Journal of the Andaman Science Association Vol. 28(2):128-142 (2023)
ISSN 0970-4183, Printed in India

Exploring Promising Species and Innovative Techniques for Sustainable Island Mariculture: A Comprehensive Review

Imelda-Joseph*, Sekar Megarajan and Shoji Joseph
ICAR-Central Marine Fisheries Research Institute, Post Box No.1603, Ernakulam North P.O., Kochi- 682 018, Kerala, India
***Corresponding author's E-mail:** imeldajoseph@gmail.com*

Abstract

The aquaculture sector has emerged as the fastest-growing food-producing industry globally, meeting the escalating demand for aquatic food sources. In India, this phenomenon is mirrored, with the aquaculture sector experiencing remarkable growth, positioning the country as the second leading producer of cultured fish. Notably, during the 2021-22 period, India exhibited an impressive average annual growth rate of 8.0%, underscoring the sector's robust expansion. With an extensive coastline spanning 8129 km across nine coastal states and three union territories, including the scenic Andaman Islands, India possesses a diverse landscape for aquaculture development. The Andaman Islands, renowned for their natural beauty, boast a rich marine ecosystem with potentially undiscovered fish species, contributing significantly to India's total coastline, approximately a quarter thereof. Mariculture technologies tailored for island ecosystems encompass a spectrum, ranging from onshore aquaculture with facilities like tanks, ponds, and aquaponics systems that afford precise control over conditions, to inshore aquaculture providing a more natural environment for farmed species, and offshore aquaculture featuring enclosed sections in open water, exposing species to varied natural conditions such as currents and nutrient cycles. The present review delves into the specific realm of mariculture in island ecosystems, shedding light on global trends, issues within these unique environments, and considerations imperative for the successful and sustainable development of mariculture. The paper extends beyond India, encapsulating the broader context of island mariculture worldwide, offering insights into the challenges and opportunities inherent in these ecosystems. Ultimately, the review strives to contribute to the discourse on fostering sustainable practices, balancing environmental conservation with economic viability, and addressing social considerations to ensure the long-term resilience of island mariculture.

Key words: *Aquaculture, mariculture technologies, islands, species, sustainability*

Introduction

Mariculture is a component of aquaculture that involves the cultivation, management, and harvesting of marine organisms in their natural habitats (like estuarine, brackish, coastal, and offshore waters) or within controlled enclosures such as cages, pens, tanks, or channels. This form of aquaculture specifically focuses on marine species, including fish, shellfish, seaweed, and other aquatic organisms. In contrast, commercial fishing involves the harvesting of wild fish from natural marine or freshwater environments. This distinction is essential to understand the methods and approaches involved in both practices, one being controlled cultivation and the other involving the capture of wild aquatic species. Mariculture is crucial for meeting the growing global demand for seafood while reducing the pressure on wild fisheries. It allows for controlled and sustainable production of marine organisms, offering economic benefits to coastal communities and contributing to food security.

Aquaculture is the fastest-growing food-producing sector on a global scale, displaying immense potential to address the ever-increasing demand for aquatic food. In 2020, the sector made a substantial contribution, yielding 214 million tonnes of fish, aquatic animals, and algae for human consumption, with 178 million tonnes comprised of aquatic animals and 36 million tonnes of algae (FAO, 2022). Asia, in particular, has been a key player in this growth, boasting a significant surge in aquaculture activities. It's vital, however, to emphasize sustainable practices and responsible resource management to ensure

the long-term viability of this essential industry. Declining in fish catch from wild in recent past, has led to rapid growth of fish farming in different farming systems across the world to meet the fish requirement. Consequently, the world food fish aquaculture production has increased considerably at an average growth rate of 6.7% in the year 2022.

The aquaculture sector in India is mirroring the global trend of remarkable growth. The substantial increase in aquaculture production has positioned India as a leading producer of cultured fish, showcasing an impressive average annual growth rate of 8.0% during the 2021-22 period. This growth is significant and underlines the importance of aquaculture in meeting the rising demand for aquatic food. Aquaculture is practiced in both freshwater and coastal areas throughout India, with freshwater aquaculture contributing significantly to the overall aquaculture production in the country, as highlighted by the Food and Agriculture Organization (FAO) in 2020 . Notably, the coastal aquaculture and mariculture sector have seen substantial development, marked by the introduction of new species suitable for culture. However, there remains considerable untapped potential in coastal aquaculture and mariculture that requires focused attention to effectively utilize the available area and address the increasing demand for marine fish food in India and around the world. Striking a balance between sustainable practices, technological advancements, and responsible resource management is essential to harness this potential while ensuring the long-term sustainability of the aquaculture industry in India.

India possesses abundant resources for mariculture, including an extensive coastline spanning 8129 km, a vast Exclusive Economic Zone (EEZ) of 2.2 million km^2, substantial continental shelf area, expansive brackishwater and inland saline areas, as well as 20 million ha designated for sea farming (Modayil *et al*., 2008). Despite this rich potential, mariculture production in the country remains in its nascent stages compared to global standards.

The estimated marine fish landings along the coast of the mainland of India during 2022 was 3.49 million tonnes, showing an increase of 14.53% compared to the landings in 2021 (CMFRI, 2023). However, India's mariculture production lags significantly behind its potential, estimated at 4 to 8 million tonnes annually. Presently, mariculture production stands at less than 0.01 million tonnes, accounting for approximately 2.0% of the total aquaculture production (Gopalakrishnan *et al*., 2019). This emphasizes a substantial opportunity for growth and development within the mariculture sector in India. Efforts to leverage these resources effectively and develop sustainable mariculture practices can significantly contribute to meeting the increasing demand for aquatic food and enhancing India's position in the global aquaculture landscape.

At present, coastal aquaculture contribute major share in production, which is mainly from shrimp culture. In India only 13% of total available potential area is under coastal aquaculture operation. In 2019, the production of both vannamei and monodon shrimp was less than 600,000 tonnes. Production rose to 650,000 tonnes in 2020 and by 2021, the volume escalated to 930,000 tonnes and expected a production decline to 902,525 tonnes in 2023 (SAP, 2023). The major species groups farmed in the Asia-Pacific region are: finfishes, crustaceans, molluscs, echinoderms and aquatic plants. Marine finfish aquaculture in the Asia-Pacific region is exceptionally diverse. The commonly cultivable brackish and marine water fish species/group in Asia and Pacific region includes milk fish, sea bass, jacks, sea bream, flat fishes, groupers, cobia, mullets, snappers, pompanos and other marine fishes such as eels, croakers & drums. Japanese amberjack (*Seriola quinqueradiata*) makes up 17% of regional marine finfish production. Most of the species are available in Indian seas, and they can be domesticated and cultured in India.

Understanding the importance of the mariculture potential in Indian seas, ICAR research institutions, such as Central Marine Fisheries Research Institute and Central Institute of Brackishwater Aquaculture had initiated different mariculture programmes and has developed seed production and farming technologies of different species including Cobia (*Rachycentron canadum*), Orange-spotted grouper (*Epinephelus coioides*), Silver pompano (*Trachinotus blochii*), Indian pompano (*T. mookalee*), Pink- ear sea bream (*Lethrinus lentjan*), banded grunter

(*Pomadasys furcatus*), John's snapper (*Lutjanus johnii*), Vermiculated spine foot (*Siganus vermiculatus*) and picnic seabream (*Acanthopagrus berda*) (Shinoj *et al.,* 2023, Gopalakrishnan *et al.,* 2019; Anuraj *et al*., 2021; Suresh Babu *et al*., 2022). Aiasn seabass, (*Lates calcarifer*), and mangrove red snapper (*L. argentimaculatus*) (CIBA) (Arasu *et al*., 2009). Apart from the above, a recent publication from ICAR-CMFRI has prioritized 76 finfish and shellfish species that could be targeted for future expansion of mariculture production in the country (Ranjan *et al*., 2017). Using these potential cultivable species, mariculture production from Indian seas can be considerably improved by adopting hatchery based and capture based culture methods both mainland and island in Indian coastal areas.

Island Ecosystems

Island ecosystem is very unique in terms of its biodiversity, physical environment and threat by various natural and anthropogenic factors. However the diversity is not uniformly distributed among the tropical islands, which are conditioned by the natural forces and the influence of human activity significantly altered it. Tropical islands are known to have uniquely naturally variable ecosystems, including tropical rainforests, open woodlands and grass savannahs, freshwater lakes and streams, salt marshes and mudflats, mangrove and coastal littoral forests, seagrass, fringing and offshore coral reefs, and deep sea trenches and abyssal plains (SPREP, 2012). Due to favourable climatic and edaphic conditions, the tropical region ecosystems have high species turnover and an unusual richness of endemic terrestrial and freshwater species. Isolation of islands promotes high endemism and specialised flora and fauna (MacArthur and Wilson, 1967). Because the isolation of islands over a period of time exerts unique evolutionary forces that result in the development of a distinct genetic reservoir and the emergence of highly specialised species with entirely new characteristics and the occurrence of unusual adaptations. In the same context patterns of species diversity on islands have also yielded significant insights into evolutionary and ecological processes such as immigration, speciation and extinction (Witt and Maliakal-Witt, 2007).

India has an 8129 km long coastline which extends across nine coastal states and three union territories, namely the Damn & Diu, Andaman and Nicobar and Lakshadweep islands. According to the bio-geographic classification of India (Rodgers et al 2002), the coasts fall under zones 8A (west coast), 8 B (east coast), 8 C (Lakshadweep), 10A (island-Andamans) and 10B (island-Nicobars). The Lakshadweep and Andaman & Nicobar Islands form the major groups of islands in the country, comprising 36 and 572 islands, respectively. The Lakshadweep Islands lie 220 to 440 km off the coast of Kerala in the Arabian Sea, with an area of 32 km (http://lakshadweep.nic.in/). The Andaman & Nicobar archipelagos are situated between latitudes 6° and 14° N and longitudes 92° and 94° E, in the Bay of Bengal (http://www.andamans.gov.in/). These islands have received much attention in the recent past due to their rich coastal and marine ecosystems, which harbour many critical species and provide a source of livelihood to over 4,00,000 people (Census of India, 2011). Several studies in the past have focused on the biodiversity of both the island groups, the Lakshadweep Islands (Murty 2002; Apte 2009; James 2011; Venkataraman, *et al*., 2012; Reddy *et al.,* 2013) and the Andaman & Nicobar Islands (Sankaran 1998; Ravindran *et al*., 1999; Davidar *et al*., 2002; Padalia *et al*., 2004; Ramachandran *et al*., 2005; Andrews et al 2006; Aul *et al*., 2014).

There are 21 islands and 3 groups of islands of peninsular India, their biodiversity and related threats. In addition to these two main islands, Lakshadweep and Andaman & Nicobar groups of islands all these small islands are having greater scope in developing fisheries and, mariculture in peninsular India. Many islands dot the east coast of India from West Bengal to Tamil Nadu, of which some are, Halliday Island, Lothian Island, Dhanchi Island, Sagar isaland, in West Bengal; Wheeler Island in Odisha; Hope Island, Sriharikota Island in Andhra Pradesh: Pamban Island, Gulf of Mannar group of islands in Tamil Nadu. Similarly, the western coastal plain is a 50-100 km wide strip of land sandwiched between the Western Ghats and the Arabian Sea. It extends from Gujarat, in the north, through Maharashtra, Goa and Karnataka to Kerala. Which includes, Gulf of Kutch group of islands, Diu (union territory of Daman &

Diu) in Gujrat; Elephanta island, Murud-Janjira Islands, Padamdurg islands, Khanderi islands, underi islands, islands at Vengurla rocks in Maharastra; Chorao islands, Divar islands, Jacinto islands, cumbarjua islands in Goa: Netrani islands, St. Mary's islands in Karnataka; Kochin group of islands in Kerala. Islands that are close to mainland India are critical in many ways to human beings. These islands are breeding grounds for several fishes, protect the sea shore from natural calamities such as cyclones and Tsunamis and provide livelihoods to thousands of coastal communities (Pande et al., 2014). The rich island potential as the breeding ground for many marine fishes can be used judiciously with the advent of some of the mariculture technologies.

The Andaman Islands, known for their unparalleled natural beauty, boast a rich marine ecosystem with an abundance of fish species, some of which may still be undiscovered. The islands are blessed with a vast coastline spanning 1,962 km, accounting for about a quarter of India's total coastline. Furthermore, the exclusive economic zone (EEZ) of the Andaman Islands extends across approximately 0.6 million km², encompassing about 30% of the Indian subcontinent's EEZ. Given these expansive marine resources and the favourable environmental conditions, the Andaman Islands are highly conducive to mariculture. Mariculture, which involves the cultivation of marine organisms in controlled environments, can thrive in the Andaman's pristine coastal waters. The region's diverse marine habitats, including coral reefs, seagrass beds, and coastal areas, provide an ideal setting for cultivating various species of fish, shellfish, and other marine organisms. Efforts to harness the mariculture potential in the Andaman Islands should focus on research, technological advancements, and sustainable practices. Collaboration between research institutions, government agencies, local communities, and the private sector is essential for realizing the full potential of mariculture in this unique and ecologically valuable region. It is also reported that after the post-tsunami earthquake, an area of about 1,300 ha was permanently inundated, of which 830 ha is suitable for brackish water aquaculture apart from the abundant freshwater sources where freshwater aquaculture is also possible.

Mariculture in Island ecosystems

Utilizing mariculture in the Island ecosystems offer several benefits, including:

Diversification of Aquaculture: Mariculture diversifies the aquaculture sector by incorporating a wide range of marine species, reducing reliance on traditional forms of aquaculture.

Sustainable Seafood Production: Cultivating marine species through mariculture contributes to sustainable seafood production, meeting the increasing demand for high-quality marine products.

Economic Growth and Employment Opportunities: Mariculture can drive economic growth by creating employment opportunities in coastal communities and supporting related industries.

Food Security: By enhancing seafood production, mariculture contributes to food security by providing a steady supply of nutritious marine products.

Conservation and Sustainable Use: Mariculture allows for the conservation and sustainable use of marine resources, reducing pressure on wild populations and promoting responsible aquaculture practices.

Tourism and Eco-Tourism: The success of mariculture can attract eco-tourists interested in sustainable practices, benefiting the local tourism industry.

Suitable Mariculture Species for Island ecosystems

Shrimp and Prawns (Penaeid Shrimp)

Black tiger shrimp (*Penaeus monodon*), Pacific white shrimp (*Litopenaeus vannamei*), Indian white shrimp (*Penaeus indicus*)

Finfishes

Asian Seabass (*Lates calcarifer*), Grouper (*Epinephelus* spp.), Pompano (*Trachinotus mookalee* and *Trachinotus blochii*), Milkfish (*Chanos chanos*), Cobia (*Rachycentron canadum*)

Bivalve Molluscs

Green mussel (*Perna viridis*), Indian *backwater oyster (Crassostrea madrasensis), Pearl oyster (Pinctada fucata, Pinctada* margaritifera)

Seaweeds

Kappaphycus alvarezii, *Gracilaria* spp.

Mariculture Technologies for Island ecosystems

Mariculture, encompasses various cultivation methods. Well-planned and managed mariculture can also contribute positively to coastal environmental integrity. However, future development of mariculture will occur, in many areas, with increasing pressure on coastal resources caused by rising populations, and increasing competition for resources. Thus, considerable attention will be necessary to improve the environmental management of aquaculture through environmentally sound technology and better management, supported by effective policy and planning strategies and legislation.

Mariculture can be done on land in artificial facilities like tanks, ponds, or aquaponics systems (onshore aquaculture), offering precise control over conditions. In well-sheltered shallow waters near the shore (inshore aquaculture), species experience a more naturalistic environment. Offshore aquaculture involves enclosed sections in open water, where species are kept in cages or racks, exposed to diverse natural conditions such as currents and nutrient cycles. Each approach has unique advantages and challenges, influencing the choice based on species, environmental factors, sustainability, and technology. Responsible mariculture practices are essential for industry sustainability and environmental preservation.

