory, antioxidant and neuroprotective effects by inhibiting pro-inflammatory cytokines and oxidative stress marker[4] such action can modulate hyperexcitability and demyelination in the trigeminal nerve, stabilizing neural conduction and alleviating pain. Boswellia (bosnea) tablets: in trigeminal neuralgia, where perineural inflammation and vascular compression trigger demyelination of the trigeminal root, boswellia's anti-inflammatory and membrane-stabilizing properties help prevent further neural injury.[1,8] Its *katu-tiktarasa*, *ushnaveerya* and *vata-kaphahara* properties relieve stiffness (*stambha*) and pricking pain (*toda*). *Triphalachurna*: It supports *agniDeepana* and *srotoshodhana* while serving as a mild *rasayana*. It regulates ama *pachana* and maintains *koshthaShuddhi*, indirectly aiding *vataanulomana*. It has antioxidant and neuroprotective effects that protect neuronal cell from oxidative stress and mitochondrial dysfuction, both implicated in chronic neuropathic pain.[12]*Godanti Bhasma* is known for its ability to balance all three doshas, with a particular emphasis on *rakta* and *pitta*.[8]*Akikapishti* acts as *raktapittahara* reduced burning and tingling sensation often associated with neuralgia. in chronic trigeminal neuralgia, where anxiety and insomnia are common, *akikapishti* calming and adaptogenic effects, improving pain perception and quality of life. Together these formulations acted synergistically to relieve pain and improve functional integrity. conventional treatment with carbamazepine or gabapentin primarily aims to block

Table-3 :

Before treatment	After treatment
Seven	Two

sodium channels and suppress neuronal firing but these medicines have side effects like dizziness, drowsiness and hepatotoxicity. The ayurveda approach, in contrast targets root cause (*moolanidana*) and maintain doshas *samyak* and does dhatu *poshana*, without any adverse effects.

Limitations

- Single-patient case report
- Short treatment duration (8 days)
- No MRI to exclude secondary causes
- No standardized quality-of-life instruments
- No long-term follow-up
- Lack of laboratory safety monitoring

Conclusion

In this single case, an 8-day course of *Ayurvedic* interventions coincided with short-term improvement in trigeminal neuralgia symptoms without observed adverse effects. These findings are preliminary and hypothesis-generating. Controlled clinical studies with standardized diagnostics, adequate follow-up, and objective outcome measures are required before conclusions regarding efficacy or safety can be drawn.

Ethics Statement

Written informed consent for publication was obtained from the patient. Institutional ethics approval was not required for this single case report.

Conflict of Interest

The authors declare no conflict of interest.

Funding

No external funding was received.

Data Availability

All relevant data are included in the manuscript.

CARE Checklist

The CARE checklist has been completed and submitted as supplementary material

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https://doi.org/10.33451/florafauna.v32i1pp150-152
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 150-152 ISSN 0971 - 6920 (Print)

Maintaining Smile by Non Invasive Methods : Short Communication

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Received **:** 11.03.2026; ***Accepted*** : 11.04.2026

How to cite : Srivastava R, Srivastava R. Maintaining Smile by Non Invasive Methods : Short Communication. *Flora and Fauna* 2026. 32(1) : 150-152.

ABSTRACT

Esthetics, which is derived from the Greek word for "perception", deals with beauty and the beautiful. It has two dimensions: objective and subjective. Artificial tooth pontic can be used as an esthetic step towards the treatment of pathological migration in patients having poor bone support in the anterior region. As it causes splinting of the mobile teeth along with replacement of space with esthetically pleasing appetence which is readily accepted by the patient.

Figures : 06 References : 05 Table : 00

KEY WORDS : Chronic Periodontitis, Esthetics, Splinting

Introduction

Esthetics, which is derived from the Greek word for "perception", deals with beauty and the beautiful. It has two dimensions: objective and subjective.[3] Objective (admirable) beauty is based on consideration of the object itself, implying that the object possesses properties that make it unmistakably praiseworthy. Subjective (enjoyable) beauty is a quality that is value-laden, relative to the tastes of the person contemplating it.[4] Careful technique in dentistry should lend objective esthetics (admirable beauty) to the entire orofacial complex, involving unity, form, structure, balance, color, function, and display of the dentition. On the other hand, the creation of subjective beauty may enhance cosmetic value.[2]

Esthetics plays an important role in periodontal practice in today's clinical world and is amongst the necessity of any treatment procedure, and also the primary need of the patient apart from relief of the chief complain.[3]

The Replacement of missing anterior teeth enhances the smile as well as appearance of the patient which is easily appreciated by the patient and the population.[2]

The Replacement can be by Implants, Crowns, Bridges or Cast Partial Dentures amongst this treatment modality is natural and artificial tooth pontic. This article deals with the replacement of anterior teeth by an artificial tooth which helps in enhancement of esthetics and phonetics.[5]

Case Report

A female patient age 45 yrs attended Dental Wing, Savitri Hospital Jhansi with the chief complaint of bleeding from gums and bad breath on Clinical examination. It was found that patient was having localized chronic periodontitis. A radiographic examination was made for the same (Figs. 1 & 2)

ACKNOWLEDGEMENTS : The Authors thank the Director Savitri Hospital Jhansi, Prof R.P Srivastava for providing opportunity and necessary facilities for performing this case. No Funding is involved in this case report.

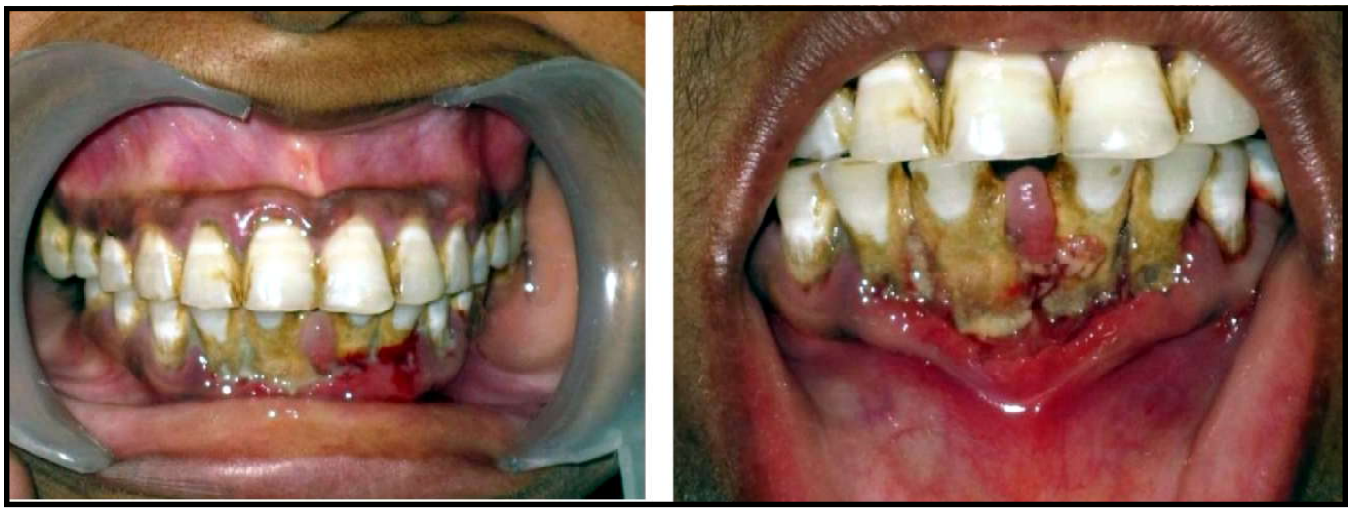

Fig. 1 **Fig. 2**

Figs. 1& 2 : Chronic periodontitis with heavy calculus deposit

A treatment plan was formulated for the patient which included oral hygiene instruction followed by Scaling and root planning. Patient was recalled after one week.(Fig. 3)

As the lower anterior was having grade II mobility, next mode of treatment was splinting the lower anterior. A fiber reinforced composite splint restoration was planned for the same. On examination it was seen that the patient was having spacing due to pathological migration in lower anterior (Fig. 3)

An Acrylic tooth of the shape and shade of mandibular central incisor was selected from the teeth set (Fig. 4) and it was placed in the spacing created due to pathological migration with the help of fiber splint (Hager Werken) (Fig. 5)

Discussion

Periodontitis is one of the commonest diseases of oral cavity effecting majority of population. Various treatment modalities are available for the treatment of the same.[3] One of the features of Periodontitis is Pathological migration which causes unwanted spaces which if present in the anterior region causes esthetic problems, after the treatment such spaces can be treated orthodontically (by aligning the tooth) or prosthodontically (by crowns or veneers).[5]

As the patient was having tooth mobility modality of orthodontically aligning teeth would not have been possible. Artificial tooth pontic proved to be a novel approach towards cosmetic/esthetic approach for the treatment of spacing present in lower anterior which was appreciated by the patient along with solving the purpose of splinting the mobile tooth along with improvement in phonetics.[1] (Fig. 6)

Conclusion

Artificial tooth pontic can be used as an esthetic step towards the treatment of pathological migration in