Farming technologies

The geographical advantage of being surrounded by the sea, coupled with abundant brackishwater and freshwater areas, positions islands as highly conducive for various types of aquaculture. Inland aquaculture, which employs diverse culture techniques and facilities worldwide, finds a favourable environment on islands due to their proximity to water bodies. The following farming technologies are feasible and successful in island settings:

Land-based Systems: Land-based systems such as pond culture systems, raceways, and different types of intensive and semi-intensive tank cultures, including modern Recirculating Aquaculture Systems (RAS), can be effectively operated on islands. These systems offer controlled conditions for aquatic organisms, ensuring optimal growth and production.

Open Water-based Culture Systems: Open water-based culture systems, including pens and cages, are suitable for islands. These systems allow aquatic animals to grow in their natural environments within enclosed structures. By selecting appropriate sites and species, open water-based systems can yield successful aquaculture production.

Traditional, extensive, semi-intensive, and intensive are the existing farming practices. Mussel farming is an example of an extensive method of mariculture used around the globe, whereby the farmer provides a rope or a stake for the juveniles to attach to and undertakes some culling so that the density does not get too high, but otherwise leaves the mussels to grow without further interference. Some of the noted and well-developed mariculture technologies across the world (Imelda-Joseph and Asha-Augustine, 2020) are given below:

Ponds: Suitable for semi-intensive or intensive culture in ponds for shrimp, prawns, and fish. The advantages include controlled environment, efficient space utilization, water quality management, and ease of monitoring.

Raising finfish and other aquatic species in constructed earthen ponds is a widespread and highly effective culture method globally. Coastal aquaculture, typically practiced in constructed ponds either onshore or in intertidal zones, is a cornerstone of livelihoods, employment, and economic development in many developing countries, particularly across Asia. This farming technology is well-suited for adoption in the inland waters of islands, offering flexibility in terms of operational practices, input

intensity, technological sophistication, and integration with other farm activities. The selection of species for cultivation depends on factors such as availability, salinity, water quality parameters, and biological characteristics. Shrimps and various marine fish are commonly cultivated in these ponds. The production of young shrimp and fish often begins in hatcheries, although in some cases, young animals may be collected from natural sources. Ponds can be filled with suitable water either by pumping or through tidal flow, where farmers regulate water levels by opening and closing floodgates based on tides. The time required for the cultivated species to reach market size varies depending on the species and stocking size, ranging from a few months to a year. A significant advantage of this system is the ability to monitor and manipulate culture conditions from zero to 100%, allowing for a range of culture intensities based on cost-effectiveness. The culture methods can vary from extensive water exchange systems, depending on tidal influx, to zero water exchange systems where conditions are meticulously controlled. Additionally, farmers have the flexibility to choose between polyculture with minimal screening or monoculture of selected species based on their preferences and objectives. This adaptability and versatility make pond-based coastal aquaculture a crucial tool in sustainable aquaculture practices.

Although coastal ponds for aquaculture, whether modern or traditional, can be found in regions across the globe, they are more concentrated in South, Southeast, and East Asia, as well as Latin America. These areas primarily focus on raising crustaceans, finfish, molluscs, and, to a lesser extent, seaweeds through aquaculture. Many countries, especially in Asia, have developed expertise and support institutions dedicated to marine and coastal aquaculture.

Some of the most popular species cultured in marine ponds include sea bass, grouper, red sea bream, yellowtail, rabbitfish, and marine shrimps. In Asia, which accounts for a significant portion of global aquaculture production, fish ponds predominantly use freshwater or brackish water, with marine ponds being less common. This highlights the prevalence and preference for brackish or freshwater systems in the region for aquaculture practices.

Raceways

Controlled flow of water through raceways or tanks for fish culture, particularly suitable for fast-growing species. The advantages are: better water quality control, efficient feeding, and reduced disease risks.

A raceway, also known as a flow-through system, is a commonly used artificial channel in aquaculture for culturing aquatic organisms. It represents one of the earliest methods employed in inland aquaculture. Typically, a raceway consists of rectangular basins or canals constructed from materials like concrete and is designed with an inlet and outlet system. In its simplest form, a raceway is essentially a flume designed for water transportation. Raceways used for fish culture are relatively shallow tanks that depend on a high water flow in proportion to their volume to maintain the aquatic life within. In successful aquaculture, it's vital for the inflowing water to align with the temperature tolerance of the species being cultured. Ideally, the temperature should closely match the optimum temperature required for the target species.

In addition to temperature control, the incoming water into the raceway serves as a source of oxygen, which is crucial for the health and survival of the cultured aquatic organisms. Proper oxygen levels are maintained through adequate water flow and management practices within the raceway system. This makes raceways a popular choice for aquaculture, providing controlled environments that support the growth and development of aquatic species.

Indoor Facilities

Fish can be effectively cultured for domestic and commercial purposes using various types and sizes of tanks, allowing for flexibility in production based on specific requirements and input levels. The highest level of technological advancement in fish farming is often seen in indoor facilities, where fish are grown in specialized tanks such as circular raceways. These tanks receive pumped seawater, which may be directly sourced from the ocean. The water can be cycled through the tanks, discarded, recirculated, or processed through advanced water treatment systems. These facilities are capable of rearing

marine species to market size, but they are frequently utilized as hatcheries and for holding broodstock (adults used for reproduction). Aquaponics systems represent another innovative approach, integrating fish culture with vegetable cultivation. This system is designed for minimal water usage while optimizing resource efficiency. The waste generated by fish serves as a nutrient source for growing plants, and the plants, in turn, help filter and purify the water, creating a sustainable and symbiotic ecosystem. Additionally, the floc system, employing suitable consortia of organisms, presents an integrated approach that can be utilized for fish production. This system involves the strategic arrangement of multiple organisms, potentially including fish, to maximize production and resource utilization.

These diverse approaches showcase the adaptability and versatility of fish farming methods, providing options for efficient and sustainable production across various scales and needs, ranging from small-scale domestic use to large-scale commercial ventures. Integration, advanced technologies, and sustainable practices are key to optimizing the potential of these systems for successful fish culture and aquaculture.

Recirculating Aquaculture Systems (RAS)

Closed-loop systems that recycle and treat water for fish and shrimp culture. The advantages are: minimizes water usage, controls water quality parameters, reduces environmental impact, and allows for land-based culture.

RAS are indoor, tank-based systems in which fish are grown at high density under controlled environmental conditions. Generally, farmers adopt a more intensive approach (higher densities and more rigorous management) than other aquaculture production systems. Recirculating aquaculture systems are indoor, tank-based systems in which fish are grown at high density under controlled environmental conditions with different levels of filtration systems (Espinal and Matulic, 2019). Recirculation is growing rapidly in many areas of the fish farming sector, and systems are deployed in production units that vary from huge plants generating many tonnes of fish per year for consumption to small sophisticated systems used for restocking or saving endangered species. It has always been recommended to use recirculation systems to produce expensive fish because a high selling price leaves room for higher production costs. A good example is the eel farming business where a high selling price allows relatively high production costs. The suitability of rearing specific fish species in recirculation depends on many different factors, such as profitability, environmental concerns, and biological suitability. It should be mentioned that for small fish the use of recirculation is always recommended, because small fish grow faster and are therefore particularly suited to a controlled environment until they have reached the size for growing. Some of the saltwater fish species cultured in worldwide in RAS based culture system are Atlantic salmon, smolt (*Salmo salar*), Grouper (*Epinephelus spp)*; Rainbow trout (*Oncorhynchus mykiss*), Seabass/ Seabream (*Dicentrarchus labrax* / *Sparus aurata*), Yellowtail amberjack (*Seriola lalandi*), Cobia (*Rachycentron canadum),* Indian pompano *(Trachinotus mookalee)*

Farming in Cages

Sea cage farming (salmon, breams, snapper, seabass, grouper): High-density, low volume system with maximum production in unit area than in any other culture systems. The sea cage farming has been expanding in recent years on a global basis and it is viewed by many stakeholders in the industry as the aquaculture system of the millennium. Cage culture has made possible the large-scale production of commercial finfish in many parts of the world and can be considered as the most efficient and economical way of rising fish. The rapid growth of the industry across the globe is attributed to (i) availability of suitable sites for cage culture (ii) well established breeding techniques that yield a sufficient quantity of various marine and freshwater fish juveniles (iii) availability of supporting industries such and feed, net manufactures, fish processors etc. (iv) strong research and development initiatives from institutions, governments and universities and (v) the private sector ensuring refinement and improvement of techniques/ culture systems, thereby further developing the industry. Industrial marine fish farming is a relatively young phenomenon but has grown to be a major industry in many regions of the world, producing some 6.6 million tons of fish per year. The standard production units,

sea-cage fish farms, are variations on a common theme, floating, surface-based structures holding large nets which contain thousands to hundreds of thousands of fish. The major species produces using the mariculture technology is the Atlantic salmon, *Salmo salar* (2.44 million metric tonnes), and 0.767 million metric tonnes of other marine fin fishes including different species of breams, snapper, seabass, grouper, pompanos, etc. (FAO,2022).

Farming in pens

Inexpensive pen structures are constructed in shallow natural bodies of water such as creeks, swamps, lagoons, lakes, and bays, with water depths ranging from 1 to 3 meters. The bottom of the pen culture sites should ideally consist of firm clay or mud, allowing for the installation of supporting poles and posts for the pen structure. Traditionally, pens were constructed using materials like wooden planks and split bamboo. However, in modern times, synthetic materials such as nylon, polypropylene, and polythene are commonly used for pen construction. The pen structure typically consists of vertical net barriers, with a portion buried in the mud or ground using a footrope and small weights. These weights are secured to a chain link between concrete sinkers, ensuring stability. Floats are provided at the upper level to keep the structure afloat. In this system, fingerlings stocked in the pens primarily feed on natural food available in the lagoon or lake, and artificial feed is usually not provided. Milkfish (*Chanos chanos*) is the principal fish species cultured in Southeast Asian countries using these pen structures. The design and setup of these pens allow for efficient milkfish farming, making use of the natural aquatic environment and maximizing the growth and development of the fish within the pens.

Bottom culture or suspended culture for bivalve molluscs (mussels, oysters)

Marine bivalves, such as mussels, oysters, clams, and cockles, represent a sustainable source of food production due to their position low in the trophic chain as herbivores, with a trophic position of 2. In contrast, the average trophic position of the total marine capture fishery is higher at 3.1 (Duarte et al., 2009). This emphasizes the ecological efficiency and sustainability of bivalve aquaculture. With challenges related to decreasing seed resources and environmental concerns associated with seed fisheries, there has been a shift towards producing seed resources for marine bivalve aquaculture within land-based hatcheries. Over the years, aquaculture production of marine bivalves has seen a substantial increase, rising from 1.18 million tonnes per year in the period 1970–1974 to 13.47 million tonnes per year in the period 2010–2015.

One of the popular methods for cultivating marine bivalves is raft culture, where seeded ropes are suspended from a raft positioned in a suitable site and depth within the inshore area. These ropes are spaced around 0.5 to 1m apart, ensuring the end of the rope is approximately 2m above the water bottom. Marine bivalve aquaculture significantly contributes to the overall aquaculture production of aquatic animals, accounting for a substantial portion. In particular, the cultivation of marine bivalves contributes approximately 17.7 million tonnes of molluscs (valued at USD 29.8 billion), with countries like New Zealand, France, Spain, the Republic of Korea, Italy, and Japan being major contributors, surpassing the world average in terms of production percentage.

Rope culture or longline culture for seaweed cultivation: Seaweed mariculture has its origins dating back to the Tokugawa (or Edo) Era in Japan (AD 1600–1868). In the contemporary era, a substantial 92% of the global seaweed supply is sourced from cultivated species. The success of seaweed cultivation is influenced by the selection of species, understanding their biology, life history, tissue specialization, and considering the socioeconomic conditions of the region where cultivation occurs. Cultivation technologies can range from low-tech, which employs simple and highly efficient culture techniques often requiring intensive manual labour at low costs, to highly advanced and mechanized systems. The latter may involve on-land cultivation for seeding certain phases of the life history before transferring the seaweed to open-sea aquaculture sites.

Global seaweed cultivation, witnessed a growth of half a million tonnes in 2020, reflecting a 1.4% increase from 2019, reaching a total of 34.6 million tonnes. In seaweed farming, a diverse array of 13 culture methods

have been employed globally, catering to different seaweed species and varying sea climatic conditions. Selection of a particular method depends on its suitability to the prevailing sea conditions. Common methods for shallow waters include off-bottom monoline, broadcast, floating bamboo, net systems, and tubular nets. On the other hand, deeper waters typically utilize methods such as multiple raft long line, spider web, hanging basket, and free swing methods. The selection of an appropriate method is crucial for optimizing the productivity and sustainability of seaweed cultivation operations.

Integrated Multi-trophic Aquaculture (IMTA)

Combining different species with complementary ecological niches to optimize resource use and reduce environmental impact. For example, integrating fish, shrimp, and seaweed culture in the same area. The advantages include enhanced sustainability, nutrient recycling, and increased production efficiency.

IMTA represents a significant shift in aquaculture practices, providing a more sustainable and environmentally friendly approach. The conventional intensive monoculture systems have posed challenges related to sustainability, environmental degradation, and disease problems. IMTA offers a solution by integrating commercially important species from different trophic levels to create balanced systems that promote environmental stability, economic stability, and social acceptability.

In IMTA, the cultivation of fed aquaculture species (e.g., fin fish, shrimp) is combined with organic extractive aquaculture species (e.g., shellfish, herbivorous fish) and inorganic extractive aquaculture species (e.g., seaweed). This integrated approach aims to mitigate the environmental impact caused by excess nutrients and organic matter generated by intensive aquaculture activities, especially in marine waters. By incorporating species from various trophic levels into the same system, IMTA helps in maintaining a more harmonious and balanced ecosystem.

IMTA is aligned with the principles of the ecosystem approach to aquaculture (EAA) advocated by the Food and Agriculture Organization (FAO). It not only addresses environmental concerns but also diversifies products and reduces risks, contributing to economic stability. Furthermore, IMTA can enhance the production capacity of a specific site, making it a holistic and efficient method of aquaculture.

Integrated Mangrove-Shrimp Farming

Cultivating shrimp in ponds with integrated mangrove areas for ecological balance and waste assimilation. Advantages are, natural filtration, improved water quality, and additional income from mangrove products.

Status of mariculture in island ecosystem

Mariculture, or marine aquaculture, takes place in the sea for the entire cycle or only during the grow-out phase. In the first case, the production cycle takes place entirely in the seas for those species dependent on wild seeds from the sea, especially bivalve culture. Otherwise, mariculture refers only to the grow-out phase of the production cycle when a species is produced from a land-based hatchery and sometimes even in freshwater (FAO, 2022). Mariculure has been extensively practised in different ecosystems, where the protected coastal ecosystem considered to be the one the most suited for the mariculture practices due to its inherent advantageous. The islands are having protected sea, mostly grouped and are also moderately indented. As a result there are numerous bays, lagoons, creeks and inlets with varying depths and different substrata which are optimal for several types of mariculture operations. Ideal situations exist for raft culture and cage culture in the bays; shallow lagoons are suited for pen culture; the water bodies in the creeks and inlets with the adjoining land area can be used for the development of costal fish farms and land based farming methods and hatchery establishment due to its contamination free water quality

Presently, there is a continuous increase in demand for seafood across the globe, and it is estimated that aquaculture will dominate global fish supplies by 2030, with less than half the fish consumed coming from capture fisheries. Thus, emphasis has been given to island ecosystem by the different nations on the aquaculture development plan for island regions is to provide a sound basis for developing a

new aquaculture industry in the islands. While at the same time conserving the unique environment of the islands for present and future generations, and minimizing conflict between aquaculture and existing and future users of the islands. Also considering the growing population, static levels of wild capture fisheries, an increasing recognition of seafood as part of a healthy diet and growing affluence among the populations of some key export markets. The island ecosystem provides an ample opportunity mainly towards the production of premium species. According to the United Nations Sustainable Development Goals (SDGs), aquaculture activity can permeate several of these objectives, namely SDG2 (zero hunger), SDG12 (responsible consumption and production), and S14 (life below water). Target 14.7 specifically states that by 2030 it is necessary to increase the economic benefits for small island developing states and countries of lower relative development from the sustainable use of marine resources, including through sustainable management of fisheries, aquaculture, and tourism (The Global Goals 2022).