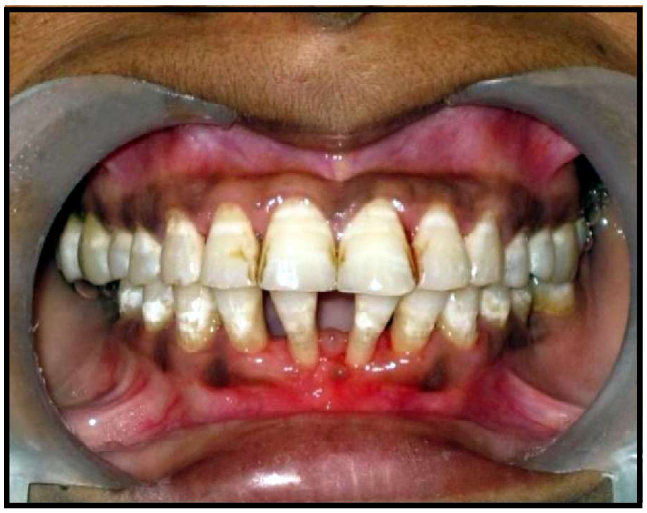

Fig. 3 : Post Scaling and Root Planing

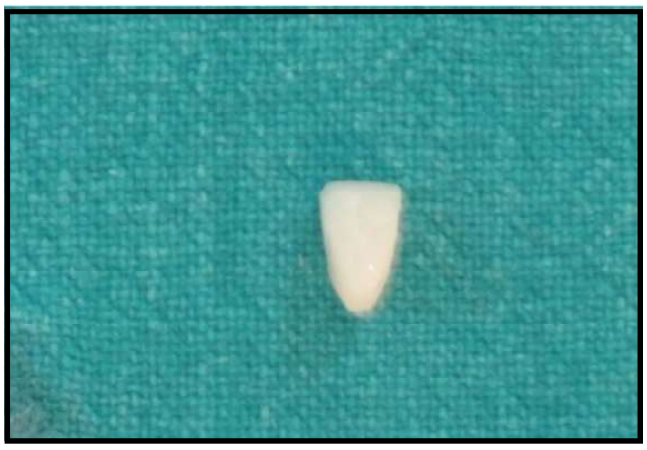

Fig. 4 :Acrylic Tooth

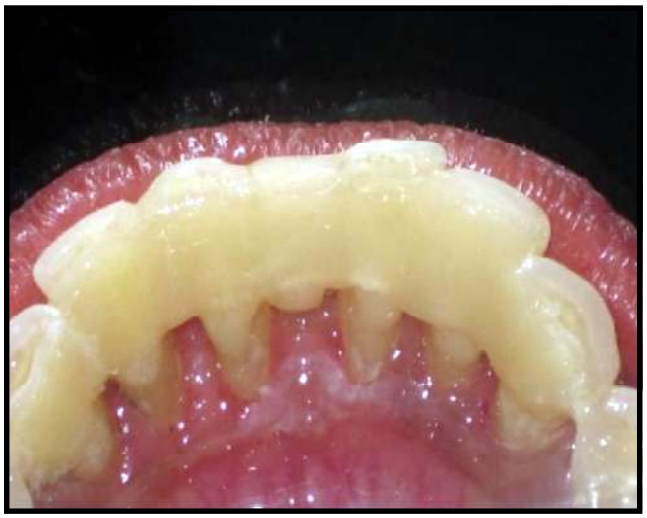

Fig. 5 : Acrylic tooth stabilized with Fiber Reinforced Composite Splint

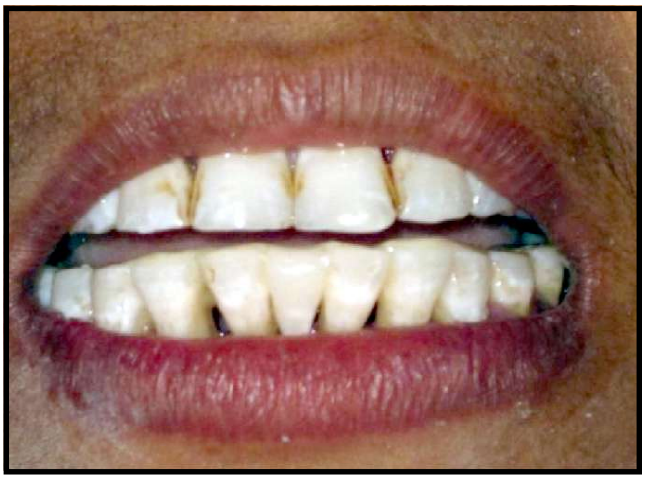

Fig. 6: Post Treatment Smile

patients having poor bone support in the anterior region. As it causes splinting of the mobile teeth along with replacement of space with esthetically pleasing appetence which is readily accepted by the patient. This modality is non invasive and economic solution to the patients with low socioeconomic status who demand esthetics, as beauty is deserved by all.

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https://doi.org/10.33451/florafauna.v32i1pp153-160
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 153-160 ISSN 0971 - 6920 (Print)

Interrelationships between BMI and Behavioural Health Factors in Young Adult Collegiate: A Cross-Sectional Analysis

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Received **:** 11.03.2026; ***Accepted*** : 08.04.2026

How to cite : Gholap AB. Interrelationships between BMI and Behavioural Health Factors in Young Adult Collegiate: A Cross-Sectional Analysis. *Flora and Fauna* 2026. 32(1) : 153-160.

ABSTRACT

Microorganisms, especially fungi and bacteria cause biodeterioration of our cultural heritage including paper, cloths, wood and leather. Cellulolytic Fungi cause decomposition of papers and cloths present in our cultural heritage *etc.* In the present investigation, fungi invading books of RajkThis study investigates the interrelationships between Body Mass Index and various behavioural and lifestyle factors among a young adult collegiate population. BMI is utilized as a primary measure to assess nutritional status, categorizing individuals into underweight, normal, overweight, and obese classifications. The analysis reveals that although most students (1,729) having normal BMI range *i.e.* (18.5"25 kg/m2 18.5"25 kg/m2) significant portions of the population exhibit both under nutrition and over-nutrition. This coexistence of health issues is described as malnutrition. A gender-wise students analysis female are more in the under nutrition and over-nutrition categories compared to their male Student. The study correlates BMI with several lifestyle factors. Students having normal BMI are engage in regular physical activity, get proper sleep, and adequate water intake. Conversely students in the underweight categories tend to have lower physical activity and inadequate sleep, while those in the obese and overweight categories show very low physical activity and higher consumption of fast food. Overall, the findings highlight that both under nutrition and obesity are significant concerns within the student population strongly associated with lifestyle behaviour. The study suggests a need for targeted, gender-sensitive health and nutritional interventions within educational institutions to address these dual challenges.

Figures : 03 References : 29 Tables : 03
KEY WORDS : Behavioural Health Factors, Body Mass Index, Nutrition, Obesity.

Introduction

The Body Mass Index is used to measure and calculates an individual's height in relation to their body weight[4]. It used to screen for macro-nutritional status whether under nutrition, normal range or over nutrition[10]. The importance of health to humans is incomparable. Longevity is promoted by living a health-aware way of life. A person's basal index of metabolism would be enhanced because of proper eating. Holding a healthy lifestyle and a proper weight is a duty for all. An unhealthy lifestyle would be bad for holistic growth. The world has changed the way it lives in industry; this lifestyle change has brought about changes in dietary practices that require an increased dietary consumption of fat, sweet, and non-nutritive foods, together with a reduction of healthier eating practices[23]. An individual's weight in kilograms divided by height in meters squared was devised, in the 1830s to enable the comparison of White middle-aged European men of varying heights *via* a common parameter. This Quetelet index was termed the body mass index[10] Four categories of BMI are in common use today to estimate patient conditions: (1) underweight or undernourished status (<18.5 kg/m2); (2) normal BMI (18.5–<25.0 kg/m2); (3) overweight status (25.0–<30.0 kg/m2); and (4) persons with obesity as e"30.0 kg/m2[1]. Obesity status can be subcategorized further to capture severe BMI variance[21]. BMI serves as a valuable tool for assessing the overall health of

ACKNOWLEDGEMENTS : The author sincerely thanks the institutional authorities for permission and support, and I also acknowledge the students and staff for their guidance and assistance during the study.

TABLE-1: BMI Distribution Age and Academic Level-wise Among Students

BMI range - kg/m2	Total No of Students	First year Undergraduate (19 yr. Age)	Second year Undergraduate (20 yr. Age)	Third year Undergraduate (21 yr. Age)	Postgraduate (22 to 23 yr. Age)
Mild Thinness < 16	230	71	56	57	46
Moderate Thinness 16 - 18.5	57	21	14	14	11
Normal 18.5 - 25	1729	531	415	433	348
Overweight 25 - 30	298	91	71	75	60
Obese Class I 30 - 35	29	9	7	7	6
Obese Class III 35 - 40	9	3	2	2	2
Severe Thinness > 40	144	44	35	36	29

populations and identifying trends in weight distribution across different demographic groups[22]. It provides a standardized measure that aids in monitoring the prevalence of underweight, normal weight, overweight, and obesity within a cohort of subjects. By using BMI data, public health officials can develop targeted interventions to address obesity-related health issues such as type 2 diabetes, cardiovascular diseases, and joint problems[7]. Health care providers and public health agencies alike recognize obesity to be a risk factor for multiple outcomes such as cardiovascular disease, diabetes, chronic orthopaedic problems, accidents, collagen vascular diseases, lower quality of life, osteoarthritis, and autoimmune disorders[8] Health Effects of Overweight and Obesity. High BMI is also associated with an increased risk of colorectal cancer[17] primary liver cancer, and cancer mortality[27]. Obesity is a risk factor for death from SARS-CoV-2 infection (COVID-19 disease), perhaps related to stress on lung capacity and complexities in ventilator management in intensive care settings[26]. Besides compromising physical health, obesity very often leads to psychological and social distress. Overweight status is associated with an increased risk of depressive disorder among adults[24]. Individuals with excess weight are subject to social devaluation and discrimination, termed “fat shaming” by some. Such negative stereotypes because of body weight, better termed weight stigma, affect people with obesity; persons feeling ashamed or embarrassed may hesitate to seek timely professional help, may have trouble finding needed social support, and can impede weight loss maintenance[24]. although guidelines exist, many studies show that students’ engagement in physical activity is low. In the study[14] a prevalence of 52% of insufficiently physically active students was recorded, while the research[11] found that 53.1% of female students met the recommended level of physical activity. Studies examining the level of physical activity in Bosnia and Herzegovina have demonstrated inconsistencies in the data. A research[9] indicate that the student population in Bosnia and Herzegovina is predominantly engaged in moderate-intensity physical activities at an adequate level, whereas other studies have concluded that physical activity is maintained at a satisfactory level[19]. Furthermore, female students have been found to be less physically active than their male counterparts[18]. Physical activity has a positive and significant impact on the quality of life of students, with a clear connection to various domains of quality of life[5]. These findings suggest that physical activity is a key factor in improving quality of life, while a low level of physical activity is often associated with an increased presence of psychosomatic disorders, motor function impairments, and reduced social functioning abilities[15]. For this reason, many researchers emphasize the importance of assessing the quality of life in the university student population[12]. The present study was conducted in a population of age ranging between 40 and 60 years. This typically qualified for a middle-aged population. This age group marks a transitory phase between an adventurous, thrill-seeking young adulthood and a retired life, accompanied by a sense of fulfilment of duties and

TABLE-2: Gender-wise Analysis of BMI distribution among students

BMI range - kg/m2	Total No of Students	Male	Female
Mild Thinness < 16	230	102	128
Moderate Thinness 16 - 18.5	57	25	35
Normal 18.5 - 25	1729	768	958
Overweight 25 - 30	298	133	165
Obese Class I 30 - 35	29	12	17
Obese Class III 35 - 40	9	4	5
Severe Thinness > 40	144	63	81

responsibilities, contentment from successfully achieving one's life goals, and gratitude for the divineness of life and its splendour. The various changes that set in are carefully investigated, as those serve as important markers in determining the changed life-path trajectory of individuals[25]. The present study is aimed at investigating the existence of any potential relationship between BMI measure and PWB and memory functioning of middle-aged adults.

Materials and Methods

Study Area

Study Population and Sampling: This paper is a correlational cross-sectional study. The sample comprised 2496 collegiate students belonging to urban communities, having 1107 male and 1389 female students located at P.V.P. College, Pravaranagar.

Sampling Technique: A purposive sampling technique was used to construct the sample at the undergraduate and postgraduate levels.

Age Range: The study included both males and females within the age range of first-year undergraduates (19 yr. ages). Second-year undergraduate (20 yr. age) Third-year undergraduate (21 years old) Postgraduate (22 to 23 yr. age)

Grouping: The sample was divided into seven groups based on BMI range in kg/m² as follows: Mild Thinness < 16, Moderate Thinness 16–18.5, Normal 18.5–25, Overweight 25–30, Obese Class I 30–35, Obese Class III 35–40, and Severe Thinness > 40.

Data Collection Period: Data were gathered over a Six-month period, from Jane to November 2025

Tools used

Behavioural Health Factors and clinical data sheet This consists of the preliminary information of the concerned individual, including name, age, sex, education, Diet Type, Physical Activity, Fast Food Consumption, Sleep Duration, Screen Time (per day) and Water Intake (lit/day) Such personal details were obtained exclusively to investigate the difference between the respective Behavioural Health Factors determining an individual's likely performance. No significant personal information was revealed hence anonymity was maintained throughout.

Height and weight measuring scales: The height and weight measures of everyone were measured using a standard weighing machine and inch-wise calibrated measuring tape

Results and Discussion

The BMI distribution among students across different academic years (Table and Graph No. 1) show that the majority fall within the normal BMI category (18.5–25 kg/m²), totalling 1,729 students, and indicating overall satisfactory nutritional status in the population, consistent with WHO standards for adult BMI classification[1]. However, a notable proportion of students exhibit mild thinness (n = 230) and moderate thinness (n = 57), while 144 students fall under severe thinness, reflecting the continuing burden of under nutrition among young adults, which aligns with patterns reported in earlier studies on Indian college populations. Simultaneously, the presence of overweight (n = 298) and Obese Class I and III categories (n = 29 and n = 9) demonstrate the coexistence of rising overweight and

TABLE-3: BMI Categories with Dietary and Lifestyle Factors among Students

BMI range - kg/m2	No of Student s	Diet Type		Physical Activity		Fast Food Consumption		Sleep Duration		Screen Time (per day)		Water Intake (lit/day)	
		Vege-tarian	Mixed	Yes	No	Daily	Weakly	<6 hr.	>6 hr.	<2 hr.	>2 hr.	<3 Lt	>3 Lt
Mild Thinness < 16	230	97	133	35	195	32	198	88	142	136	94	62	168
Moderate Thinness 16 - 18.5	57	22	35	07	50	09	48	35	22	33	24	23	34
Normal 18.5 - 25	1729	720	1009	955	774	135	1594	1123	606	846	883	1089	640
Overweight 25 - 30	298	103	195	89	209	27	271	109	189	147	151	126	172
Obese Class I 30 - 35	29	10	19	03	26	02	27	18	11	21	08	12	17
Obese Class III 35 - 40	9	03	06	00	09	05	4	05	04	01	08	04	05
Severe Thinness > 40	144	62	82	23	121	21	123	62	82	93	51	100	44

obesity trends, a phenomenon often described as the "double burden of malnutrition" in developing nations[23]. The distribution across First, Second, Third-year undergraduates and postgraduates remain relatively balanced in all BMI categories, suggesting that nutritional challenges persist consistently across ages 19–23. Overall, the data indicate that although normal BMI predominates, both under nutrition and overweight/ obesity are significant concerns, highlighting the need for targeted, year-wise health promotion and nutritional interventions in educational institutions

The gender-wise BMI distribution (Table and Graph No. 2) show that most students fall within the normal BMI range (18.5–25 kg/m²), comprising 1,729 students (768 males and 958 females), and reflecting generally adequate nutritional status as per WHO BMI guidelines[1]. However, under nutrition remains significant, with mild thinness (<16) in 230 students and moderate thinness (16–18.5) in 57 students, where females (128 and 35) outnumber males (102 and 25), suggesting that female students may be more susceptible to under nutrition an observation consistent with national patterns reported[20]. Additionally, severe thinness (>40) affects 144 students (63 males and 81 females), further reinforcing a gender gap that aligns with earlier research indicating higher rates of chronic energy deficiency among young women in India[28]. At the same time, overweight and obesity also appear in the population, with 298 students overweight and smaller proportions in Obese Class I (n = 29) and Obese Class III (n = 9), again showing slightly higher representation among females, reflecting the rising burden of overweight among young adults as highlighted[23]. Overall, the data demonstrate a dual

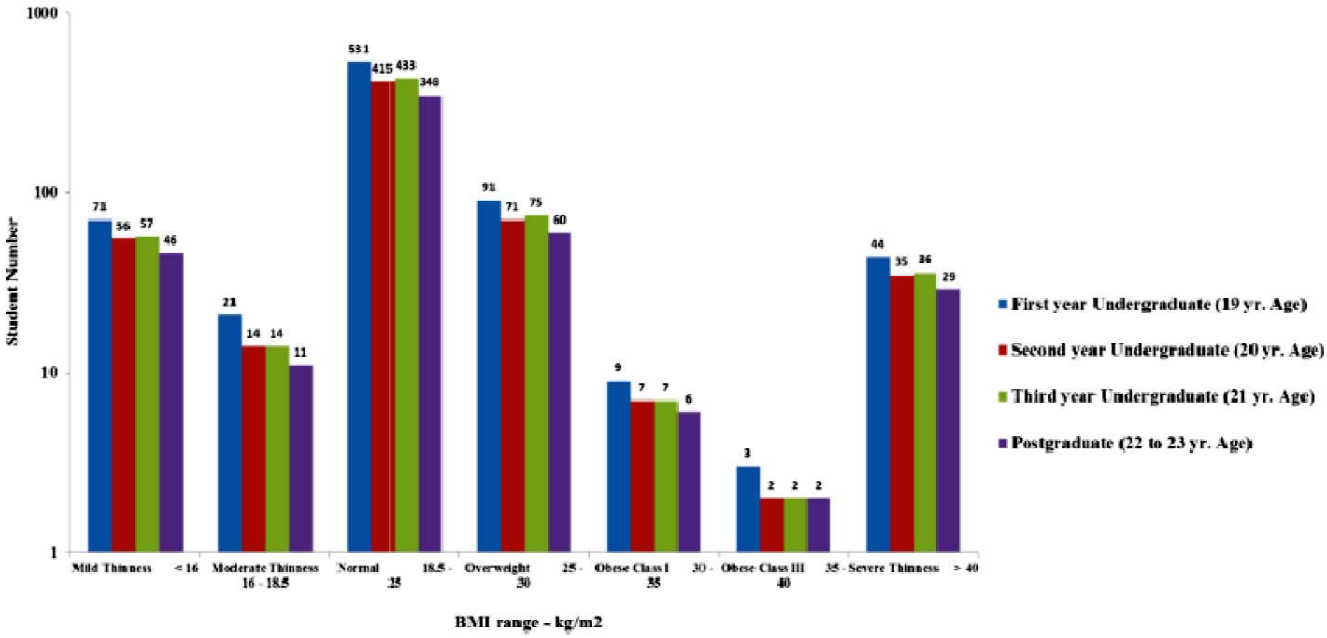

Fig. 1 :BMI Distribution Age and Academic Level-wise Among Students

burden of malnutrition, with both under nutrition and overweight/obesity coexisting, and indicates a need for gender-sensitive nutrition and health interventions within student populations.

The relationship between BMI categories and lifestyle behaviours(Table and Graph No. 3) show that students with a normal BMI (n = 1,729) generally adhere to healthier patterns, including higher levels of physical activity (955), adequate sleep (>6 hr = 1,123), moderate screen time (<2 hr = 606), and sufficient daily water intake (>3 L = 640), which aligns with evidence that balanced sleep, hydration, and activity levels support healthy weight maintenance[1]. In contrast, students with mild thinness (n = 230) and severe thinness (n = 144) show markedly lower physical activity (35 and 23), higher screen time (>2 hr = 142 and 82), shorter sleep duration (<6 hr = 88 and 62), and inadequate hydration (<3 L = 94 and 51), indicating lifestyle deficiencies that contribute to under nutrition, consistent with findings from adolescent nutritional studies in India[20,28]. Meanwhile, students in overweight (n = 298) and obesity categories (Class I = 29; Class III = 9) demonstrate low physical activity (89, 3, and 0), higher fast-food consumption, increased screen time (>2 hr = 189, 21, and 1), and mixed dietary patterns, reflecting behavioural risk factors linked with weight gain and metabolic imbalance, as highlighted in global research on lifestyle-associated obesity among young adults[23]. Overall, the data reinforces that healthy BMI is strongly associated with balanced sleep, limited screen exposure, regular physical activity, and adequate hydration, whereas deviations in these behaviour correlate with both under nutrition and obesity, underscoring the need for targeted lifestyle-based interventions within student populations.