Understanding importance of the aquaculture development in increasing fish food requirement, different countries have made and rode map or action plan for the developing island eco system for augmenting fish production using different mariculture technologies. Among which, cage culture is considered to be the most popular culture operation in different island in different regions. Some of the major islands in Atlantic and pacific oceans are well developed with mariculture activities, and contribute major share in mariculture production in FAO production data base.

Atlantic islands

Marine aquaculture activity in Madeira started in 1996 with a pilot project of floating cages for sea bream production. In recent years, efforts have been made to expand the activity, where in 2016, the Regional Government defined 5 coastal areas with the greatest potential for the development of marine aquaculture, so-called ZIAs (Zones of Interest for Aquaculture), all of which are located on the south coast of Madeira Island in Atlantic islands (Machado et al., 2023). The Faroe Islands is a self-governing archipelago, part of the Kingdom of Denmark. It comprises 18 rocky, volcanic islands between Iceland and Norway in the North Atlantic Ocean, connected by road tunnels, ferries, causeways and bridge. The Faroe Islands rely heavily on fisheries and aquaculture activities. Fish and fish products make up almost 95% of the total export income and 20% of the total Faroese gross national product. Alongside wild capture fisheries, aquaculture has become an important Faroese industry, both economically and socially. Atlantic salmon dominates production, with some small production of seaweed. Economically, aquaculture is an important industry in the Faroe Islands. Over the past decade, it has grown to represent 40–45% of total goods exported (8% of the gross national product). With 5% of the total labour force employed within aquaculture, the industry has a much stronger influence on society and the economy when compared to other International Council for the Exploration of the Sea (ICES) ecoregions. In the year 2020, the Faroe Islands alone has produced around 89, 000 tonnes of marine finfishes alone majorly contributed by Atlantic salmon and Atlantic cod (*Gadus morhua*). Most salmon are raised in very large floating fish farms located in the narrow straits between islands. These are quite vulnerable to storms, but well managed with a high degree of mechanization. Salmon farming rapidly became an important export activity for the Faroe Islands, channelling most of its products through Denmark to the European markets (FEAP, 2002).

The Canary Islands are an autonomous community of Spain and an outermost region of the European Union. They form an archipelago located off the northwest coast of mainland Africa. The Canarian jurisdictional waters have three national maritime borders, with Portugal, Morocco and Western Sahara. The aquaculture development in the island ecosystem has been supported by the European Maritime and Fisheries Fund (EMFF). Due the favourable climatic conditions in the island, sea bass and sea bream have long been produced, soles and shrimps are cultivated slightly less quantities. Floating sea cages and Race way system are the two major production systems contribute for fish production. In a 2022 report on the blue economy of the Canary Islands authored by the Technological Centre for Marine Sciences (CETECIMA), data from the Ministry of Agriculture, Livestock and

Fisheries of the Government of the Canary Islands show that of the eight islands in the archipelago four have fish farming production and/or marketing activities. Production capacity on the four islands has remained stable since2014 at about 11,000 tonnes. Both sea bass and sea bream are cultivated in the islands, but only from the grow-out systems. Juveniles typically weighing 5-15 g are obtained either from the Spanish mainland or imported from other countries and introduced into cages in the sea where they grow to market size (FAO, 2020)

Pacific Islands

Aquaculture operation is currently of little commercial significance to the Pacific Islands. Shrimp (*Penaeus* spp.) farming has been a focus of commercial development in several islands with varying degrees of success; tilapia (*Oreochromis niloticus*) aquaculture has entered the subsistence economy in some areas, and seaweed (*Kappaphycus spp*.) is a well-developed industry. Profitable aquaculture of penaeid shrimps and black lip pearl oysters has now been established in some areas of the Pacific islands by commercial interests. Stand-alone enterprises producing penaeid shrimps for export markets are firmly established in New Caledonia, Fiji and Solomon Islands. These enterprises are applying technology developed originally in Japan, Taiwan and France, and now common place throughout the tropics. A large, sustainable, industry for culturing pearls using the black lip pearl oyster (*Pinctada margaritifera*) has been established in the Tuamotu Archipelago, French Polynesia, and on a couple of atolls in the Cook Islands (Fassler, 1995). In French Polynesia, the value of cultured pearls exceeds US$150 million per annum. In Cook Islands, the industry is currently worth US$5 million and is the second largest source of revenue for the country after tourism. Black pearl farming in French Polynesia and Cook Islands, and shrimp aquaculture in New Caledonia represented more than 98% of the total value of aquaculture production. Solomon island is the one the major player in production of culture seaweed, occupied 12th position in seaweed producing countries with 5.5 thousand tonnes of seaweed during 2018-19 (Gillett and Tauati, 2018). In the Pacific Islands, there are few traditional collection fisheries for juvenile fish to support grow-out operations. The exception is collection of milkfish (*Chanos chanos*) for pond grow-out in several Pacific Island countries, including Kirabati and Nauru

The NOAA Fisheries Pacific Islands Regional Office works with the aquaculture industry and research partners to develop, evaluate, and transfer appropriate technologies to the pacific islands. NOAA Fisheries has prepared a Programmatic Environmental Impact Statement (PEIS), in coordination with the Western Pacific Regional Fishery Management Council, to analyse the potential environmental effects of a potential Pacific Islands aquaculture management program and alternatives. Such a program would support an environmentally sound and economically sustainable aquaculture industry in Federal waters of the Pacific Islands. In 2016, the NOAA Fisheries Pacific Islands Regional Office completed its environmental review and issued a Special Coral Reef Ecosystem Fishing Permit (SCREFP) to Kampachi Farms, LLC., to allow the culture and harvest of palmaco jack (*Seriola rivoliana*), or kampachi, using a net pen system with production capacity of approximately 60 tonnes.

Islands in Indonesia

Grow out is carried out in many areas of Indonesia, and grouper farming in particular is growing fast, especially in the Lampung area of southern Sumatra. Cage culture can be found throughout Indonesia, including the islands Sumatra, Bangka, Bengkulu, Lampung, Kepulauan Seribu, Banten, Java, Lombok, Kalimantan and Sulawesi. However, much of this culture is based on wild fish seed. Recent developments in Lampung have been largely driven by the availability of hatchery-reared grouper seed (Halwart, et al., 2007**)**.

Addressing the issues in Island based ecosystems

The islands in spite of their wide diversity, are all confronted to a greater or lesser extent with similar problems including, isolation and remoteness, limited natural and human resources, difficulties in terms of competitiveness and economic development, and fragile environments. Work in island regions involves consideration of potentials and constraints in various aspects of development. Island ecosystem required

a special agro-ecological approach in the pursuit of sustainable development (Brooks, 2002). Agriculture, fisheries and forestry have provided for centuries the main source of livelihood for the population of the islands. Their sustainable management remains crucial for the future. Mariculture development activities in the island ecosystem islands should involve consideration of potentials and constraints in the various aspects of development, including:

Economic issues: narrow resource base, isolation from major markets, vulnerability to volatile international markets, erosion of preferential trade arrangements, high external debt, high level of foreign aid and remittances, difficulties in conforming to sanitary regulations, importance of tourism and dominance of public sector.

Ecological issues: rich marine and terrestrial biodiversity, vulnerability to natural hazards, harsh competition for scarce natural resources, particularly fresh water, degradation of coastal habitats, loss of traditional agricultural systems and over-exploitation of forest and coastal resources.

Social issues: high population growth and mobility, limited variety of dietary intakes and nutritional problems, institutional "brain drain", scarcity of skilled manpower and weak institutional capacities.

Mariculture initiatives in Indian Islands

The Andaman and Nicobar Islands, with their vast coastline and extensive Exclusive Economic Zone, present a prime opportunity for mariculture and aquaculture development in India. Despite abundant marine fisheries potential and high demand for fish products, current harvest levels remain considerably below the capacity. Challenges such as limited seed availability, transportation constraints, adverse climatic conditions, and a lack of awareness about alternative livelihoods like cage culture impede the sector's growth. However, recent research initiatives and policy recommendations emphasize the potential of cage farming for various marine finfishes. Government support, including the establishment of a marine fish hatchery and grow-out farm, aims to overcome challenges and further unlock the region's mariculture potential, crucial for sustainable economic development and meeting the growing demand for seafood (Kiruba-Sankar *et al.,* 2019).

Considerations for Successful Mariculture

Environmental Impact Assessment (EIA): Conduct thorough assessments to ensure that mariculture activities do not harm the surrounding ecosystem.

Water Quality Management: Regular monitoring and management of water parameters like temperature, salinity, dissolved oxygen, and nutrient levels are crucial.

Biosecurity Measures: Implement measures to prevent and control **disease outbreaks, including quarantine procedures and disease-free seed stock.**

Sustainable Feed Management: Optimize feed formulation and feeding practices to minimize waste and reduce reliance on wild fish for feed ingredients.

Community Engagement and Training: Involve local communities, provide training, and promote sustainable mariculture practices to ensure long-term benefits and acceptance of mariculture initiatives.

India, like many other countries, faces the necessity to increase seafood production to meet the rising demand. Projections indicate a need to produce about 18 million tonnes of fish by 2030, compared to the approximately 13 million tonnes produced at present. This implies that aquaculture production needs to escalate from about 5 million tonnes to about 12 million tonnes to bridge this gap. While enhancing fish production from the inland sector has limitations in terms of scope, the bulk of the additional demand is expected to be met through the development and expansion of mariculture.

Sustainable mariculture practices in Indian island ecosystems can contribute to food security, economic development, and environmental conservation, provided they are implemented with careful planning and adherence to best practices.

Acknowledgement

The authors acknowledge Director CMFRI for facilitation and necessary administrative support.

References

Andrews, H.V., S. Krishnan and P. Biswas. 2006a. Distribution and status of marine turtles in the Andaman and Nicobar Islands. In: Marine Turtles of the Indian Subcontinent (eds. Shanker, K. & C. Choudhury), pp. 33-57. Universities Press, Hyderabad. India.

Anuraj, A ,Suresh Babu, P P , Loka, Jayasree , Ignatius, B , Santhosh, B , Ramudu, K R ,Sonali, S M , Rao, K Srinivasa , Dube, Praveen ,Kumbhar, N P ,Joseph, Shoji ,Imelda, Joseph , Gopalakrishnan, A and Shirdhankar, M M (2021) *Induced breeding and larval rearing of vermiculated spinefoot, Siganus vermiculatus (Valenciennes, 1835) in indoor conditions*. *Aquaculture*, 539. pp. 1-8.

Apte, D. 2009 Opisthobranch fauna of Lakshadweep Islands, India, with 52 new records to Lakshadweep and 40 new records to India: Part 1. *Journal of the Bombay Natural History Society*, 106(2):162-175.;

Arasu A, R T, Kailasam M. and Sundaray J. K.. 2009. Asian Seabass, Fish Seed Production & Culture Sponsored By National Fisheries Development Board, Hyderabad. Training Manual, 145 p.

Aul, B., Bates P.J.J.,.Harrison ,D.L and M Arimuth G . 2014. Diversity, distribution and status of bats on the Andaman and Nicobar Islands, India. *Oryx* 48(02):204-212.

Brooks, S.S., Palmer, M.A., Cardinale, B.J., Swan, C.M and Ribblett, S. 2002. Assessing Stream Ecosystem Rehabilitation: Limitations of Community Structure Data. *Restoration Ecology*, 10(1): 156-168. https://doi.org/10.1046/j.1526-100X.2002.10117.x

Davidar, P, K. Yogananad, T. Ganesh and Soubadra Devy. 2002. Distributions of Forest Birds and Butterflies in the Andaman Islands, Bay of Bengal: Nested Patterns and Processes. Ecography, 25, (1): 5-16.

Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J. & Jordi, A. (2009). Will the oceans help feed humanity? *BioScience,* 59(11), 967-976.

Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J. and Jordi, A. (2009). Will the oceans help feed humanity? *BioScience*, 59(11), 967-976.

Espinal, C.A. and Matulić, D. 2019. Recirculating Aquaculture Technologies. In book: Aquaponics Food Production Systems (pp.35-76). DOI:10.1007/978-3-030-15943-6_3.

FAO Fisheries Technical Paper, No. 498. FAO, Rome. 255 pp.

FAO, 2022. The State of World Fisheries and Aquaculture 2022.Towards Blue Transformation.

FRAEED, CMFRI, 2023. Marine Fish Landings in India-2022. Technical Report, CMFRI Booklet Series No. 31/2023. ICAR-Central Marine Fisheries Research Institute, Kochi.

Gillett, R., and Tauati, M. I. 2018. Fisheries in the Pacific. Regional and national information FAO Fisheries and Aquaculture Technical Paper No. 625. Apia, FAO.

Gopakumar, G., Abdul Nazar, A. K., Jayakumar, R., Tamilmani, G. and Sakthivel, M. 2014. Strategies and way forward to augment seafood production through finfish mariculture, *Marine Fisheries Information Service*, T & E Ser., No. 219, 3-7.

Gopalakrishnan, A, Kirubagaran, R, George, J., Ponniah, A G , Gopakumar, G , Mohamed, K S , Krishnan, P , Imelda, Joseph , Ignatius, Boby , Jayakumar, R , Raju, M S ,Sreepada, R A ,Shinoj, P and Rajesh, N (2019). CMFRI Marine Fisheries Policy Series No.17; Draft National Mariculture Policy 2019 (NMP2019) Report of the Committee constituted by the National Fisheries Development Board (NFDB), Ministry of Fisheries, Animal Husbandry & Dairying, Govt. of India. [Report of the Committee constituted by the National Fisheries Development Board (NFDB) Ministry of Fisheries, Animal Husbandry & Dairying, Govt. of India]. CMFRI Marine Fisheries Policy (17). ICAR - Central Marine Fisheries Research Institute, Kochi.

Halwart, M., Soto, D. and Arthur, J. R. 2007. Cage aquaculture, Regional reviews and global overview.

Hayashi, L., Yokoya, N. S., Oliveira, E. C., and Ostini, S. 2010. Seaweed farming in Brazil: a review of annual changes in seaweed crops. *Aquaculture International*, 18(3): 279-288.

Hurtado, A. Q., Agbayani, R. F., Sanares, R., and de Castro-Mallare, M. T. R. 2014. Advances in cultivation technology of commercial eucheumatoids: progress, constraints, and prospects in the Philippines. *Journal of Applied Phycology*, 26(4): 2209-2225.

Hurtado, A. Q., Yunque, D. A., Tibubos, K. R. and Critchley, A. T. 2015. Development of the seaweed industry in the Philippines: present status, bottlenecks and opportunities. *Journal of Applied Phycology,* 27(5): 2121-2130.

Imelda –Joseph and Asha Augustine 2020. Marine biotechnology for food, *In* Genomics and Biotechnological Advances in Veterinary, Poultry, and Fisheries, 271-283

James, P. S. B. R. 2011. *Lakshadweep: Islands of Ecological Fragility, Environmental Sensitivity and Anthropogenic Vulnerability. Journal of Coastal Environment*, 2 (1): 9-25.