To address the dual burden of malnutrition among students, educational institutions should implement integrated nutrition education and regular health screening programs to identify both under nutrition and overweight at an early stage, in line with WHO recommendations[29]. Promotion of physical activity, adequate sleep, reduced screen time, and proper hydration should be prioritized through structured campus-based lifestyle interventions, as these behaviours are strongly associated with healthy BMI[6]. Gender-sensitive strategies, particularly focusing on female students who are more vulnerable to under nutrition, are essential, along with improving access to nutritious and affordable food options on campus to discourage unhealthy dietary patterns[20,23] reproduction and survival. Similar seasonal patterns, with monsoon minima and winter maxima, have been reported, indicating that seasonal environmental dynamics play a crucial role in regulating copepod populations in freshwater ecosystem

Conclusion

This study demonstrates that although most students fall within the normal BMI range, a considerable proportion experience both under nutrition and overweight/obesity, reflecting a clear double burden of malnutrition. This pattern is consistent across academic years and is influenced by gender, with females showing higher levels of thinness and a slightly greater tendency toward overweight. Lifestyle behaviours strongly

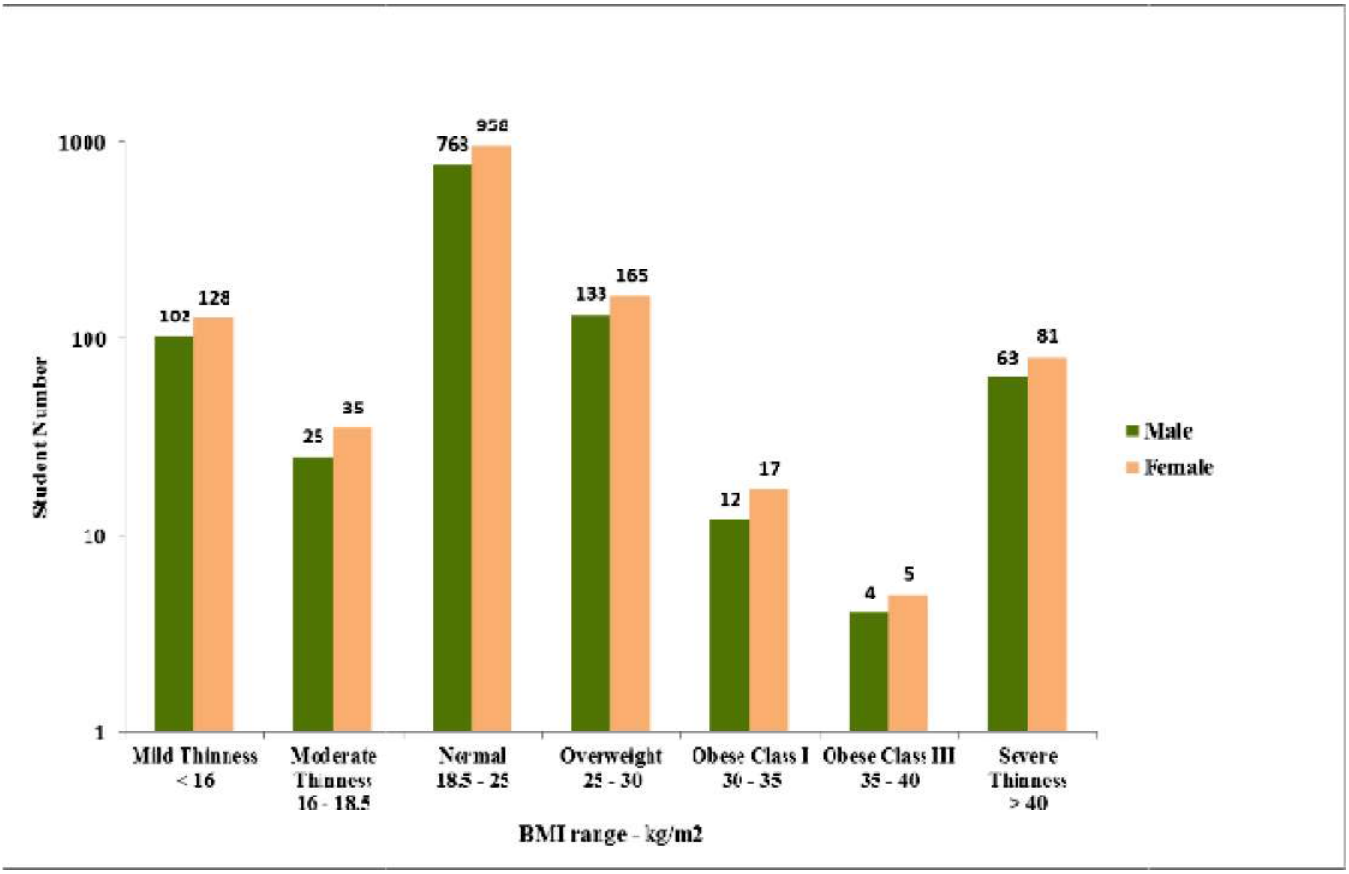

Fig. 2 : Gender-wise Analysis of BMI distribution among students

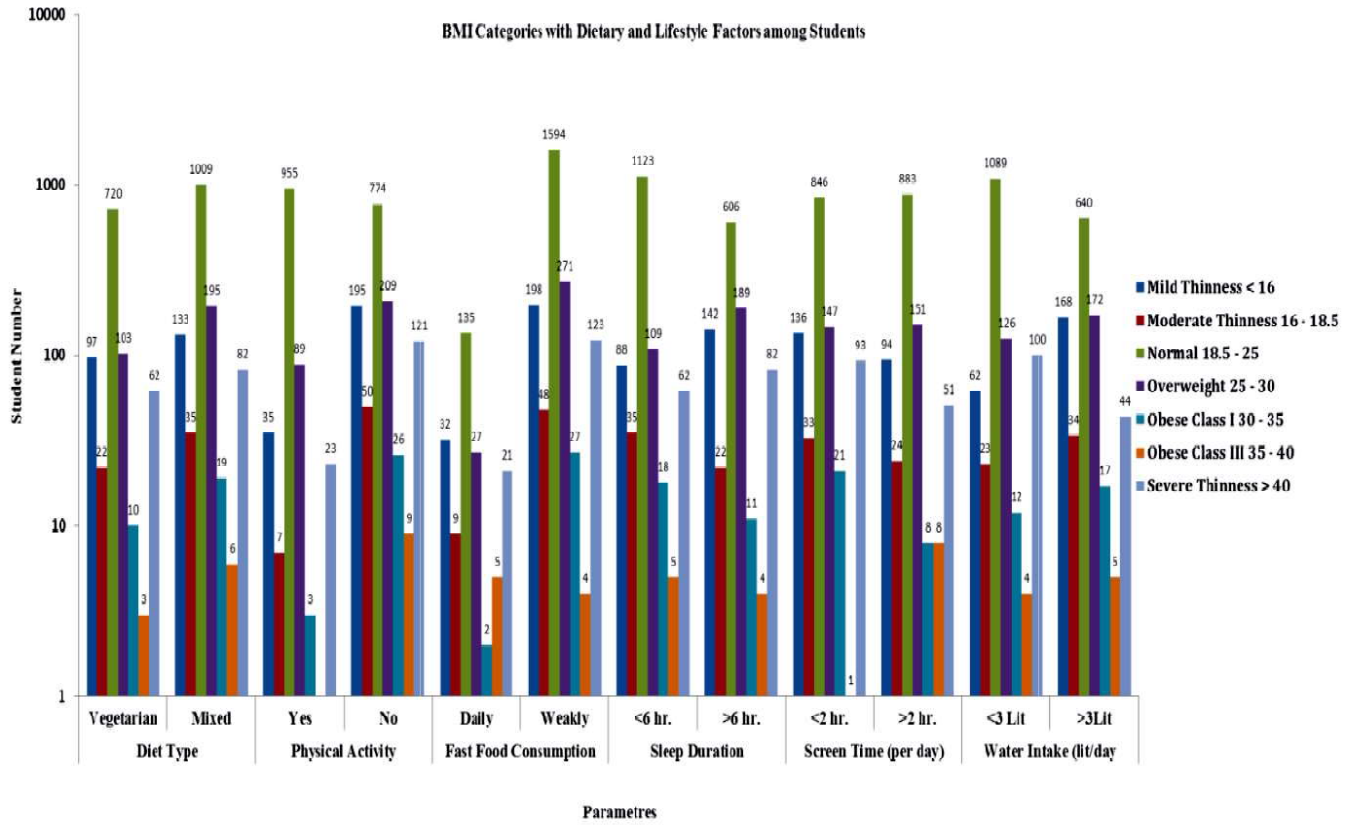

Fig. 3 : BMI Categories with Dietary and Lifestyle Factors among Students

correlate with BMI status, as normal BMI is associated with adequate physical activity, sleep, hydration, and lower screen time, whereas unhealthy lifestyle patterns are linked to both thinness and obesity. Overall, the findings highlight the interrelationship between BMI, gender, academic stage, and lifestyle behaviours, emphasizing the need for integrated, gender- and year-specific lifestyle and nutrition interventions among students

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https://doi.org/10.33451/florafauna.v32i1pp161-167
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 161-167 ISSN 0971 - 6920 (Print)

Fluoride impact as carcinogen: a case evidence in *Zaprionus indianus* (Diptera, Drosophilidae)

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Received **:** 25.03.2026; ***Accepted*** : 28.04.2026

How to cite : Rani S, Gupta KK. Fluoride impact as carcinogen: a case evidence in Zaprionus indianus (Diptera, Drosophilidae). *Flora and Fauna* 2026. 32(1) : 161-167.

ABSTRACT

Fluoride, a trace element, maintains bone and dental well-being at trace levels. However, high levels of fluoride are associated with systemic toxicity, genotoxicity, and carcinogenic effects. Drosophila melanogaster has become a widely used genetic model organism to study fluoride induced toxicity. In this study, the invasive fruit fly *Zaprionus indianus*, a robust species, was used to study the potential carcinogenic effects of fluoride. Larvae were grown on fluoride containing food media (0.0-4.0 ppm) prepared by dissolving sodium fluoride, and the mortality response was analysed using probit analysis to determine the median lethal concentration (LC… €). The LC… € value for fluoride in *Z. indianus* was estimated at 2.5 ppm, and total mortality occurred at e" 3.0 ppm. Hypertrophy of cerebral lobes and structural alterations in the ventral nerve cord were found using morphometric examination of third instar larval brains. Cytological assays using trypan blue and propidium iodide showed lowered apoptotic cell populations, suggesting that programmed cell death was disrupted. These results reinforce *Z. indianus* suitability as a viable invertebrate model for fluoride toxicity studies by providing the first evidence of fluoride induced neuromorphological and cytological changes compatible with early carcinogenic processes.

Figures : 04 References : 25 Tables : 03
KEY WORDS : Apoptosis, Carcinogenesis, Fluoride toxicity, Genotoxicity, *Zaprionus indianus*.

Introduction

Fluoride is a naturally occurring trace element that is vital for bone and dental mineralisation in trace amounts. However, chronic excessive exposure to fluoride has been associated with numerous toxicological impacts in humans and animals. Both natural and anthropogenic activities contribute to increased fluoride levels in the environment[13]. In India, excessive fluoride contamination has been observed in 23 states, with concentrations exceeding the WHO permissible limit of 1.5 mg/L[7, 17, 20, 25].

Although the manifestations of skeletal and dental fluorosis are the most obvious signs of the toxic effects of fluoride, recent reports have shown that fluoride can produce systemic toxicity[15]. Research has shown that excessive fluoride intake can lead to renal toxicity[12], reproductive toxicity[3], bone diseases[24] and alterations in the activities of critical enzymes, including phosphorylase, adenosine triphosphatase, and alkaline phosphatase, in invertebrates[16]. Mechanistic studies on fluoride toxicity have shown that it can induce oxidative stress by increasing the formation of reactive oxygen species (ROS), leading to alterations in the redox balance of cells and contributing to its genotoxic and carcinogenic effects[19].

Drosophila melanogaster is one of the fruit fly

ACKNOWLEDGEMENTS : The authors express sincere thanks to the Department of Zoology, Vinoba Bhave University, Hazaribagh, for providing the laboratory facilities for the experimental work on Zaprionus indianus and Mr. Satish Kumar, research scholar, Dept. of Biotechnology, Vinoba Bhave University, for assisting the fluorescence microscopy.

TABLE-1:Preparation of fluoride solutions from 100 ppm stock solution using dilution method.

S. No.	Target concentration (ppm)	Vol. of Stock Solution (mL)	Volume of Distilled water(mL)
1	1	1.00	99.00
2	2	2.00	98.00
3	2.5	2.50	97.50
4	3	3.00	97.00
5	4	4.00	98.00

species that has been used to study the genetic and toxicological effects of different environmental pollutants, including fluoride[14]. Past research has shown that sodium fluoride (NaF) exposure can lead to effects on development timing, compound eye development, and melanotic tumour formation in adult *Drosophila melanogaster*[8]. Other research has shown that there is a significant association between water fluoridation and cancer prevalence[6] in 9 fluoride prone sites in Japan[23]. Other fluoride compounds, such as fluoride pesticides containing Fipronil (FP), have shown that they can lead to mutagenic, recombinogenic, and carcinogenic effects on the somatic cells of *Drosophila melanogaster*[4,5,21]. From all the research and literature reviewed on the effects of fluoride and other fluoride compounds on organisms, there is sufficient evidence to support the proposed hypothesis that carcinogenesis can be caused in organisms through different pathways.

Despite extensive studies on *D. melanogaster*, little is known about fluoride toxicity in other members of the family Drosophilidae. Extension of such studies is essential to confirm species-specific effects and to find alternative model organisms for comparative carcinogenicity studies. With this aim, *Z. indianus*, a species widely distributed and easily adapted to laboratory conditions within the family Drosophilidae, has been used to assess the carcinogenic effects of fluoride exposure, especially regarding morphometric changes in brain ganglion larvae[9] and cytological features of apoptosis. The results would give us basic information about this new model organism for studying fluoride induced carcinogenesis.

Materials and Methods

Preparation of Fluoride Solutions

Anhydrous sodium fluoride (NaF; analytical grade) was used to prepare the stock solution following the protocol described[18]. Briefly, 0.015 g of NaF was dissolved in 500 mL of distilled water in a volumetric flask to obtain a 100 ppm (100 mg/L) stock solution. Working solutions of desired concentrations were prepared by serial dilution from the stock using the standard formula M V = M, V, (Table 1). All fluoride solutions were freshly prepared to ensure chemical stability and prevent precipitation.

TABLE-2 : Probit analysis for LC_{50}

Concentration (ppm)	log 10 (concentration)	% mortality	Probit of kill	SUMMARY OUTPUT	
0.5	-0.301029996	13	3.87	*Regression Statistics*	
1	0	18	4.08	Multiple R	0.904904
1.5	0.176091259	30	4.48	R Square	0.818852
2	0.301029996	37	4.67	Adjusted R Square	0.782623
2.5	0.397940009	48	4.95	Standard Error	0.423454
3	0.477121255	77	5.74	Observations	7
4	0.602059991	92	6.41		

Rearing of *Zaprionus indianus*

Z. indianus (Family: Drosophilidae) is morphologically characterised by two pairs of silvery white, zebra-like stripes on the thorax and abdomen (Fig. 1). Collected and identified species of *Z. idianus* were reared on standard *Drosophila* food medium in the genetics lab. of the department. Cultures were maintained at 25 ± 1°C, 60 ± 5% relative humidity.For experimental exposure, food media were prepared in fluorinated water with different concentrations of fluoride solutions (0.00–4.00 ppm). Six replicate culture vials were maintained for each concentration, resulting in a total of 36 replicates (6 × 6 = 36).

Determination of LC

A single mated female was allowed to oviposit overnight in a Petri dish containing standard food medium. The bifilamentous eggs were observed under a stereomicroscope, and 10 eggs were carefully transferred into each vial containing freshly prepared fluoride-supplemented food at concentrations ranging from 0.0 to 4.0 ppm, along with a control (untreated Group). All the vials were incubated in a BOD chamber at 25°C and 60% relative humidity. The parental female was removed after a week, and 14 days later, the number of adult flies that successfully emerged was recorded. The median lethal concentration (LC… €) value was determined as the fluoride concentration that inhibited the development of 50% of the eggs (5 out of 10). Percentage mortality at each concentration was recorded, followed by probit analysis. Probit analysis was conducted to determine LC_{50} statistically as described[2]. using MS –EXCEL.

Neoplasia assessment

Tumour formation (neoplasia) and tissue hypertrophy are hallmark indicators of carcinogenic transformation. To assess these endpoints, a culture of *Z. indianus* was established by rearing a naturally inseminated wild female on food medium containing fluoride at the LC… € concentration (2.5 ppm). Third instar larvae were dissected in Poels' salt solution (pH 6.8) under a stereoscopic binocular microscope. Brain ganglia were isolated, and the size of the right & left cerebral lobes and the ventral nerve cord was measured using an ocular micrometrecalibrated with a stage micrometre.Morphometric alterations were statistically compared with those of control larvae.

Cytological Assessment of Apoptosis

To evaluate fluoride-induced alterations in apoptosis, cytological assays were performed using the Trypan Blue exclusion assay[22] and the propidium iodide (PI) fluorescencedye exclusion method[11]. Larval brain ganglia were incubated in 0.4% Trypan Blue for 5 min, washed in phosphate-buffered saline (PBS, pH 7.4), and examined under a compound microscope. For PI staining, tissues were incubated in 10 µg/mL PI for 5 min, rinsed with PBS and visualised under a fluorescence microscope. The proportion of stained

TABLE-3:Comparative brain lobe size in control and fluoride-exposed (2.5 ppm) larvae of *Zaprionus indianus*.

Replica	Control (0.0 ppm) Right lobe (µm)	Control (0.0 ppm) Left lobe (µm)	Treated (2.5ppm) Right lobe (µm)	Treated (2.5ppm) Left lobe (µm)	% Increase (Right lobe)	% Increase (Left lobe)
1	26.5	26.5	30.8	35.42	16.2	33.6
2	26.5	26.5	33.24	35.30	25.43	33.20
3	26.5	26.42	28.2	29.00	6.41	9.76
4	25.8	26.25	30.8	35.42	19.37	34.93
5	26.43	26.52	33.24	35.30	25.76	33.10
6	26.8	26.5	33.5	35.20	25.00	32.83
Mean ± SD	**26.42 ± 0.33**	**26.44 ± 0.05**	**31.63 ± 2.09**	**34.27 ± 2.58**	**19.71 ± 7.57**	**29.57 ± 9.73**

Fig. 1 : Experimental model: *Zaprionus indianus* showing characteristic zebra-like silvery white thoracic and abdominal stripes

(non-viable) and unstained (viable) cells was visualised (Fig. 4) under a fluorescence microscope and an Olympus light microscope.

Results

Mortality of *Zaprionus indianus* at different fluoride concentrations used for LC... € determination, which indicated that 50% of flies died at the fluoride concentration of 2.5 ppm, indicating LC_{50}. (Table-2).Further probit analysis was performed to estimate the LC_{50}, which revealed a value of 1.91 ppm.