Kiruba-Sankar, R., Sar U.K, Vinod Kumar, Nazar, A.K., Raymond, J.A, Dam Roy, S, K., Saravanan, K., Zameer A and Kundu, A. 2019. Policy brief: expanding open sea cage culture in Andaman and Nicobar Islands: blue growth perspective. Technical Report, CIARI, Port Blair.

MacArthur and Wilson, 1967. The theory of Island Biogeography, Princeton University Press, Princeton

Machado L.P. and Almeida, A. 2014. The On-going Process of Reinventing Classic Tourism Destinations – The Case of Nordic Tourists in Madeira Island, *Scandinavian Journal of Hospitality and Tourism*, 13: sup, 24-43, DOI: 10.1080/15022250.2014.961242

Modayil, M.J., Sathiadhas, R. and Gopakumar, G. 2008. India. In A. Lovatelli, M.J. Phillips, J.R. Arthur and K. Yamamoto (eds). FAO/NACA Regional Workshop on the Future of Mariculture: a Regional Approach for Responsible Development in the Asia-Pacific Region. Guangzhou, China, 7–11 March 2006. FAO *Fisheries Proceedings*. No. 11. Rome, FAO. 2008. pp. 145–171.

Murty, V.S. 2002. Marine Ornamental Fish Resources of Lakshadweep. CMFRI, Spl. Pub. 72: 134 pp

Padalia,H., Nidhi Chauhan, M. C. Porwal and Roy P. S. 2004. Phytosociological observations on tree species diversity of Andaman Islands, India. *Current Science*, 87 (6): 799-806

R. Sankaran 1998. An annotated list of the endemic avifauna of The Nicobar Islands. Forktail 13: 17-22

Ramachandran, S., S. Anitha, V. Balamurugan, K. Dharanirajan, K. Ezhil Vendhan, M. Irene, P. Divien, A. Senthil Vel, I. Sujjahad Hussain & A. Udayaraj. 2005. Ecological impact of tsunami on Nicobar Islands (Camorta, Katchal, Nancowry and Trinkat). *Current Science*, 89(1): 195–200.

Ranjan, R, Megarajan, Sekhar, Xavier, Biji , Dash, B , Ghosh, S , Muktha, M and E, Loveson 2017. Conditioning, maturation and year□round natural spawning of orange□spotted grouper, Epinephelus coioides (Hamilton, 1822), in recirculating aquaculture system. *Aquaculture Research*, 48: 5864-5873.

Ravindran, J., Raghukumar C and S Raghukumar, 1999. Disease and stress-induced mortality of corals in Indian reefs and observations on bleaching of corals in the Andamans. *Current Science*, 76(2):12-20

Reddy, S. C., B Debnath, H K Peddi and C.S.Jha. 2012. Landscape level assessment of critically endangered vegetation of Lakshadweep Islands using Geo-spatial techniques. *Journal of Earth System Science*,122(2). DOI:10.1007/s12040-013-0272-4

Rodgers W A, Panwar H S and Mathur V B 2002 Wildlife protected area network in India: A review; Wildlife Institute of India, Dehradun.

SAP, Society of Aquaculture Professionals. 2023. Shrimp crop 2022 review: Farmed shrimp production in India. *Aquaculture Asia-Pacific,* March/April 2023, 4-5p.

Shinoj, P , Muktha, M , Jeeva, Charles J ,Johnson, B , Anuraj, A ,Swathi Lekshmi, P S , Ramachandran, C ,Padua, Shelton, Aswathy, N , Ghosh, Shubhadeep , Divu, D , Megarajan, Sekhar ,Geetha, R ,Vinuja, S V , Ignatius, Boby , Suresh, V V R , Narayanakumar, R , Gopalakrishnan, A and Chand, Prem (2023) Sustainable intensification of small-scale mariculture systems: Farm-level insights from the coastal regions of India. *Frontiers in Sustainable Food Systems*, 7: 1-27.

SPREP, Secretariat of the Pacific Regional Environment Programme 2012. Annual Report

Suresh Babu, P P ,Anuraj, A , Shilta, M T , Asokan, P K , Vinod, K , Dube, Praveen , Rao, K Narayana , Vaidya, N G , Sonali, S M , Pal, Mahendra , Loka, Jayasree , Santhosh, B , Jeena, N S , Imelda, Joseph , Ignatius, Boby and Gopalakrishnan, A (2022) Broodstock development, breeding and larval rearing of Acanthopagrus berda (Forsskal, 1775), a suitable species for farming in tropical waters. *Aquaculture Research*. 1-15.

Venkataraman K., Rajkumar, R., Sathyanarayana Ch., 2012. Fauna of the ecosystem of India. Coral reefs (Zoological Survey of India, Kolkotha) 1-30.

Witt, C.C. and Maliakal-Witt, S. (2007) Why Are Diversity and Endemism Linked on Islands? *Ecography*, 30: 331-333.

Received: 01st October 2023 Accepted: 25th October 2023

Journal of the Andaman Science Association Vol. 28(2):143-157 (2023)
ISSN 0970-4183, Printed in India

Improved Practices for Harnessing the Potential of Tree Spices

P. M. Haldankar[1]*, S. B. Thorat[1], H. Cheriyan[2],Y. S. Saitwal[1] and N. V. Dalvi[1]
[1]*College of Horticulture, Dr. BSKKV, Dapoli.*
[2]*Directorate of Arecanut and Spices Development, Kozhikode, Kerala.*
***Corresponding author's E-mail:** paraghaldankar5@gmail.com*

Abstract

India is known as 'land of spices'. Tree spices are an important group among spices and about 17 tree spices are grown in India. There is need to harness yield potential of tree spices owing to its economic importance and demand. The improved varieties with good yield capacity and quality are developed in some tree spices viz. Nutmeg- Konakn Swad, Konkan Sungadha, Konkan Sanyukta, Keralashree, Viswashree; Cinnamon- Konkan Tej, Konkan Tejpatta, Navashree, Nithyashree; Kokum-Konkan Hatis, Konkan Amrita. Remarkable quality planting material of tree spices is being supplied to the stakeholders due to standardization of nursery and propagation techniques such as soft wood grafting in nutmeg and kokum; air layering in cinnamon etc. The tree spices are best suited mixed and inter crops for coconut, arecanut plantations as well as in kitchen garden. These crops are important component of agro and eco tourism. Developments of appropriate practices of post harvest management are important for marketing of tree spices. The various processed products adds the value of tree spices and some of them are nutmeg- pickle, candy, sweet chutney, powder; cinnamon - powder, quills, bark oil, leaf oil, bark oleoresin; kokum- kokum syrup, RTS, amsul, seed oil, butter, agal etc. The effective production technology must be coupled with proper dissemination technology. The initiative of Government of India through Mission for Integrated Development of Horticulture (MIDH) has helped for remarkable impact for area expansion under tree spices. Total 7 accredited and 15 implementing centers are participating in MIDH on Spices under jurisdiction of Dr. Balasabeb Sawant Konkan Krishi Vidyapeeth, Dapoli for catering the objectives to produce genuine and quality planting material of tree spices recommended by university, front line demonstrations and transfer of technology through farmer training programmes in entire Konkan region.

Key words: *Nutmeg- Konakn Swad, Konkan Sungadha, Konkan Sanyukta, Keralashree, Viswashree; Cinnamon- Konkan Tej, Konkan Tejpatta, Navashree, Nithyashree; Kokum-Konkan Hatis, Konkan Amrita, Improved tree species*

Introduction

India is known as 'land of spices' and history of Indian spices dates back to the beginning of human civilization. Among the various groups of spices, tree spices are unique owing to their nature. As the name indicates, tree spices are tall plants with more canopy compared to other spices like rhizome, seed and herbal spices, seed spices, bulbous spices, aromatic spices, leafy spices. There are different tree spices are grown in different parts of India shown in Table 1. They are the best components for intercropping systems particularly those of coconut and arecanut. Among the tree spices clove, cinnamon and tamarind are relatively more important and they are grown on area of 67304 with a production of 176136 tonnes (Table 2).

Table 1. Tree spices grown in India.

Sr. No.	Botanical Name	Family	Common Name	Part used as spice
1.	*Averrhoa bilimbi* L.	Averrhoaceae	Bilimbi	Fruit
2.	*A. carambola* L.	Averrhoaceae	Carambola	Fruit
3.	*Cinnamomum aromaticum* Nees	Lauraceae	Chinese cassia	Bark, leaf
4.	*C. tamala* Nees	Lauraceae	Tejpat, Indian cassia	Leaf, bark
5.	*C.verum* Bercht & Pres l.	Lauraceae	Cinnamon	Bark, leaf
6.	*Garcina gummi-gutta* (L.) Robs.	Clusiaceae	Garcinia, Cambogia	Pericarp of fruit
7.	*G. indica* (Thouars) Choisy	Clusiaceae	Garcinia, kokum	Pericarp of fruit
8.	*Illicum verum* Hook	Illiciaceae	Star anise	Fruit
9.	*Juniperus communis* L.	Cupressaceae	Juniper	Fruit
10.	*Laurus nobillis* L.	Lauraceae	Bay leaf	Leaf
11.	*Mangifera indica* L.	Anacardiaceae	Mango	Rind of immature fruit
12.	Mu*rraya koenigii* (L.) Sprengel	Rutaceae	Curry leaf	Leaf
13.	*Myristica fragrans* Houtt.	Myristicaceae	Nutmeg	Kernel, Aril
14.	*Pimenta dioica* (L.) Merr.	Myrtaceae	Allspice, Jamaica pepper	Immature fruit, leaf
15.	*Punica granatum* (L.)	Punicaceae	Pomegranate	Dried seed (with flesh)
16.	*Syzigium aromaticum* (L.)	Myrtaceae	Clove	Flower bud
17.	*Tamarindus indica* L.	Fabaceae	Tamarind	Fruit

(Haldankar *et al*. 2013)

2. Area, production and productivity of tree spices in India

Table 2. Area, Production and Productivity of tree spices in India 2021-22

Sr. No.	Crop	Area (ha.)	Production (toones)	Yield (kg/ha)
1.	Tejpatta	1682	4089	2432
2.	Nutmeg	23353	18429	789
3.	Clove	1924	1209	628
4.	Tamarind	40345	152409	3778

(Source-DASD, Calicut, 2023)

Table 3. Area and Production of Nutmeg in India 2021-22

Sr. No.	States	Area (ha.)	Production (toones)
1.	Kerala	22152	17435
2.	Karnataka	490	619
3.	Andaman & Nicobar	4	1
4.	**Total including other states**	**23353**	**18429**

(Source-Spices Board, Government of India)

Table 4. Area and Production of Clove in India 2021-22

Sr. No.	States	Area (ha.)	Production (toones)
1.	Tamilnadu	1085	1049
2.	Karnataka	115	86
3.	Kerala	715	53
4.	Andaman & Nicobar	9	21
5.	**Total including other states**	**1924**	**1209**

(Source-Spices Board, Government of India)

Table 5. Area and Production of Tamarind in India 2021-22

Sr. No.	States	Area (ha.)	Production (toones)
1.	Tamilnadu	14395	44415
2.	Karnataka	11012	41877
3.	Kerala	8232	28317
4.	Andhra Pradesh	3810	13811
5.	Telagana	54	147
6.	Maharshtra	1652	12592
7.	**Total including other states**	**40345**	**152409**

(Source-Spice Board, Government of India, 2023)

3. Improved varieties of tree spices in India

Various research institutes, Agricultural Universities are involved on improvement for tree spices and few varieties are released for commercial production of tree spices in India. The detail of the varieties along with their characteristics is given in Table. 6.

Table 6. Improved Varieties of Tree Spices in India

Variety	Characters	Developed by
Nutmeg		
Konkan Sanyukta (2018)	Monoecious nutmeg bearing 500 fruits per plant per year with bold nuts (9.20 g), high nut oil (27%) and mace oil (17.75%). Recommended for release during 2018-2019.	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli
Keralashree (2013)	1. First nutmeg variety developed by Farmers participatory breeding 2. Very bold nut with thick and entire mace 3. Wide adaptability. 4. The mace and nut oils are rich in sabinene and myrcene with low myristicin and elemicin.	ICAR - Indian Institute Of Spices Research, Kozhikode, Kerala
Viswashree (2001)	A high yielding, high quality variety with bushy and compact plant type.	ICAR - Indian Institute Of Spices Research, Kozhikode, Kerala
Konkan Shrimanti (Konkan Chaul) (2005)	A high yielding variety of nutmeg having bold nut and thick mace.	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli
Konkan Swad (2003)	A high yielding female variety with medium size fruits.	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli

Konkan Sugandha (1998)	First variety of nutmeg released in India. High yielding and hermaphrodite	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli
KAU Mundathan	Single mace weight (dry)-2.5 g, SingL nut weight (dry)-l2.6 g, Average no. fruits per tree: 1800	Kerala Agricultural University
KAU, Punnathanam	Single mace weight (dry)3.02 g, Single nut weight (dry)-13.9 g, Average no. fruits per tree: 1500	Kerala Agricultural University
KAU Pullan	Single mace weight (dry)1.4 g, Single nut weight (dry)-10.9 g, Average no. fruits per tree: 2100	Kerala Agricultural University
KAU Poothara	Single mace weight (dry) 2.1 g, Single nut weight (dry)-10.0 g, Average no. fruits per tree: 2000	Kerala Agricultural University
KAU Kochukudy	Single mace weight (dry)2.5 g, Single nut weight (dry)-11.6 g, Average no. fruits per tree: 1800	Kerala Agricultural University
Cinnamon		
PPI (Ci) 1 (2003)	It is a selection from the germplasm of open pollinated seedlings maintained at HRS, Pechiparai. The tree can be retained upto 50 years. It is tolerant to drought and resistant to pest and diseases. It is suitable for coppicing at an interval of 18 – 24 months and has good regeneration capacity. June – July is found to be the best season. It yields about 980 kg bark / ha (248.42 kg of quills and 731.58 kg of chips and dust) which is 25 percent higher than Pechiparai local. It is well adopted at lower elevation (100 – 500m), high rainfall with wide range of soil.	Tamil Nadu Agricultural University, Coimbatore
Sugandhini (2001)	Selection from germplasm maintained at AMPRS Odakkali	Kerala Agricultural University
Navashree (1996)	A selection with high shoot regeneration capacity. Higher cinnamaldehyde and oleoresin in bark.	ICAR - Indian Institute Of Spices Research, Kozhikode, Kerala
Nithyashree (1996)	A selection with high shoot regeneration capacity. Gives quality quills. Bark oil, leaf oil and oleoresin contents are high giving good aroma and taste.	ICAR - Indian Institute Of Spices Research, Kozhikode, Kerala
YCD 1 (1995)	It is a selection from the germplasm of open pollinated seedlings from Yercaud. It come to harvest from third year and can be maintained economically for 20 years. High dry bark yield of 359.75 quills and 3800 kg of dried leaves /ha with high bark recovery (35.3%). It has a 2.8 % volatile oil and in 3% quills and leaves. High regeneration capacity of 19.2 harvestable shoots in a harvest. The quills are sweet and light pungent. It is well adapted under 500m to 1000m above MSL and areas receiving 1000 to 2000 mm rainfall.	Tamil Nadu Agricultural University, Coimbatore
Konkan Tej (1993)	A high quality and high yielding variety of Cinnamon (*Cinnamomum verum* Presl.).	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
KonkanTejpatta (1993)	High yielding cinnamon variety for leaves	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth

Kokum		
Konkan Hatis (2006)	A high yielding kokum variety having very bold fruits.	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
Konkan Amrita (1997)	A variety of Kokum released for the first time in India. It is early, high yielding and fruits have long shelf life.	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
Clove		
PPI (CL) 1-	First clove variety with dry flower bud yield of 5.2 kg/ tree, 6% oil content and 34.22% bark recovery. It is suitable for Tamil Nadu conditions. Recommended for release during 2012-2013.	Tamil Nadu Agricultural University, Coimbatore
Cassia		
Konkan Cassia	First cassia variety with low coumarin content and dry bark yield is 262.94 kg ha-1. Suitable for cassia growing regions of the country. Recommended for release during 2017-2018.	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
Curry leaf		
Suwasini Dharwad-1	The leaves are dark green (0.1629 mg of chlorophyll/ gram of fresh leaf), shiny and highly aromatic. It is sensitive to low temperature in winter season and hence the bud burst is poor. The leaves have oil content of 5.22 % and can be dehydrated at 50^{o}C without loss of quality and made into powder.	University of Agricultural Sciences, Dharwad
Dharwad-2	The leaves are slightly pale green and less aromatic. It is not very sensitive to low temperature and much superior in number of bud burst, inter nodal length and 8 times higher in growth of shoot than DWD-1	University of Agricultural Sciences, Dharwad
Tamarind		
Shiwai (2018)	1. TSS-41.6% 2. Acidity of fruits-31.2% 3. Regular bearer	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
Goma Prateek (2013)	It is an early with spreading growth habit, regular bearer, semi-dwarf and starts flowering in 4th year. Fruit shape is slightly curved with reddish pulp, suitable for processing.	ICAR-CHES, Godhra, Gujarat
Ajintha (2006)	1. Sweet tamarind 2. Acidity-5.19% 3. Regular bearer	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
No.263 (1987)	1. Acidity-18% 2. Regular bearer	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
Prathistan (1985)	1. Acidity-18% 2. Regular bearer	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
PKM 1	Grafts come to flowering three years after planting and give commercial yield from fifth year. Nine year old trees give a mean yield of 263 kg. It has 35% pulp, 17.10 % and 3.90 mg/100 g ascorbic acid.	Tamil Nadu Agricultural University, Coimbatore
DTS 1	The pods are straight having semi-curved shape, 23.6 cm length, 3cm width, 19.5 gm weight, 51.00 % pulp and 13.60 % acidity. It is a late variety and takes 310 days from fruit set to maturity	University of Agricultural Sciences, Dharwad

DTS 2	This is a selection made at College of Horticulture, Arabhavi, UAS, Dharwad.The pods are straight having semi- curved shape, 17.60 cm length, 2.60 cm width, 18.00 gm weight, 53.00 % pulp and 12.20 % acidity. It is an early variety and the pods mature in 280 days after fruit set.	University of Agricultural Sciences, Dharwad
Malbar Tamarind Garcinia Cambogia		
Nithya (2018)	Acidity 53.67 %; tannin 520 mg/100 g; driage 9.76 %, intensive branching with spreading canopy; 740 fruits/tree/ year. Average fruit weight 88.25 g. Suitable for loamy to laterite soil. Dry Rind – 10.11 kg/tree; HCA -16.96 %	Kerala Agricultural University
Haritham (2015)	A HY Malabar tamarind ; spreading trees; golden yellow oblate fruits; good quality fruit rind; dry rind yield of 9.91 kg/ tree/ year; HCA content 52.99%	Kerala Agricultural University
Amrutham (2015)	Compact res , golden yellow colored global shaped fruits, average yield of 16.38 kg dry ring/ tree/year; mean HCA content 51.58%	Kerala Agricultural University

4. Propagation technologies for tree spice-

Propagation methods are standardized in some tree spices which resulted in to rapid multiplication and increase in the area of tree spice. For seedling production seeds are used for raising rootstock. However, scions of desired varieties are procured from mother orchard which gives true to type variety. The details propagation methods are given in Table. 7.

Table 7. Propagation techniques of Tree Spices in India

Sr. No.	Tree Spice	Propagation method	Advantages
1.	Nutmeg	Epicotyl grafting, Softwood grafting, top working	Female plants are propagated conservation of male plants to female plants. Rapid multiplication of elite plants per unit area.
2.	Cinnamon	Cottage, air layering (rapid multiplication techniques)	Earliness, More plants per unit area
3.	Cassia	Air layering, cutting	True to type plants
4.	Allspice	Cutting, layering	Management of rhizome rot
5.	Kokum	Soft wood grafting	True to type plants, early bearing
6	Clove	Seed, Inarching on clove seedling	Earliness, dwarfness and high productivity
7.	Tamarind	veneer grafting, Softwood grafting	Earliness, dwarfness

(Haldankar *et al*., 1999; Varghese and Jose, 2019)

Nutmeg-

It is a dioeciously in nature, therefore sexual propagation may lead to male plants and these male plants are unproductive. Commercial plantations are raised mostly using grafts or buds to circumvent the sex problem. A ratio of 1:10 male: female trees are recommended in the plantations. Currently, femaleness is ensured by grafting or budding, which needs good skill and experience for reasonable success. The dimorphic branching pattern of the tree is another issue of relevance in case of vegetative propagation. The scions or buds extracted from the orthotropic shoots only exhibit vigorous erect growth and canopy development similar to the seedling trees and the availability of such scions from mother trees is limited. Hence, commercial nurseries generally propagate nutmeg

through budding rather than grafting as the bud-wood provides maximum numbers of orthotropic propagules. However, the success rate in budding is a matter of skill, experience and season and hence budded plants are priced very high by the nurseries.

Softwood grafting is possible in nutmeg (*Myritica Fragrans* Houtt.) it was revealed that may was the best season for softwood grafting with maximum success (80.00%) followed by June (54.00%) and July (50.00%). were preferred for softwood grafting. Retention of one terminal leaf on except the terminal leaf, for apical bud swelling was advantageous and recorded 70.00 per cent success. The retention of the leaves on rootstocks did not influence the success of softwood grafting (Haldankar *et al*., 1997).

Nissar *et al*. (2019) reported air layering in nutmeg and attempted in matured trees for the first time in the India. They reported that, Air layering was successful in plagiotropic and orthotropic shoots with 100 per cent survival. This method is cost effective and is easy for adoption.

5. Planting systems for tree spice-

Tree spices are with compact canopy and tall in nature, so they are the best component for intercropping and can be utilized for planting with various crops as a intercrop. Nutmeg, Clove, Cinnamon are the best intercrop tree spices with coconut and arecanut. 'Laki Baug' at Dr. BSKKV, Dapoli is the best example for the same. 'Laki Baug' techniques utilize the potential of tree spices, land, resources etc. which resulted in improvement in economy of farmers.

Mixed cropping of spices in coconut

The excellent growth and good bearing capacity of cinnamon, nutmeg, black pepper and clove planted in coconut as intercrops proved that these crops can be cultivated on commercial scale in the Konkan region of Maharashtra.

Multi-storeyed Cropping of Tree Spices in Coconut

Among the various spices the tree spices are highly remunerable crops. These crops can sustain the climatic changes experienced in the region. Tree spices viz., nutmeg (*Myristica fragrans* Houtt.), cinnamon (*Cinnamomum verum* Bercht. and Presl.), clove (*Syzygium aromaticum* (L.) merr. and Perry), kokum (*Garcinia indica* Thouars), and all spice (*Pimenta dioica* (L.) Merr.) are suitable intercrops in coconut plantation. The air space, partial shade, solar radiation and available irrigation water in the plantation provide scope of growing intercrops. The multi-storeyed cropping with tree spices in coconut is a solution for sustainability with greater income to counter the climatic fluctuations. Various production models of multi-storeyed mixed cropping are developed for small farmers through rigorous research for suitable varieties, propagation protocols, densities of various spices in the coastal region of Maharashtra. The integrated multi-storeyed cropping system with tree spices continuously for ten years contributed higher returns per hectare elevated the nut productivity from 6300 to 9800 nuts acre^{-1} which helped for the sustainability of coconut plantations under aberrant climate of coastal region.

The planting of cinnamon, nutmeg, black pepper, kokum and clove under coconut as mixed crops were recorded excellent growth and bearing under coastal conditions (Patil *et al*. 1991, Nagwekar *et al*. 2014). The mixed cropping of tree spices resulted in increase of the average yield of coconut palm from 23 to 96 per cent at the end of 26th year as compared to the average yield of previous years (Table 8). Nutmeg proved to be the best intercrop in coconut plantation. (Nagwekar *et al*. 2014).

Table 8. Average yield of coconut per palm before and after planting spice crops and per cent increase in different blocks of spices crops.

Block Particulars	Cinnamon Block	Nutmeg Block	Black Pepper	Allspice Block	Clove Block	Garcinia Block	Control Block
Average yield before planting spices	69	71	83	49	47	64	77 Average yield of 26 years
Average yield after planting spices 1982 to 2003	118	119	102	93	92	96	
Percent increase (%)	71	69	23	90	96	50	-

(Anonymous 2015)

High density multistoried system in coconut plantation with nutmeg, cinnamon, banana, black pepper and pineapple contributed very high returns (Table 9 and Figure 1) (Anonymous 2005). The yield of coconut before planting with different component crops was 5,320 nuts acre-1 which was increased to 6,300 to 9,800 nuts acre^{-1} after planting of component crops. Whereas, nutmeg yield commenced from 4th year and it was 15,000 nuts and 7.5 kg mace (aril) per acre respectively from 10th year onwards (Anonymous 2005). The cinnamon started yielding from 3rd year and it was 50 kg dried bark acre-1 and 370 kg dried leaves acre-1 at 10th year whereas black pepper recorded 105 kg dried black pepper yield at the age of 10th year (Table 10). Inter cropping with nutmeg, kokum, black pepper, cinnamon and clove in the coconut plantations proved beneficial in Konkan region of Maharashtra (Table 11).

Table 9. Plant population in one acre of coconut plantation.

Name of crops	Variety	No. of plants
Coconut	D X T	70
Nutmeg	Konkan Swad	54
Cinnamon	Konkan Tej	246
Banana	Safed Velachi	246
Black pepper	Panniyur-1	140
Pineapple	Kew	4320

(Source: Nagwekar *et al*. 2014)

Table 10. Yield of coconut and different components crops in Lakhi Baug (in one acre).

Name of the crop	Year									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Coconuts (no.)	6300	7000	8400	8400	9100	9100	9100	9100	9100	9800
Nutmeg										
i) No. of Nuts	-	-	-	250	500	1000	2500	5000	10000	15000
ii) Mace (kg)	-	-	-	0.100	0.200	0.500	1.250	2.500	5.000	7.500
Cinnamon										
i) Dried bark (kg)	-	-	-	12.3	-	24.6	24.6	37	37	49
ii)Dried leaves (kg)	-	-	-	196	-	370	370	370	370	370
Banana (t)	-	2.2	1.76	2.2	1.76	2.2	1.76	2.2	1.76	2.2
Pineapple (t)	-	6.4	4.0	-	6.4	4.0	-	6.4	4.0	-
Black pepper (kg)	-	-	-	-	14	70	70	70	70	105

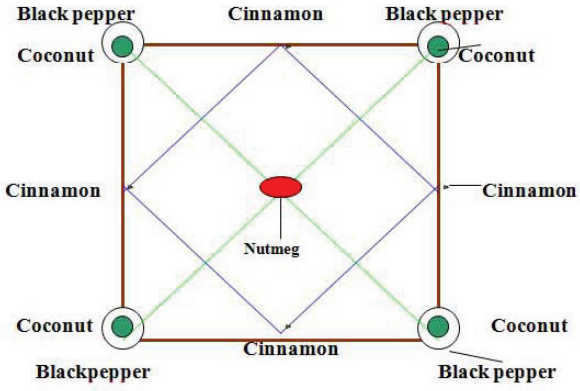
Source: Nagwekar *et al.* 2014.

Table 11. Economics of Coconut-based high density multi species cropping system (per hectare average of 5 years: 1999-2004).

Block	Total Cost ₹	Total Returns ₹	Net Profit ₹	B:C Ratio
Cinnamon	83449	137877	54428	1.65
Nutmeg	86417	179995	93578	2.08
Black Pepper	79313	130213	50900	1.64
All spice	68087	105952	37865	1.55
Clove	77017	109496	32479	1.42
Garcinia	81483	136976	55493	1.68
Control	55207	81194	25987	1.47

Lakhi Baug

Model -1



Plant population

Coconut: 70; Cinnamon: 123; Nutmeg: 54; Black pepper: 140

Lakhi Baug Model - 2

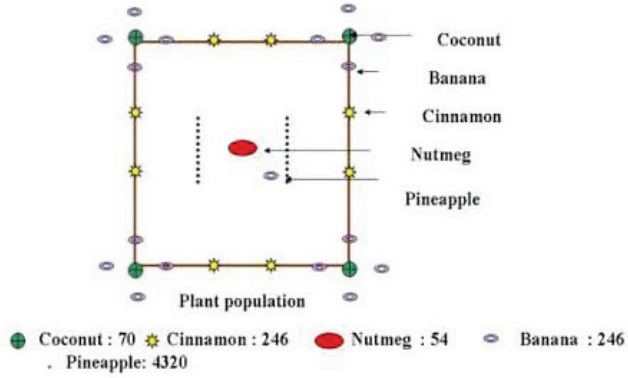


Fig. 1. High density multispecies cropping system model in coconut.

Expected Expenditure/Acre	**Rs. 2,30,000/-**
Expected Incomer/Acre	**Rs. 3,75,000/-**

Success Stories for Tree Spices as a mixed crop

The various farmers from Konkan region of Maharashtra adapted mixed cropping of tree spices instead of monocropping and successful in production of tree spice.

Mrs. Priyanka Nagwekar (34), a resident of Hatis village in Ratnagiri Tahsil of Ratnagiri District ventured into family farming. She was cultivating subsistence crops like rice, finger millet, vegetables, etc. in the traditional way in her 22 ha farm. Her farm income was very limited. Under the guidance of RCRS, Bhatye started to cultivate spice as mixed crops in coconut garden and successfully planted the spices Nutmeg, and Arecanut as mixed crop in their coconut garden which could get her additional income. Mrs. Priyanka Nagwekar received net profit Rs. 87860/- due to mixed cropping of coconut and Cinnamon (Indian Coconut Journal, May, June, July 2021). The few other success stories are enlisted in Table 12.

Table 12. Success story for mixed cropping of Tree spices in coconut garden

Sr. No.	Name of Farmer	Address	Crops grown
1.	Mr. Raja Bhargav Padhye	Nate, Tal. Rajapur, Dist. Ratnagiri.	Coconut, Nutmeg, Cinnamon
2.	Mr. Hemant Phatak	At-Someshwar, Post- Chinchkhari, Dist. Ratnagiri.	Coconut, Areacnut, Cinnamon
3.	Mr. Mahadev Naravane	Post- Nakhare, Tal. & Dist. Ratnagiri.	Coconut, Nutmeg, Cinnamon, black pepper, Banana
4	Mr.Abdul Majgaonkar	A/P- Karla, Tal. & Dist. Ratnagiri.	Coconut, spices
5	Mr. Amrut Ravji Desai	A/P- Kudase, Tal. Dodamarg, Dist. Sindhudurg.	Coconut, Arecanut, Kokum, spices, Coffee, Cashewnut, Pineapple
6	Mr. Vivekanand H. Naik	A/P- Dodamarg, Dist. Sindhudurg.	Mango, Coconut, Arecanut, Kokum, balck pepper, Coffee, Cashewnut, Pineapple
7	Mr. Raghunath V. Naik	A/P- Oros, Tal. Sawantwadi, Dist. Sindhudurg.	Coconut, Arecanut, Kokum, Clove
8	Mr. Vijay Waman Gogate	A/P- Mavlange, Tal. & Dist. Ratnagiri.	Coconut, Arecanut, Spice
9	Dr. Vivek Y. Bhide	A/P- Malgund, Tal. & Dist. Ratnagiri.	Coconut, Arecanut, clove

(Source- Success stories, Dr. BSKKV, Dapoli, 2009)

6. Post harvest of tree spice-

Tree spices in addition to adding taste, flavour, scent, and colour, also serve as preservatives by preventing food and beverage goods from getting deteriorated. There is a vast source of aromatic compounds and essential oils, which are in high demand in the pharmaceutical and cosmetics industries in both domestic and international trade. They are recognized as one of the most functionally significant food ingredients since; they also have nutritional, antibacterial, antioxidant, and medicinal qualities. Therefore, efforts are to be focused on post harvest management of tree spices in view of minimizing their losses along with the upgrading the area, raising productivity, and enhancing tree spice quality.