Neoplasia Assessment after measuring the size of the enlargement of the brain and metastasis revealed significant size variation at 2.5 ppm of fluoride (Fig. 3)

Apoptotic (programmed cell death)

Apoptotic (programmed cell death) assessment was performed to visualise the cell's fate. Dye exclusion with both dyes, PI (3-1) and trypan blue (3-2), clearly

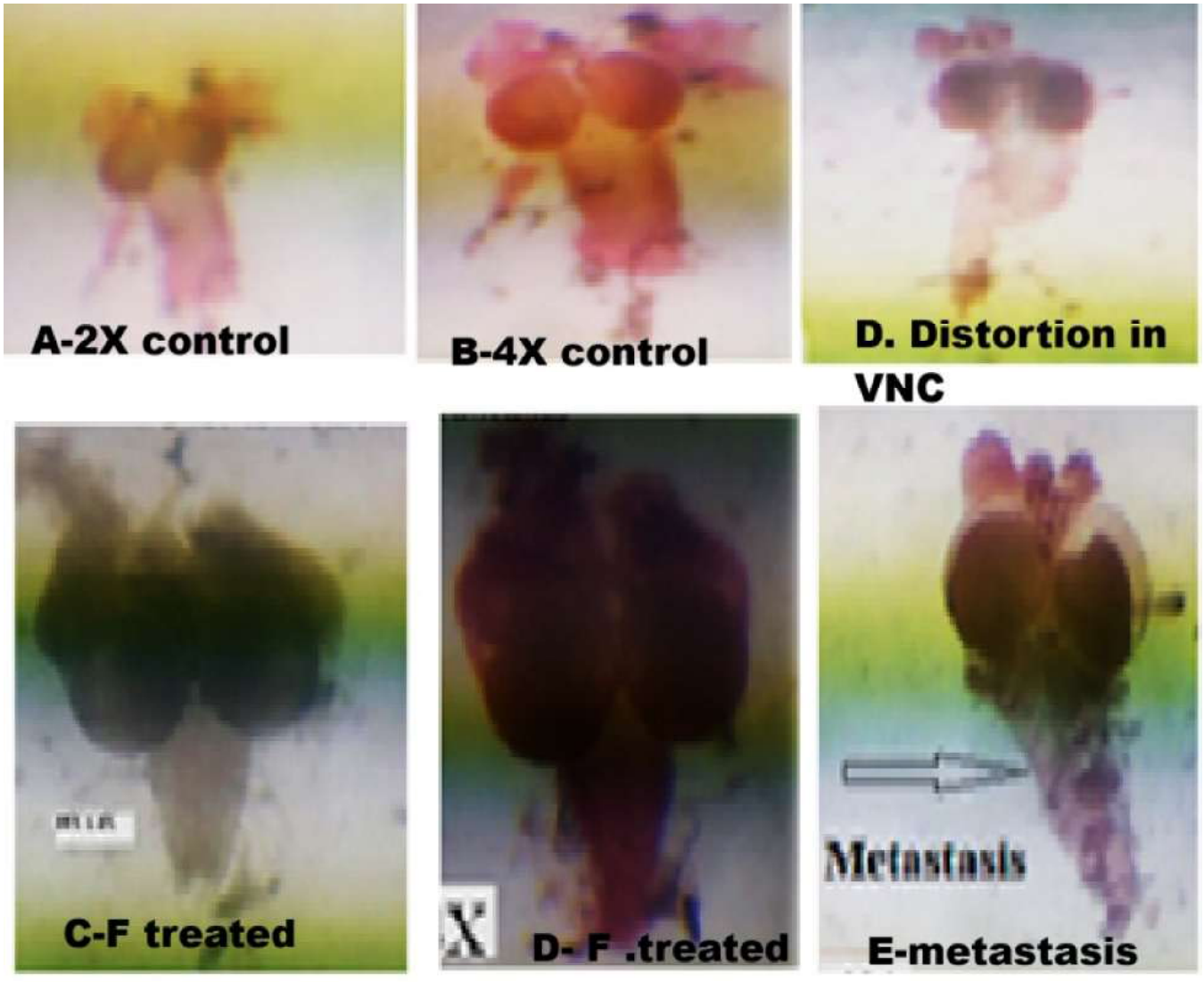

Fig. 2 :Neoplasia in the cerebral ganglion. (A-B) Control, (B-C) Neoplasia, (D) Distortion in Ventral Nerve Chord (VNC),(E)Metastasis in VNC

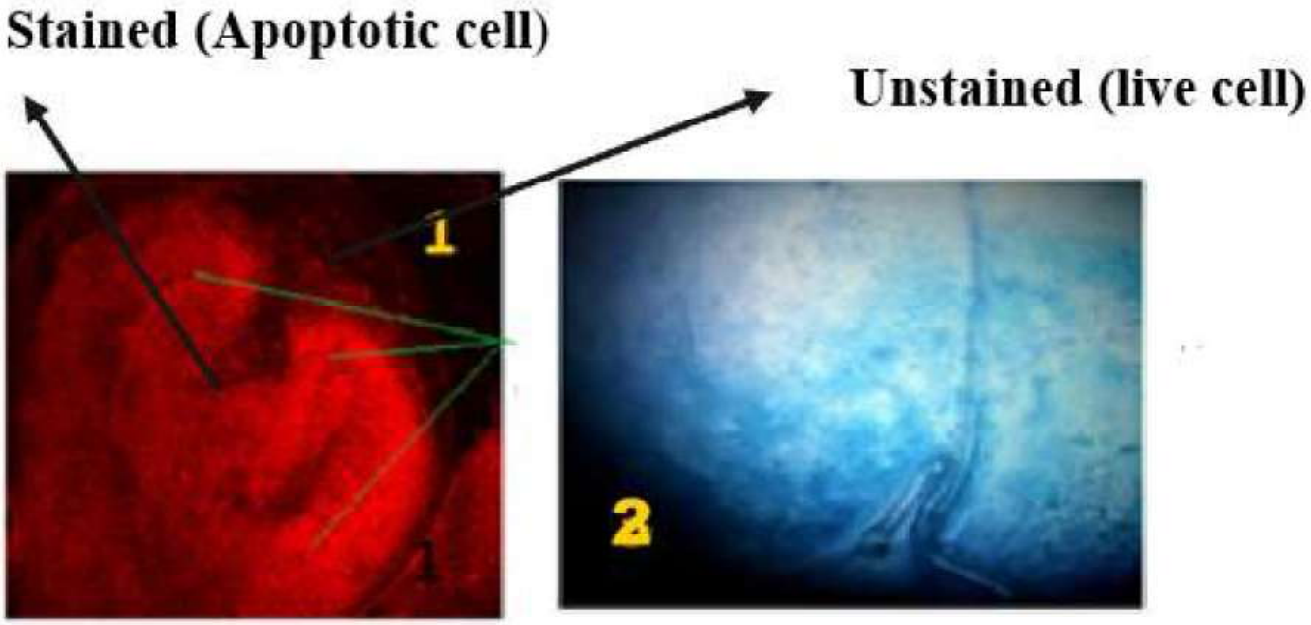

Fig. 3 :Dye exclusion for apoptosis (1) with Propidium iodide, (2) with trypan blue (stained dead cell and unstained region live cell)

showed stained regions corresponding to dead cells and unstained regions indicating live cells.

Discussion

The current work highlights *Z. indianus*'s high susceptibility to fluoride stress by showing that fluoride exposure causes notable toxic, morphological, and cytological changes in the organism. Strong toxicity even at relatively low concentrations is indicated by the observed concentration-dependent increase in mortality and the LC_{50} value of 2.5 ppm (Figure 4). Complete mortality (100%) was reported at 3.0 and 4.0 ppm, whereas approximately 51.7% mortality was observed at 2.5 ppm, which corresponds to the LC_{50}. Fluoride also exhibits developmental toxicity and promotes mortality.The log-transformed concentration and the probit of mortality showed a strong positive linear association, with a correlation coefficient value of 0.905.Similar results have been documented in other biological systems, where high fluoride levels cause damage to vital organelles such as the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus, as well as alter cellular homeostasis by blocking important enzyme processes[10]. Increased cellular stress and compromised metabolic processes are frequently linked to these disturbances.

Severe neuro developmental abnormalities are suggested by the significant enlargement of brain lobes and distortion of the ventral nerve cord seen in larvae treated with fluoride. Chemically induced carcinogenic-like alterations in *Drosophila* species have previously been linked to such structural defects[1]. The underlying dysregulation of cell proliferation and differentiation processes, which are crucial steps in the start of carcinogenesis, may be reflected in these morphological alterations.

Apoptosis is vital for maintaining cell stability because it eliminates defective cells. Apoptosis is considered fundamental for maintaining cell balance and is vital for removing defective cells. The blockage of apoptosis can lead to the preservation of defective cells. Results from cytological analysis showed that there was a reduction in the number of apoptotic cells within the brain tissue of larvae exposed to fluoride. This was shown through a reduction in Trypan Blue and propidium iodide stain uptake, implying that there was an inhibition of apoptosis due to fluoride treatment.

Conclusion

The results of the study suggest that fluoride exposure has the capacity to induce carcinogenic changes in *Z. indianus* at both the cellular and organismal stages. The increased mortality rate, structural deformation of the brain tissue, and suppression of apoptosis are all suggestive of the possibility of fluoride poisoning inducing the early stages of carcinogenesis. *Z. indianus* has thus been found to be a viable and cost-effective substitute for the study of fluoride-induced carcinogenic changes and the development of treatment possibilities.

Declarations

Conflict of interest

The authors declare no conflict of interest.