Nutmeg-Fruits of nutmeg are harvested when they split open on ripening. Fruits of nutmeg ripen 6 to 9 months after blossoming. (Nazeem, 1979)

The split fruits are either plucked from the tree with a hook or collected soon after they drop onto the ground. Since this crop is harvested during rainy season in Kerala, sun drying is difficult resulting in improper drying of nutmeg and mace. Drying of medium to large quantities of nutmeg is done in drying rooms. The harvested nutmeg is spread on raised wire mesh floor and heated air is passed through pipes inside the drying room. Continuous drying of nutmeg is not recommended as the oil oozes out without proper drying. The local practice of drying nutmeg at Kalady, a prominent nutmeg growing area is to pass the hot air for one or two days (8 hours each) and then allow to dry at ambient conditions for one week. The heated air is again passed for a day or two and then dried for aweek or 10 days. The nutmegs are dried until the seeds inside rattle on shaking. This takes about 15 days or more. The seed cover is removed by breaking the hard seed coat manually or mechanically.Some of the nutmegs are dried improperly by this process have lot of fungal infections and the problems of aflatoxins are also reported by the exporters. Nutmeg samples dried in solar tunnel drier showed a higher reduction in drying time. It took about 8 hours for drying from an initial moisture content of 42.6% to 7.2%, while the conventional drying practice took about 13 days (Joy etal. 2000). Nutmeg is usually packed in double layered jute or polythene bags. If other packing materials are used, care must be taken to avoid materials, which might lead to 'sweating' and mould development. Powdered nutmeg is prepared by grinding at ambient temperature. Mace is detached from the nut carefully soon after harvest, washed, flattened by hand or between boards and then sun dried until they become brittle. Hot air ovens can be used for drying and the colour retention is much better than sun dried mace. Studies conducted at IISR, Calicut showed that blanching of mace in hot water at 75oC for two minutes preserved the qualities of mace during drying (Amaladhas etal., 2002). Dried mace is graded and packed.

Kokum- Fruit is mature when its colour changes from green to light green and from red to purplish red. Kokum fruit has a shelf life of 4-5 days when stored at room temperature. It can be extended to 15 days with application of waxol 12 percent and stored in a cool environment. Paddy straw and CFB boxes are the excellent kokum packaging materials. (Shirke and Pinjarkar, 2023)

Cinnamon-Cinnamon is harvested twice a year. Due to the high humidity during the monsoon makes it easier to peel the bark. It attains maturity after three years. The side stems are cut off by removing the bark. Bark with a diameter of between 1.2 and 5 cm is preferred for cinnamon. Cinnamon quills are dried in the shade on coir rope racks. The quills are rolled on a board to tighten the bark after 4-5 days of drying, and then placed in gentle sunshine to continue drying. Since, the weather is humid during the rainy season; a mechanical dryer is required to dry the cinnamon. Different dryers are used, which includes those are powered by electricity, gas, biomass, etc. Cinnamon quills are divided into pieces up to 10 cm long and placed in moisture-proof polypropylene bags before sold. Bag sealing is crucial to preventing moisture gain. Product labels must be visually appealing. The label should include all necessary product and legal information, including the product's name, brand, manufacturer's contact information (including name and address), date of manufacture, expiry date, weight of the contents, and any additional ingredients that may be needed (a barcode, producer code and packer code are all extra information that is required in some countries for traceability).

7. VALUE ADDITION OF TREE SPICES

Nowadays, many value-added spices are used and they impart a special taste to food preparations. Value addition has several plus points, viz. the value added products are simple to carry, having long-lasting flavours, with low bacterial contamination, having higher income from food industry, used as preservatives and also in pharmaceutical industry. Some prominent value-added products accredited globally are black pepper powder, pepper oleoresin, cardamom oil, curcumin, turmeric oleoresin, bleached ginger, garlic paste, onion powder, coriander oleoresin, etc. Big entrepreneurship to be

developed in large scale, and year round production of the value-added product for meeting the international demand is feasible (Mani and Kabiraj, 2019).

Some value-added products of tree spices

A. Ready-made spice powder and paste

Powdered spice is air tight packaging material is of enormous demand. Increasing urbanization paired with a rise in number of working women has reduced the time of cooking. Consequently, home-makers have started demanding readymade spice powder that includes chilli powder, cumin powder, fennel powder, black pepper powder, turmeric powder. Also popular are ready made paste of onion, garlic, ginger in packet form. An official report from Everest Spices Ltd. Reveals their exports about 10 per cent of its products to the US, West Asia, Singapore, Australia, New Zealand and East Africa, said: "The total market size of branded spices is estimated at 6,600 crore, and is growing at 14 per cent annually

B. Spices extractives.

Essential oil

Essential oils are major flavouring constituents of spices, highly concentrated about 75-100 times than the fresh spice.

Crop wise Value added products of major tree spices

A. Nutmeg and mace - Nutmeg oil and mace oil, Nutmeg oleoresin, Nutmeg butter are the main value added products. By utilizing nutmeg pericarp (rind), many value added products have been developed viz., Nutmeg (rind) pickle, Nutmeg(rind) preserve from slices, and Nutmeg (rind) preserve from shreds, Nutmeg (rind) candy, Nutmeg (rind) sweet chutney and Nutmeg (rind) powder.

B. Cinnamon - In addition to cinnamon bark, various other products are obtained from the tree namely, powder, quills, bark oil, leaf oil, bark oleoresin etc.

C. Clove - Clove oil, ground clove, oleoresins, clove-stem oil, clove-leaf oil, oil of mother of cloves and clove-root oil are some of the value-added products of clove.

D. Allspice - Pimenta berry oil, Pimenta leaf oil, Pimenta oleoresin, Pimenta bark and wood.

E. Kokum- Kokum Syrup, RTS, Amsul, seed oil, butter, agal etc.

8. Challenges and threats to Indian spice and its value added product export -

Production of spices pay important role in Indian economy. Following the challenges and threats to Indian spice and its value added product export:

i. Lack of planting material -

In India (other than south India), the availability of quality planting material is always an issue. Even though planting material is available they are not that superior in quality as that of the elite one. Hence the production is quite low.

ii. Small size of spice growing farms -

India is a nation with several small land holdings. This is the main reason why the production is scattered and hard to be computed and estimated in a single platform.

iii. Lack of technical skills -

In India, it is very tough to ensure that the spice growers in every corner are trained with the latest skills needed for optimum cultivation, management and processing of spice. In India, farming as a profession is accepted by the least educated section. Hence, the best technologies are hard to be transmitted at grass root level.

iv. Residual toxicity of chemicals-

Usually the developed qualities which are the potential market of spice and value-added products of spice are very much aware and concerned about residual toxicity in the food product they import. Quality of spice crops are ensured by proper PGR applications. Pest management seldom needs spraying of chemicals. These agro-chemicals have residual effects. Due to lack of proper organic practice and absence of Organic certification, export is limited to organic spices only.

v. Lack of value addition and quality upgradation –

Developed countries do import spices at a decent rate and extract the oil and oleoresins to obtain a huge margin of profit. Spices produced in India are rarely value added in India due to lack of proper platforms and little MNC/s intervention. They are imported and value added in different country. As soon as bigger companies and MNC's start investing on spice value added products, spice industry would show an upsurge.

vi. Adoption of improper post-harvest practices –

Some spice growers do not follow proper post-harvest practices for their spice crop. This includes right from the optimum stage of harvesting to proper handling, storage packaging, etc. Proper packaging is also necessary for ensuring optimum quality after harvest. Rough handling during interstitial stages can endure rottenness to tree spices. Improper drying of cinnamon and nutmeg could lead to loss of flavor and destruction of oil glands.

9. Impact of MIDH (Spices) for sustainable agriculture

The effective production technology must be coupled with valid dissemination technology. It helps to improve the impact of production technology. The initiate of Government of India through Mission for Integrated Development of Horticulture (MIDH) has created excellent impact as per as the spices production technology is concern.

Mission for Integrated Development of Horticulture (MIDH) is a Centrally Sponsored Scheme for the holistic growth of the horticulture sector and also covering spices. Under MIDH, Government of India (GOI) contributes 60%, of total outlay for developmental programmes in all the states except states in North East and Himalayas, 40% share is contributed by State Governments. In the case of North Eastern States and Himalayan States, GOI contributes 90%. MIDH also provides technical advice and administrative support to State Governments/ State Horticulture Missions (SHMs) for the Saffron Mission and other horticulture related activities Rashtriya Krishi Vikas Yojana (RKVY)/NMSA.

Main objectives of the Mission for Integrated Development of Horticulture (MIDH) are: a) Promote holistic growth of horticulture sector, including bamboo and coconut through area based regionally differentiated strategies, which includes research, technology promotion, extension, post harvest management, processing and marketing, in consonance with comparative advantage of each State/region and its diverse agro-climatic features; b) Encourage aggregation of farmers into farmer groups like FIGs/FPOs and FPCs to bring economy of scale and scope. c) Enhance horticulture production, augment farmers, income and strengthen nutritional security; d) Improve productivity by way of quality germplasm, planting material and water use efficiency through Micro Irrigation. e) Support skill development and create employment generation opportunities for rural youth in horticulture and post harvest management, especially in the cold chain sector. (Operational guidelines, Horticulture Division Department of Agriculture & Cooperation Ministry of Agriculture Krishi Bhavan, New Delhi. www.midh.gov.in, April, 2014).

MIDH (Spices) at Dr. BSKKV, Dapoli

In 2014, the MIDH (Spices) scheme under Directorate of Arecanut and Spices Development, Calicut was established at Department of Horticulture under the aegis of Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli. The objective of scheme was to produce genuine and quality planting material of tree spices recommended by university, establishment of nursery centre, transfer of technology through farmer training programme, seminars and front line demonstrations in entire Konkan region. There are 7 accredited and 15 implementing centers under the jurisdiction of Dr. BSKKV, Dapoli which are participating to fulfill these objectives,.

Accordingly the following tree spices planting material was distributed among the farmers.

Table 13. Tree spices planting material distributed by MIDH (Spices), DBSKKV, Dapoli

Sr. No.	Name of tree spices	Planting material	Estimated area covered (ha)
1	Nutmeg grafts	36,000	202
2	Cinnamon layers	57,000	8
3	Kokum grafts	96,000	345
	Total	1,89,000	555

About 2515 farmers have been trained for various technologies on package of practices like propagation techniques, nutrient management, integrated pest and disease management, harvesting techniques and value addition on tree spices through above centers of Dr. BSKKV, Dapoli in entire Konkan region. Also, new initiatives like participatory demonstration of cinnamon on farmers' field has initiated through this scheme to develop cinnamon tree spice village. Therefore, this scheme has help the farmers for area expansion of tree spices like nutmeg, cinnamon and kokum in entire Konkan region about 1.62 lakh ha with 40 lakh MT (fnbnews.com, 2023) and many entrepreneurs, nursery growers and processors on small scale has established.

The area under tree spices in Kokan region is expanded due to distribution of quality planting material, transfer of technology, farmers training. The mixed cropping of tree spices resulted in double the farmers income which resulted in sustainability of the coconut and arecanut growers. The tree spices also gaining the popularity through agro tourism. Similarly; the tourist from the city area are demanding more and more spices grown in Konkan region. New varieties, advanced propagation and production technologies, expansion in area, mixed intercrops of tree spices, MIDH initiatives, agro-tourism aspects are fruitful in to rural employment which diverted the flow of youth towards sustainable agriculture. The new variety, propagation techniques, mixed cropping, lakhibaug model, post harvest handling, value added products and MIDH is privilege for tree spices.

References

Amaladhas H P, Rajesh P & Shinoj Subramanian 2002 Get better quality mace by blanching, Spice India, 15(2): 8-10.

Anonymous 2005. A final report of coconut-based high density multi-species cropping system. Regional Coconut Research Station, Bhatye, Ratnagiri, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India, 8-9.

Arghya Mani and Arpan Kabiraj, 2019. Export potential of spices and its value added products. AGRICULTURE & FOOD: e- Newsletter. Volume 1 (8).

Haldankar P. M., Nagwekar D. D. and Desai A. G. 1997. Studies on softwood grafting in nutmeg. Indian J. Cocoa, Arecanut and Spices. 2: 23.

Haldankar, P. M. & Lawande, K.E. and Parulekar, Y. R. (2013). Tree spices-Problems and prospects. SYMSAC VII Post-Harvest Processing of Spices and Fruit Crops.

Haldankar, P.M., Nagwekar, D.D., Desai, AG and Rajput J. C. 1999. Factors influencing epicotly grafting in nutmeg. Indian Journal of arecanut spices and medicinal plants. 21: 940-944.

Joy C M, George Peter Pittappillil & Jose K P 2000 Quality improvement of nutmeg using solar tunnel dryer, J. Plantn. Crops 28(2): 138-143.

Nagwekar D. D., Haldankar P. M., Arulraj S, Maheshwarappa H. P. 2014. Lakhi baug for realizing maximum income from coconut. Indian Coconut J. 57: 23-26.

Nazeem PA. 1979. Studies on growth, flowering, fruit set and fruit development in nutmeg. MSc. (Hort.) Thesis Kerala Agricultural University, Thrissur. p. 136.

Nissar V. A., Muhammed & Bhas, Sasikumar & Sounderarajan, Aarthi & Rema, Rema. (2019). Air layering in nutmeg (Myristica fragrans Houtt.). Journal of Spices and Aromatic Crops. 28. 66–69.

Patil J. L., Haldankar P. M., Jamdagni B. M. and Salvi M. J. 1991. Influence of intercropping with tree spices on yield of coconut (Cocos nucifea L.). Indian Coconut J. 22: 15-18.

Sanu Varghese and Mathew Jose K. 2019. Nutmeg-'The Twin Spice': An Overview. Journal of Emerging Technologies and Innovative Research. 6 (6): 37-46.

Shirke G. D. and Pinjarkar M. S. 2023. Post-harvest technology of tree spices. Journal of Pharmacognosy and Phytochemistry. 12(2): 88-102.

Received: 01st October 2023 Accepted: 20th October 2023

Journal of the Andaman Science Association Vol. 28(2):158-165 (2023)
ISSN 0970-4183, Printed in India

Studies on electrocardiographic changes in xylazine-butorphanol and dexmedetomidine-butorphanol premedicated goats with tiletamine-zolazepam induction and total intravenous anaesthesia (TIVA) and partial intravenous anaesthesia (PIVA) protocol

Sanjaykumar Vithalrao Udharwar[1], John Martin K. D.[2], Aziz Zarina[3], Sudheesh S. Nair[4], S. Anoop[5], C. Jayakumar[6] and Syam K. Venugopal[7]

[1]Ph. D. Scholar, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, KVASU, Mannuthy, Thrissur, Kerala-680651.
[2]Professor and Head, University Veterinary Hospital, KVASU, Kokkalai, Thrissur, Kerala- 680021.
[3]Assistant Professor, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, KVASU, Mannuthy, Thrissur, Kerala-680651.
[4]Assistant Professor, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala-673576.
[5]Professor and Head, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala-673576.
[6]Associate Professor and Head, Department of Animal Reproduction, Gynecology and Obstetrics, College of Veterinary and Animal Sciences, KVASU, Mannuthy, Thrissur, Kerala- 680651.
[7]Professor and Head, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, KVASU, Mannuthy, Thrissur, Kerala-680651.