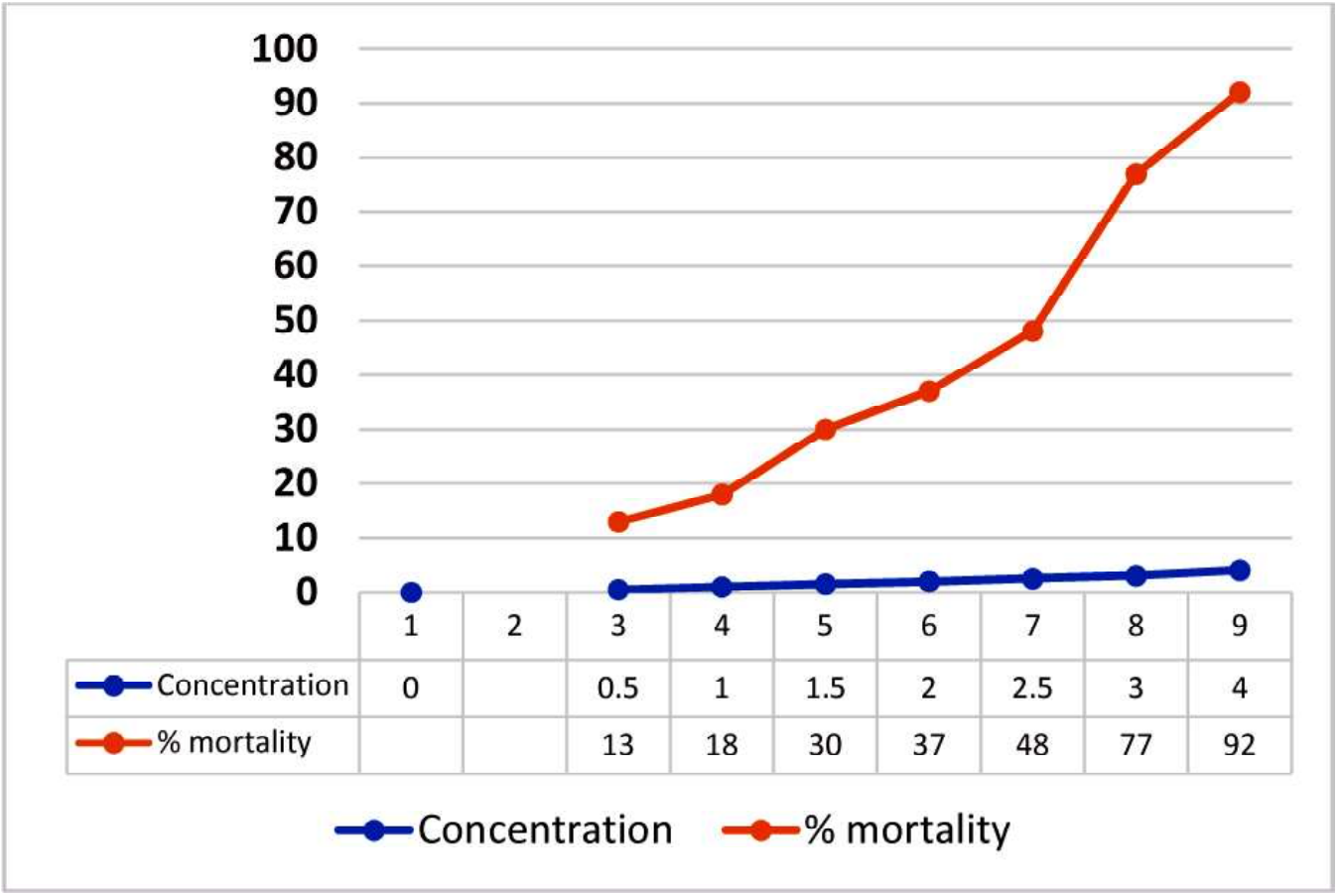

Fig. 4 :Positive Correlation between Fluoride & mortality percentage

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https://doi.org/10.33451/florafauna.v32i1pp168-172
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2026 Vol. 32 No.1 PP 168-172 ISSN 0971 - 6920 (Print)

Entero-Metabolic Imbalance Theory: Redefining Gut Microbiota Alterations in Diabetic Disorders : A Review

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Received **:** 11.03.2026; ***Accepted*** : 08.04.2026

How to cite : Srivastava S, Jain D. Entero-Metabolic Imbalance Theory: Redefining Gut Microbiota Alterations in Diabetic Disorders : A Review. *Flora and Fauna* 2026. 32(1) : 168-172.

ABSTRACT

Diabetes is a multifactorial pathological condition characterized by sustained high blood glucose level. It may be due to defective insulin dynamics, beta cell dysfunction or gut derived metabolic modulators. Specifically, Type 2 diabetes mellitus is marked by a combination of progressive dysfunction of pancreatic â-cells and diminished insulin responsiveness which tends to develop chronic hyperglycemia. Though new research points out that gut microbial colony and diabetes are closely related. The concept of entero-metabolic imbalance theory proposes that fluctuations in the commensal microbes contribute in the development of diabetes. In this systematic review, we emphasized on the potential action of different bacterial taxa undermined or afflicted diabetes. The concept of entero-metabolic imbalance theory proposes that variations in the intestinal microbiome play a crucial role in the development and course of diabetes. The intestine of healthy person is colonized by beneficial bacterial phyla such as Firmicutes and Bacteroidetes, with important genera including Roseburia, Bifidobacterium. By means of the production of short chain fatty acids, immunological regulation and epithelial integrity, these microorganisms preserve the function of gut barrier and improve insulin tolerance. This review examines microbial shifts in diabetes, investigate the biological connection between the development of diabetes and gut dysfunction and also evaluates therapeutic proposal.

Figure : 00 References : 28 Table : 00

KEY WORDS : Bifidobacterium, Gut Microbiota, Metabolic Dysfunction, Roseburia, Type 2 Diabetes.

Introduction

The complicated, diversified metabolic disease known as type 2 diabetes (T2D) is typified by persistent hyperglycemia driven by low levels of insulin and progressive β-cell malfunction.

The pancreatic centric model has been used to characterize the pathogenesis of this disease which is caused by a combination of two main factors: impaired insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin[18]. Recent data show that metabolic regulation extends beyond endocrine tissues and involves dynamic link between the gut ecosystem and the host.

Trillions of bacteria like Bifidobacterium, Roseburia, Actinobacteria[20] and many other are present in human gut. By providing short chain fatty acids they promote intestinal health and aids in nutrient metabolism, immune modulation[10 ,15]. They exert systemic effects on glucose and lipid homeostasis. This host–microbe symbiosis maintains epithelial barrier integrity and limits inflammatory activation.

Recent researches show that people with T2D frequently exhibit microbialdysbiosis characterized by reduction of butyrate-producing bacteria, and enrichment of endotoxin-producing Gram-negative bacteria. These kinds of variations are linked with impaired tight junction function, increased intestinal permeability, and translocation of lipopolysaccharide (LPS) into systemic circulationa phenomenon known as metabolic endotoxemia[3]. LPS-mediated activation of innate immune pathways promote chronic low-grade inflammation, a recognized driver of insulin resistance. These findings state that gut-derived inflammatory signaling may serve as a mechanistic bridge linking microbial dysbiosis to metabolic impairment and in progression of diabetes.

Composition of Normal Gut Microbiota

The human intestinal microbiota is a biome of bacteria, archaea, viruses, and fungi. Bacteria is the most dominant component among them. The majority

of gut bacteria belong to several major phyla, primarily Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria[21].

A large array of human metabolic disorders like obesity and diabetes are linked to disturbance in intestinal microbiota[11]. The class Firmicutes and bacteroidetes make up the vast majority of healthy gut microbes[8]. The overall microbial diversity of the gastric flora is established by Helicobacter,themost prevalent genera in the stomach. In particular, when *Helicobacter pylori (H. Pylori)* is present in the stomach as a commensal bacterium, there is a rich diversity comprised of other favoured genera, such as *Streptococcus* (most dominant), *Prevotella, Veillonella* are most closely associated to disease states[13]. A well-functioning gut microbiota could be suggested by the phylum proteobacteria's notably low abundance and the high abundance of the hallmark genera like *Bacteroidetes, Prevotella* and *Ruminococcus*[9]. The most common type of Bacteria found in the lumen of human intestine, also reflect in the stool are *Bacteroides, Bifidobacterium, Streptococcus, Enterobacteriacae, Enterococcus, Clostridium, Lactobacillus* and *Ruminococcus.* On the other hand, *Enterococcus* and *Akkermansia* are the predominant mucosa and mucus associated genera reflect in the epithelial crypts of the small intestine and mucosal layer[24].

Role of enterotypes for maintenance of a healthy gut

Enterotypes are intestinal microbes characterized by specific taxa and metabolicfunction.Three types of enterotypes are there, Enterotype 1 which is marked by *Bacteroides,* enterotypes 2 dominates as *Prevotella* while Enterotype 3 is characterized by greater abundance of *Ruminococcus*[9]. Enterotype 1 has propensity to break sugar protein as they code for the enzymes like proteases, hexoaminidases and galactosidases. This shows that these organisms extract energy from dietary carbohydrates and proteins. Enterotype 2 have a tendency to break the mucin glycoproteins that covers the gut mucosal layer. Enterotype 3 aids in membrane transport of sugars.

The intestinal microorganisms extract most part of their nutrients from the carbohydrates we take in the diet. Short chain fatty acids (SCFA) such as butyrate, propionate and acetate are rich sources of energy for the host[14,20], produced by the colonic bacteria such as *Bacteroides, Roseburia, Bifidobacterium, Fecalibacterium* and *Enterobacteria via* the fermentation of dietary carbs. These Short-chain fatty acids (SCFAs), play a major role in maintaining intestinal barrier integrity by acting as an energy source for colon microbes. Additionally, SCFAs exert anti-inflammatory effects and contribute to improved insulin sensitivity, thereby supporting metabolic homeostasis. Other gut microbes, such as *Lactobacillus* and *Faecali bacterium,* also help in microbial balance and anti-inflammatory processes. In healthy individuals, these microorganisms exist in a balanced proportion. Conjugated linoleic acid is known to have antidiabetic and immune boosting property, synthesized by *Bacteroides* and other gut microbes[1,5,6].

Gut Microbiota Alterations in Diabetes

Recent findings indicates that people with Diabetes show significant variations in the microbial diversity of their gut in comparison to healthy individuals. Reduced microbial variety and greater abundance of specific bacterial genera are hallmark of these changes.

The drop in beneficial SCFA-producing bacteria, including *Roseburia* and *Bifidobacterium* are one of the most consistent findings in the progression of diabetes. These bacteria are essential for controlling the host metabolism and intestinal barrier integrity. Their decline may impair intestinal health and metabolic regulation. One of the most significant microbiota related parameters linked to beginning and progression of T2D appears to be a reduction in butyrate producing species such as *Faecali bacterium prausnitzii* and *Roseburia intestinalis*[11].

The relative abundance of *Bifidobacterium, Clostridium, Firmicutes* phylum in diabetics was significantly decreased, while the proportion of *Bacteroidetes* and *β-Proteus* was significantly increased. These are the key features of development of diabetes.

The microbial community in the intestine of diabetic patient has shown a rise in the number of pathogenic bacteria such as *Entero bacteriaceae*, various *Clostridiales, Escherichia coli, Bacteroides caccae,* as well as *Prevotella copri* and *Bacteroides vulgates.* A decline in the number of LPS producing gram negative bacteria named *Bacteroidetes* is responsible for lowering the risk of metabolic endotoxemia and inflammation in the gut lining. The higher level of LPS produced by Bacteroidetes is responsible for the production of inflammatory cytokines, such as Interleukin-1 (IL-1), IL-6, and Tumour Necrosis Factor-α (TNF-α)[26], gradually drive the development of insulin resistance and T2D. *Proteobacteria* are highly pro-inflammatory[17].It is well-known that this subclinical pro-inflammatory status due to LPS-dependent production of inflammatory cytokines, such as Interleukin-1 (IL-1), IL-6, and Tumour Necrosis Factor-α (TNF-α), drives the development of insulin resistance and T2D.

However, some species display anti-inflammatory qualities. For instance, the genus *Lactobacillus* also has a positive correlation with T2D, it induces the production of the anti-inflammatory cytokine IL-10, which enhances insulin sensitivity in muscles, while inhibit the synthesis of proinflammatory cytokines like IL-1β, IL-8, Interferon-γ (IFN-γ)[18].

These changes in the intestinal microbial community lead to irregular production of beneficial metabolites and increased generation of inflammatory molecules such as lipopolysaccharides (LPS). Which ultimately leads to metabolic endotoxemia and systemic inflammation, which are key factors in the development of glucose intolerance and Insulin irresponsiveness.

Altered gut microbiota derived endotoxemia

According to this concept, gut dysbiosis leads to impaired intestinal barrier integrity and irregular microbial metabolite generation. An impaired intestinal barrier allows bacterial endotoxins, particularly lipopolysaccharides, to squeeze into the bloodstream. This condition, known as metabolic endotoxemia, triggers chronic inflammation. Persistent inflammatory signaling interferes with insulin receptor pathways and disrupts glucose metabolism. Fluctuations in the level of gut microbes is also related to metabolic disorders such as obesity, diabetes [18,27].

By controlling immunological responses, energy balance and food metabolism, intestinal microbial community plays a critical role in protecting metabolic health. Metabolic imbalance such as obesity, insulin resistance and type 2 diabetes is directly linked to alteration in gut microbial community[7].

Role of Microbial Metabolites

Short chain fatty acids act as a signaling molecule on GI cells and other tissue cells which can bind to four receptors to stimulate intracellular signaling cascade. They are free fatty acid Receptor 3 (FFAR3), free fatty acid receptor 2 (FFAR2), G protein-coupled receptor 109a (GPR109a) and G protein-coupled receptor 42 (GPR42). Furthermore, SCFAs activate specific targets through blood circulation, systemic circulation and other pathways, such as AMP-activated protein kinases in the liver (and maybe the heart), promote lipid oxidation and enhance glucose homeostasis in mice.

Bile Acid Metabolites

To order to enable metabolic signalling cascades like FXR and TGR5, are essential for glucose metabolism and homeostasis, the Gut microbiota converts primary bile acids into secondary bile acids.

Secondary bile acids generated by gut microbes can act as signaling molecules that fine-tune intestinal hormone secretion, especially glucagon-like peptide-1 (GLP-1), thereby regulating insulin sensitivity and Glucose control. However, the alterations in the microbial population of gut microbes connected with metabolic disorders, may impair bile acid composition and signaling, leading to altered FXR and TGR5 activation. Such disturbances in bile acid metabolism can disrupt glucose regulation, promote systemic inflammation, and contribute to the progression of insulin irresponsiveness and type 2 diabetes. The gut microbiota–bile acid axis represents a crucial metabolic interface linking intestinal microbial ecology with host glucose homeostasis and metabolic health.

A change in the bile acid metabolism due to microbial dysbiosis may contribute to impaired glucose regulation and metabolic disorders.

Branched-Chain Amino Acids (BCAAs)

Some gut bacteria contribute in the synthesis of branched-chain amino acids such as leucine, isoleucine, and valine. Elevated circulating levels of BCAAs have been strongly connected to insulin irresponsiveness and increased risk of type 2 diabetes[17].

Mechanically, higher level of BCAAs triggers the activation of rapamycin (mTOR) signaling cascade, may disrupt insulin signaling pathways and leads to impaired insulin receptor substrate activity and reduce glucose uptake in peripheral tissues. In addition, increased BCAA levels may contribute to mitochondrial dysfunction and lipid accumulation in skeletal muscle, further aggravating metabolic stress and insulin resistance. Gut microbial dysbiosis, characterized by enrichment of BCAA-producing bacterial taxa, has therefore been proposed as an important factor contributing to metabolic imbalance in diabetic conditions. Thus, the change in microbial composition may develop as a potential therapeutic strategy to regulate BCAA metabolism and improve metabolic outcomes in individuals with type 2 diabetes mellitus.

Disruption of gut barrier and development of Insulin Resistance

Variations in intestinal microbial composition, commonly called to as intestinal dysbiosis, have been recognized as important contributor to insulin resistance and barrier disruption. By producing beneficial metabolites such as short-chain fatty acids (SCFAs)[2] gut microbiota maintains intestinal barrier integrity and maintain host metabolic pathways. However, disruption of microbial balance leads to decreased number of beneficial bacteria including *Bifidobacterium, Roseburia* and *Faecalibacterium*, along with an increase

in opportunistic Gram-negative bacteria. This imbalance compromises intestinal barrier function and increases gut permeability, allowing bacterial endotoxins such as lipopolysaccharides (LPS) to translocate into systemic circulation. The presence of circulating LPS triggers chronic low-grade inflammation through activation of immune signaling cascades, disrupts insulin receptor signaling in metabolic tissues such as liver, adipose tissue, and skeletal muscle. Additionally, reduced production of SCFAs weakens anti-inflammatory responses and disrupts metabolic signaling mechanisms involved in glucose homeostasis. Collectively, these processes contribute to impaired insulin sensitivity and promote the progression of type 2 diabetes.

Therapeutic Perspectives: Targeting Gut Microbiota in Diabetes

Recent data suggest that Diabetes can be treated by the modulation of intestinal microbiota and this represents a promising therapeutic strategy to prevent the development of Diabetes. Restoration of microbial balance can improve intestinal barrier integrity, regulate inflammatory responses, and enhance metabolic signaling pathways involved in glucose homeostasis[2,24]. Fermentable fibers rich diet are known to enhance the growth of gut friendly bacteria such as *Bifidobacterium, Lactobacillus* and *Roseburia*, which are known to increase the production of short-chain fatty acids (SCFAs)[28,8,12]. These secondary metabolites of gut microbes, contribute to improved insulin sensitivity, maintains blood glucose, reduced systemic inflammation[4].

Probiotic Dietary support has been found as a potential approach to restore microbial equilibrium and strengthen intestinal barrier function. Several findings have demonstrated that probiotic strains belonging to *Lactobacillus* and *Bifidobacterium* can improve metabolic parameters by reducing endotoxin levels and modulating immune responses[7,26,15]. In addition, prebiotics, which serve as substrates for beneficial gut microbes, can selectively stimulate microbial populations that produce protective metabolites. Several therapeutic microbiome-targeted dietary strategies are currently being explored for their capability to restore gut microbial diversity and improve metabolic functions in individuals with diabetes. Collectively, interventions aimed at modulating gut microbiota may represent an effective adjunct policy for the management of metabolic disorders.

Future perspective

As growing evidence suggests the significant role of intestinal microbiota in the development and progression of diabetes. Upcoming studies should focus on exploring specific microbial species involved in insulin resistance and metabolic dysfunction. Techniques like sequencing, metabolomics, metagenomics may help in studying biomarkers before the onset of diabetes[7].

We can explore personalized microbiome-based therapies that integrate host metabolic profiles with microbial composition to develop targeted strategy for declining insulin resistance. A deeper understanding of host–microbiota interactions may help in developing novel microbiota-focused techniques for improving metabolic health and preventing the development of diabetes.

Advanced medical techniques such as metagenomics, metabolomics, and next-generation sequencing may help in innovating new novel biomarkers for early detection and therapeutic targeting of diabetes.

Furthermore, personalized microbiome-based therapeutic strategies integrating host metabolic profiles with microbial composition may enable targeted interventions to reduce insulin resistance and metabolic complications.

Conclusion

In this study we have observed that disturbances in the intestinal microbial ecosystem may drastically alter the generation of key microbial metabolites, compromise gut barrier integrity, and promote systemic inflammatory responses. Insulin resistance and metabolic dysfunction linked to Diabetes get worse by these inflammatory changes. The Entero-Metabolic Imbalance Theory explains how microbial imbalance, impaired intestinal barrier integrity, and fluctuations in the microbial metabolites collectively influence metabolic pathways.

A fall in beneficial bacteria may initiate many inflammatory processes lead to disruption of insulin signalling pathways. Understanding these interactions between intestinal microbiome and host metabolism may open new ideas for microbiota-targeted therapeutic strategies aimed at restoring microbial balance, improving insulin sensitivity, and preventing the progression of diabetic disorders.

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