Abstract

The present study was conducted to evaluate electrocardiographic changes of xylazine, dexmedetomidine and their combination with butorphanol in tiletamine-zolazepam induced goats with isoflurane and tiletamine-zolazepam maintenance, which underwent various surgeries. The electrocardiograms were recorded in a standard base apex lead system. Twenty- four goats were studied using four distinct anaesthetic regimens, with six goats in each group. Anaesthetic protocols produced transient changes in the electrocardiographic indices. The electrocardiographic abnormalities in goats premedicated with xylazine and butorphanol after induction included first degree heart block in one animal and modest T-wave changes in a few animals. All of these changes were corrected when the animals recovered from anaesthesia. Goats premedicated with dexmedetomidine-butorphanol showed little electrocardiographic changes after being induced; only a few transitory T-wave abnormalities were seen, and no additional abnormalities could be determined. The P-wave, QRS, and T-wave changes in the electrocardiographic indices during the anaesthetic study, i.e., before premedication, post-induction, and post-recovery were transient and within normal physiological limits. Thus, premedication with dexmedetomidine-butorphanol or xylazine-butorphanol in lower doses in a multimodal balanced anaesthetic approach with tiletamine-zolazepam induction, followed by tiletamine-zolazepam maintenance with CRI or isoflurane maintenance in goats for various surgeries was safe.

Key words: *Electrocardiograph, premedicated goat, xylazine-butorphanol, dexmedetomidine-butorphanol*

Introduction

Electrocardiography is a diagnostic procedure that records the electrical activity of the heart over time. It also identifies irregularities in cardiac rhythm. Several researchers have employed the bipolar base apex lead using limb lead I in goats and it has been demonstrated to be a suitable lead since the ECGs they recorded had clear waves and complexes. Animal movement has a minimal impact on the recording (Rezakhani *et al*., 2004).

In the present study, goats were premedicated with xylazine, dexmedetomidine and butorphanol, while tiletamine-zolazepam and isoflurane were used as anaesthetic agents. Various researchers reported electrocardiographic changes/abnormalities when

xylazine and dexmedetomidine were administered in different species. Electrocardiographic changes like increased PR and QT intervals after xylazine administration (Kinjavdekar *et al.*, 1999), lengthened RR, QT, and QRS complexes after dexmedetomidine administration (Kumari *et al.*, 2017) and depression of the T-wave, biphasic T-wave and spike of the T-wave after tiletamine- zolazepam and xylazine anaesthesia (Rajankutty, 1995) were among them.

Abalos *et al.* (2016) reported ECG abnormalities such as atrial fibrillation, ventricular premature contraction, premature P waves, ventricular tachycardia, sinoatrial block, atrioventricular block, and left bundle branch block in tiletamine-zolazepam and xylazine treated goats. The authors also reported that lower doses of tiletamine and zolazepam caused lesser ECG abnormalities and arrhythmogenic effects.

However, there are few reports comparing the electrocardiographic changes in anaesthetised goats with multimodal protocols including PIVA and TIVA . Hence, this study was conducted to assess the electrocardiographic effects of various drug combinations, including dexmedetomidine-butorphanol-tiletamine-zolazepam-tiletamine- zolazepam (D-B-TZ-TZ), xylazine-butorphanol-tiletamine-zolazepam-tiletamine-zolazepam (X-B-TZ-TZ), dexmedetomidine-butorphanol-tiletamine-zolazepam-isoflurane (D-B-TZ-ISO) and xylazine-butorphanol-tiletamine-zolazepam-isoflurane (X-B-TZ-ISO) in goats undergoing various surgical procedures.

Materials and Methods

The study was conducted in 24 goats reported with various surgical affections like abdominal hernia, overgrown and pointing horns, fracture of long bones, gangrenous mastitis, etc. at Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai, Thrissur of Kerala Veterinary and Animal Sciences University, Kerala and selected for respective surgeries under general anaesthesia.

Experimental design

The animals were randomly divided into four groups that are *viz.*, Group-I (X-B-TZ-TZ), Group-II (D-B-TZ-TZ), Group-III (X-B-TZ-Iso) and Group-IV (D-B-TZ-Iso) based on the anaesthetic agents used, each group consisting of six animals. All the animals fasted for 18 hours, and water was withheld for eight hours before the anaesthesia. The clinical status of the animals was assessed by recording heart rate, respiration rate, rectal temperature, electrocardiographic study and by conducting haematological and biochemical examinations.

For the premedication, Group I and Group III animals received a combination of xylazine intravenously at the dose rate of 0.02 mg/kg body weight and butorphanol at the dose rate of 0.05 mg/kg body weight. Group II and Group IV received a combination of dexmedetomidine intravenously at the dose rate of 2.5 µg/kg body weight and butorphanol at the dose rate of 0.05 mg/kg body weight as premedication. In all the animals, induction was done with tiletamine-zolazepam at the dose rate of 2.5mg/ kg body weight intravenously. In Group I and Group II, anaesthesia was maintained with tiletamine-zolazepam as a continuous rate of infusion (CRI) at the dose rate of 2.5mg/ kg/h. While in Group III and Group IV, animals were maintained with isoflurane in oxygen.

Electrocardiography (ECG) was recorded before premedication, 10 minutes after induction of general anaesthesia and immediately after recovery from anaesthesia using a Cardiart (6108T) ECG machine (BPL Medical Technologies). The ECG was recorded on a bipolar base apex lead using limb lead I. Animals were kept in a standing position while taking ECG before premedication and held in lateral recumbency while taking ECG after 10 minutes of induction and post-recovery. The alligator type electrodes were attached to the skin after applying ECG gel. The positive electrode of lead I (left arm) was attached to the skin at the point of the elbow. The negative electrode (right arm) was attached to the skin on the jugular furrow about the lower one-third on either side of the neck. The earthing electrode was attached away from these two electrodes. All the ECGs were obtained on a single channel ECG machine with a paper speed of 25mm/s and calibration of 10mm equal to 1 mV. A magnifying glass was used to measure the ECG traces (Varshney, 2020).

Results and Discussion

Due to the deep penetration of the Purkinje fibres in the myocardium of ruminants, the ECG in these species is primarily used to identify cardiac arrhythmias rather than cardiac chamber enlargement. Since a base apex lead possesses the characteristic electrocardiographic waves and complexes required for this task, it appears to be the best and most widely used lead for monitoring goats for cardiac arrhythmias (Rezakhani *et al.,* 2004).

In the study, the P wave was consistently positive in all ECG traces with no variation. The QRS complex was primarily negative. The T wave could have been positive, negative, or biphasic. The T wave was more variable in ruminants than in canines. Hence, it cannot be utilised as a marker for cardiac issues (Rezakhani *et al.*, 2004).

Analysis of morphologies of P, QRS, and T (Tables 1 and 2)

Table 1: ECG parameters showing P (mV). P (sec), QRS (mV), QRS (s) and T (mV)

Groups	Time points	P (mV)	P (s)	QRS (mV)	QRS (s)	T (mV)
Group I X-B-TZ-TZ	Before premedication	0.113 ± 0.009	0.043 ± 0.002	0.472 ± 0.03	0.052 ± 0.004	0.263 ± 0.043
	After induction	0.100 ± 0	0.046 ± 0.002	0.487 ± 0.046	0.058 ± 0.005	0.344 ± 0.081
	At recovery	0.105 ± 0.005	0.055 ± 0.006	0.515 ± 0.054	0.052 ± 0.004	0.385 ± 0.103
Group II D-B-TZ-TZ	Before premedication	0.097 ± 0.003	0.048 ± 0.007	0.567 ± 0.192	0.073 ± 0.018	0.233 ± 0.054
	After induction	0.100 ± 0	0.048 ± 0.007	0.658 ± 0.169	0.067 ± 0.007	0.258 ± 0.051
	At recovery	0.100 ± 0	0.048 ± 0.007	0.658 ± 0.171	0.062 ± 0.007	0.267 ± 0.054
Group III X-B-TZ-Iso	Before premedication	0.130 ± 0.016	0.052 ± 0.007	0.555 ± 0.044	0.06 ± 0.005	0.18 ± 0.031
	After induction	0.116 ± 0.017	0.046 ± 0.003	0.52 ± 0.08	0.06 ± 0.007	0.24 ± 0.042
	At recovery	0.132 ± 0.02	0.060 ± 0.005	0.59 ± 0.093	0.06 ± 0.005	0.21 ± 0.033
Group IV D-B-TZ-Iso	Before premedication	0.108 ± 0.008	0.048 ± 0.004	0.542 ± 0.125	0.075 ± 0.018	0.258 ± 0.071
	After induction	0.113 ± 0.018	0.053 ± 0.006	0.7 ± 0.167	0.063 ± 0.008	0.288 ± 0.048
	At recovery	0.1 ± 0	0.041 ± 0.001	0.667 ± 0.117	0.065 ± 0.007	0.258 ± 0.049
Normal Reference Range Varshney, (2020)		0.1-0.2	0.04-0.06	0.40-1.4	0.06-0.1	0.10-0.70

Table 2: ECG parameters showing T (s) PR (s), T(s), ST (s), QT (s) and HR (bpm)

Groups	Time points	T (s)	PR (s)	ST (s)	QT (s)	Heart rate
Group I X-B-TZ-TZ	Before premedication	0.072 ± 0.005	0.117 ± 0.003	0.173 ± 0.027[b]	0.391 ± 0.016[b]	107.31 ± 13.26
	After induction	0.075 ± 0.005	0.135 ± 0.008	0.228 ± 0.019[a]	0.472 ± 0.032[a]	78.89 ± 2.59
	At recovery	0.087 ± 0.011	0.125 ± 0.007	0.212 ± 0.014[ab]	0.484 ± 0.036[a]	90.27 ± 7.73
Group I D-B-TZ-TZ	Before premedication	0.122 ± 0.033	0.153 ± 0.019	0.153 ± 0.012	0.407 ± 0.036[b]	82.79 ± 7.55
	After induction	0.13 ± 0.037	0.167 ± 0.024	0.18 ± 0.027	0.480 ± 0.043[a]	74.5 ± 4.88
	At recovery	0.131 ± 0.043	0.173 ± 0.03	0.2 ± 0.031	0.487 ± 0.019[a]	76.72 ± 7.54
Group III X-B-TZ-Iso	Before premedication	0.06 ± 0.007	0.104 ± 0.008[b]	0.160 ± 0.018[b]	0.384 ± 0.030[b]	115.83 ± 12.66
	After induction	0.08 ± 0.01	0.128 ± 0.007[a]	0.208 ± 0.019[a]	0.472 ± 0.016[a]	81.92 ± 3.96
	At recovery	0.084 ± 0.013	0.112 ± 0.007[a]	0.192 ± 0.016[a]	0.424 ± 0.030[ab]	105.63 ± 8.38
Group IV D-B-TZ-Iso	Before premedication	0.077 ± 0.013	0.133 ± 0.013	0.193 ± 0.019[b]	0.407 ± 0.019[b]	87.01 ± 4.21
	After induction	0.09 ± 0.009	0.147 ± 0.008	0.220 ± 0.031[b]	0.493 ± 0.042[a]	80.21 ± 3.86
	At recovery	0.072 ± 0.009	0.153 ± 0.016	0.260 ± 0.031[a]	0.500 ± 0.027[a]	79.81 ± 6.51
Normal Reference Range Varshney, (2020)		0.07-0.16	0.07-0.16	0.16-0.4	0.22-0.56	60-130

The mean values of the P wave amplitude (mV) and P wave duration (s) did not differ significantly (P□0.05) within groups as well as between the groups at various time points, i.e., before premedication, post-induction, and post-recovery. They fluctuated within the normal physiological limits, 0.1-0.2 mV and 0.04-0.06 s, respectively (Varshney, 2020). The mean values of the QRS amplitude (mV) and duration (s) did not differ significantly within groups as well as between the groups at various time points, i.e., before premedication, post-induction, and post-recovery. The variations in QRS amplitude (mV) and duration (sec) observed in all the treatments were within the normal physiological limits (0.40- 1.4 mV and 0.06-0.10 s).

The mean values of the T amplitude (mV) and T duration (s) did not differ significantly within groups as well as between the groups at various time points. The variations in T amplitude (mV) and T duration observed in all the treatments were within the normal physiological limits (0.10-0.70mV and 0.07-0.16 s) (Varshney, 2020).

The P wave, QRS complex and T wave during the anaesthetic study did not change, indicating that xylazine, dexmedetomidine, butorphanol, tiletamine-zolazepam and isoflurane treatment in multimodal anaesthetic approach had no impact on atrial depolarisation, ventricular depolarisation or ventricular repolarisation. Similar findings were reported by Hamed *et al.* (2015),

who studied dexmedetomidine in goats and Sarchahi *et al.* (2009), who studied xylazine in dogs.

The mean values of the heart rate (HR) (beats / min) did not differ significantly within and between groups at various time points. A non-significant decreasing trend in heart rate after tiletamine-zolazepam induction was found in all groups. Half of the study animals were premedicated with xylazine- butorphanol and half were given dexmedetomidine-butorphanol. The reduction in heart rate in xylazine and dexmedetomidine premedicated goats could be attributed to increased vagal tone and decreased sympathetic outflow from the central nervous system (CNS). Similar findings were reported by Kinjavdekar *et al.* (1999) in goats, by Singh *et al.* (2005) in calves after administration of xylazine, and by Kumari *et al.* (2017) in goats after administration with dexmedetomidine, respectively. However, at the time of recovery, heart rate was showing an increasing trend as tiletamine-zolazepam indirectly inhibits the neuronal re-uptake of catecholamines, especially norepinephrine; thereby, increasing heart rate (Pugh and Baird, 2020). All the fluctuations in the heart rate at various observation points were within normal physiological limits (Varshney, 2020).

Mean values of the PR interval (mV) did not differ significantly between the groups at various time points. PR interval was non-significantly increased after 10 minutes of induction in Group I (X-B-TZ- TZ), II (D-B-TZ-TZ), and IV (D-B-TZ-Iso). In contrast, a significant increase was noticed in Group III (X-B-TZ-Iso) after induction. The values that fluctuated during the study were within normal physiological limits (Varshney, 2020). The study found that the heart rate was reduced after induction and simultaneously the PR interval increased as HR and PR intervals are inversely proportional. Similar findings were reported by Filippi (2011) in canines.

The mean values of S-T segment (sec) did not differ significantly between the groups at various time points. There was a significant increase in the value in Groups I, III, and IV, but a non-significant increase was noticed in Group II. The variations in S-T segment values observed in all the treatments were within the normal physiological limits. A similar finding of an increase in the S-T segment was reported by Peshin *et al.* (1979) in dogs following the administration of xylazine.

The mean values of the Q-T interval (sec) did not differ significantly between the groups at various time points. A significant increase in the Q-T interval after 10 min of induction was noticed in all the groups. There was no significant difference found in the Q-T interval noticed at 10 min after induction and recovery. Still, all the variations in the Q-T interval were within normal physiological limits (0.22-0.56 sec) as reported by Varshney (2020). Similar findings were reported by Hamed *et al.* (2015) after the administration of dexmedetomidine in goats.

Electrocardiographic alterations occurred in ECG traces during the study

After induction of anaesthesia, the following electrocardiographic alterations were observed in different groups and were as follows:

In Group I, sinus rhythm was found in all the animals. T wave alterations like tall T were noticed in three animals, biphasic T in one animal, and reverse T wave in one animal. The deviation and inconsistency of the T wave might be a result of transient changes in the acid-base balance due to carbon dioxide retention (Peshin and Kumar 1979).

Changes in the polarity of the T wave and an increase in the amplitude of the T wave and inverted T wave noticed might be due to the anesthetic induced myocardial hypoxia (Tilley,1985), similar findings were noticed by Rajankutty (1995) in dogs after the administration of xylazine-tiletamine-zolazepam.

In Group II, sinus rhythm was found in all the animals after induction. T wave alterations like tall T were noticed in two animals, biphasic T in two animals, notched T in one animal and reverse T wave in one animal. All the changes in the T wave are in accordance with the studies reported by (Tilley 1985) and Rajankutty (1995) and might be due to the anesthesia induced myocardial hypoxia. All the changes in the T wave became normal at the time of recovery. At the time of recovery, T wave changes like reverse T, notched T, and biphasic T were noticed in one animal each and such changes in T wave were recorded

by Rajankutty (1995) and found to be normalised after 24 hours of the observation period in dogs administered with tiletamine-zolazepam.

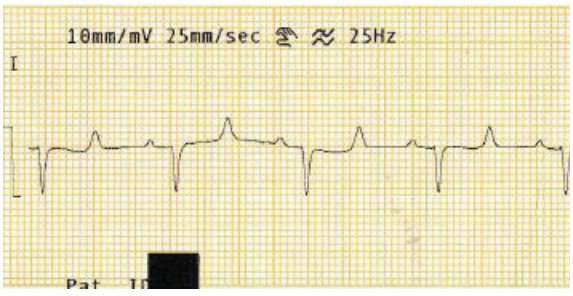
In Group III, sinus rhythm was found in all the animals after induction. Any other alterations were not noticed during induction and recovery except for the first-degree atrioventricular block in one animal, as the PR interval was found to be 0.24 seconds (more than 0.16 seconds) after induction, and the same was continued till recovery. Similar findings were recorded by Abalos *et al.* (2016) after the administration of tiletamine-zolazepam in goats. Similar findings of ECG alterations, first-degree heart block/atrioventricular block, was reported by Ramanakutty (2008) in dog after premedication with xylazine.

In Group IV, sinus rhythm was found in all the animals except for a few changes in the T wave after induction. ECG traces remained without any other alterations at all observations. T wave alterations like tall T were observed in two animals. In contrast, biphasic T was noticed in one animal, notched T was observed in one animal and reverse T wave was noticed in one animal. Carvalho *et al.* (2019) found similar T-wave changes after the administration of dexmedetomidine in cats, Rajankutty (1995) also reported similar changes after the administration of tiletamine zolazepam with xylazine in dogs. At the time of recovery, no changes were noticed in the ECG.

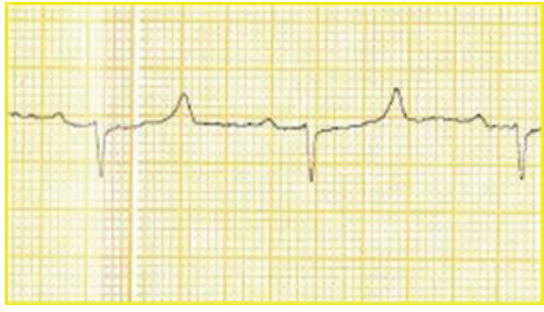
Conclusion

All anaesthetic combinations were safe in goats, producing only temporary and transient changes in the electrocardiographic indices. The recording of ECG before pre-medication and ECG changes after 10 minutes of induction and recovery helped to identify the exact nature of rhythm and heart rate under different anaesthetic protocols. Goats that had been premedicated with dexmedetomidine-butorphanol showed little electrocardiographic changes after being induced with tiletamine-zolazepam, only a few transitory T-wave abnormalities were seen, and no additional abnormalities could be determined. The results of the ECG recording indicated that life threatening myocardial abnormalities were absent with all four anaesthetic regimens under study.

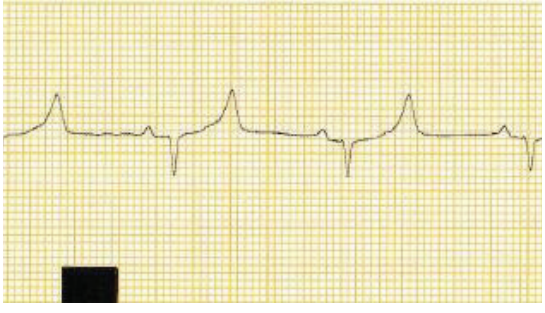
Plate 10. Electrocardiogram



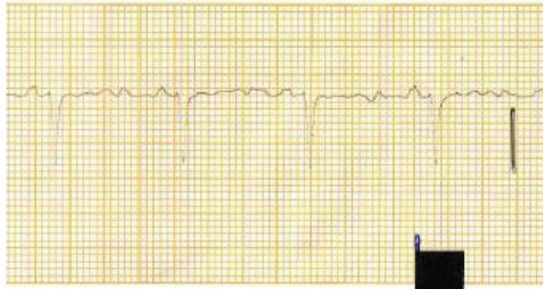
Normal electrocardiogram



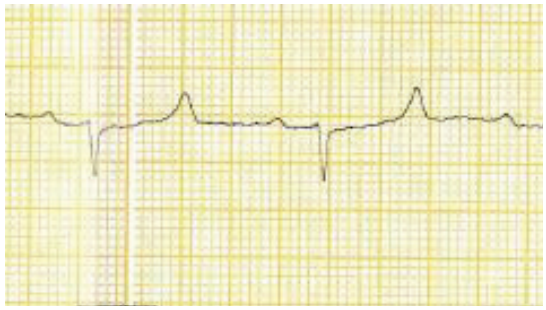
E: Increase in QT Interval (X-B-TZ-TZ)



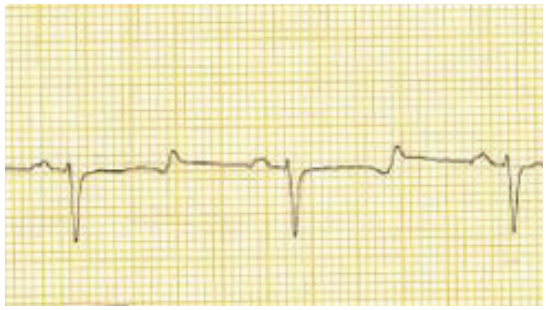
B: Tall T wave (X-B-TZ-TZ)



C: Reverse T wave (D-B-TZ-TZ)



F: 1st-degree heart block- (X-B-TZ-Iso)



G: Biphasic T wave (D-B-TZ-Iso)

Authors' declaration of interests: No conflicts of interest have been declared.

Source of funding: Ph. D. Research Fund by Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.

Acknowledgement

The authors are highly grateful to The Dean, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, for providing the necessary facilities and encouragement to conduct this study.

References

Abalos, J. H. A., Gicana, K. R. B., Brobio, E. F. and Addatu, M. J. B. 2016. Anaesthetic and cardiopulmonary effects of intravenous tiletamine-zolazepam with or without xylazine in goats (*Capra hircus*). *P. J. V. M.* 2: 118-125.

Carvalho, E.R., Champion, T., Vilani, R.G.O.C., Freitas, G.C., Ambrosini, F., Silva, G.A., Gonçalves, K.S. and Fischborn, J.C.J., 2019. Sedative and electrocardiographic effects of low dose dexmedetomidine in healthy cats. *Pesq. Vet. Bras.* 39: 142-147.

Hamed, M., Rizk, A., Mosbah, E., Abonorg, M., and Karrouf, G. (2015). Evaluation of different anesthetic drugs combination for pain management in goats undergoing tube cystostomy. *G Vet*, *14*(2), 251-261

Hugar, B., Gupta, O.P., and Singh, G. R. (1998). A note on the effects of medetomidine with and without ketamine in goats. Indian Vet. Medical J. 22: 139-140.

Kinjavdekar, P., Singh, G.R., Amarpal, Pawde, A.M. and Aithal, H. P. (1999). Effects of subarachnoid xylazine and medetomidine on haemodynamics and ECG in goats. *J. Vet. Med. A*. 46: 271-5. doi: 10.1046/j.1439-0442.1999.00215. x. PMID: 10445001.

Kumari, L., Sharma, A. K., and Sinha, M. P. 2017. Haemodynamic and Electrocardiographic Changes Following Epidural Ropivacaine with or without Dexmedetomidine in Black Bengal Goat. *J. Anim. Res.* 7: 489-493.

Peshin P.K. and Kumar, A. 1979. Physiological and sedative effects of xylazine in buffaloes. *Indian Vet. J.* 56: 864-871.

Pugh, D. G and Baird, A. N. 2020. Sheep, goat and cervid medicine, 3rd Edn. Elsevier publication, United States. pp: 461-478. https://doi.org/10.1016/C2017-0-02021-9

Rajankutty, K. 1995. General anaesthesia in dogs with tiletamine-zolazepam. Ph. D. Thesis submitted to Kerala Agricultural University, Thrissur, Kerala.

Ramankutty, S. 2008. Clinical evaluation of propofol-isoflurane anaesthesia with xylazine premedication in dogs. M. V. Sc Thesis submitted to Kerala Agricultural University, Thrissur, Kerala.

Rezakhani, A, Paphan, A. A. and Shekarfroush, S. 2004. Analysis of base apex lead electrocardiograms of normal dairy cows. *Vet. Arhiv.* 74: 351-358.

Sarchahi, A.A., Vesal, N., Nikahval, B. and Karampour, A. 2009. Comparison of the effects of different doses

of acepromazine-xylazine on the electrocardiogram in dogs. *Iran J. Vet. Res.* 10: 208-215.

Singh, P., Pratap, K., Amarpal, Kinjavdekar, P., Aithal, H. P. and Singh, G. R. 2005. Haemodynamic and electrocardiographic effects of xylazine, ketamine, lidocaine and their combinations after lumbar epidural administration in healthy buffalo calves. *J. App. Ani. Res*.28: 101-106. DOI: 10.1080/09712119.2005.9706801

Tilley, L. P. (1985) Essentials of canine and feline electrocardiography. 2nd Edn. Lea and Febiger, Philadelphia.

Varshney, J. P. 2020. Electrocardiography in veterinary medicine, 1st Edn., Springer Nature Publication, Singapore. pp:291. http://doi.org/10.1007/978-981-15-3699-1.

Venugopalan C.S., Holmes, E.P. Fucci, V., Keefe, T. J. and Crawford, M. P. (1994). Cardiopulmonary effects of medetomidine in heartworm infected and noninfected dogs. *Amer. J. Vet. Res*. 55:1148-1152.

Received: 31st August 2023 Accepted: 20th September 2023

Journal of the Andaman Science Association Vol. 28(2):166-172 (2023)
ISSN 0970-4183, Printed in India

Comparative Evaluation of the Isoflurane-sparing Effects of Premedication with Dexmedetomidine-Butorphanol and Xylazine-Butorphanol in Tiletamine-Zolazepam induced anaethesia in Goats

Sanjaykumar Vithalrao Udharwar[1], John Martin K. D.[2], Sudheesh S. Nair[3], S. Anoop[4], C. Jayakumar [5], Aziz Zarina[6] , Senthilkumar, S[7], and Syam K. Venugopal[8]

[1]Ph. D. Scholar, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, KVASU, Wayanad, Kerala
[2]Professor and Head, University Veterinary Hospital, Kokkalai, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, KVASU, Wayanad, Kerala
[3] Assistant Professor, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Pookode, Thrissur, KVASU, Wayanad Kerala
[4]Associate Professor and Head, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Pookode, Thrissur, KVASU, Wayanad Kerala
[5]Assistant Professor, Department of Animal Reproduction, Gynecology and Obstetrics, College of Veterinary and Animal Sciences, Pookode, Thrissur, KVASU, Wayanad Kerala
[6]Associate Professor and Head, Department of Veterinary Surgery and Radiology, Veterinary College and Research Institute, Salem, TANUVAS, Tamil Nadu
[7]Professor and Head, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, KVASU, Wayanad, Kerala

Abstract

Isoflurane is frequently used as an inhalant anaesthetic in small ruminants. The drug isoflurane has several adverse side effects, including respiratory depression, hypotension and diminished cardiac output. Additionally, its metabolites pollute the atmosphere. Premedicating goats with an alpha-2 agonist, such as xylazine, is a long-standing practice. Some negative consequences are associated with xylazine and each negative impact is dose-dependent. Additive effects are produced when alpha-2 agonists and opioids are combined so that the dose of the latter can be reduced.

Due to its high price and relatively new introduction dexmedetomidine, although it had advantages over xylazine. In this study, goats were premedicated with xylazine-butorphanol in one group and dexmedetomidine-butorphanol in another group. Anaesthesia was then induced with tiletamine-zolazepam and maintained by isoflurane. It was planned with the hypothesis that a combination of dexmedetomidine along with butorphanol in the partial intravenous anaesthesia protocol might have an isoflurane-sparing effect than xylazine-butorphanol premedication.

Key words: *Anaesthesia, goat*

Introduction

Goats are becoming more significant because they are used as surgical models for a range of biomedical research studies and because they are becoming popular as expensive meat-producing animals. This has led to increased interest in surgeries on goats and a corresponding demand for an effective anaesthetic protocol.

Premedication with alpha-2 agonist medications like xylazine with or without opioids, induction with injectable anaesthetics and inhalant anaesthetic maintenance with isoflurane are frequently used to induce balanced anaesthesia in goats.

The dose for the principal drug, i.e., inhalant anaesthetics, can be reduced in balanced anaesthesia protocols by the sparing action of lesser important drugs such as opioids/sedatives.

Isoflurane alone is insufficient to completely eliminate the autonomic and nociceptive responses to the surgical stimuli, which can result in inadequate analgesia during surgery. (Steffey and Mama, 2007). The

usual adverse effects of isoflurane, such as respiratory depression, hypotension and decreased cardiac output, must be minimised by reducing the amount required to maintain general anaesthesia (Hikasa *et al.*, 2000).

Isoflurane is a chlorofluorocarbon substance that might be harmful to the ozone layer of the earth and might cause global warming. Isoflurane and its metabolic byproducts pollute the atmosphere and damage it (Joubert, 1999). Therefore, any decrease in isoflurane dosage will benefit the patient, surgical team and environment.

Due to their strong analgesic impact, opioid analgesics are said to have an inhalant-sparing effect and minimise the minimum alveolar concentration (MAC) needed to maintain a surgical plane of anaesthesia (Tranquilli *et al.*, 2007). There are reports that including butorphanol in the anaesthetic protocol reduced the isoflurane requirement in goats (Kumar *et al.*, 2013) and cattle (Senthilkumar *et al.*, 2013).

Over the past few years, alpha-2 agonists have gained popularity in balanced anaesthetic approaches, mostly due to their ability to lower the MAC of volatile anaesthetic agents and support postoperative analgesia. According to Poppel *et al.* (2015), a xylazine infusion during isoflurane anaesthesia lowered the quantity of isoflurane in horses by more than 45%. Singh *et al.* (2013) reported greater isoflurane (minimum alveolar concentration) sparing effect of dexmedetomidine in comparison to xylazine and medetomidine in buffaloes.

However, there is a scarcity of literature showing the sparing effect of the combination of alpha-2 agonists and opioids on isoflurane in goats until now. Due to the possibility that premedication with a dexmedetomidine-butorphanol combination could spare the isoflurane, it was decided to compare the isoflurane-sparing effects of this combination to that of xylazine-butorphanol in goats induced with tiletamine-zolazepam and undergoing various surgical procedures.

Materials and Methods

The study was conducted on 12 goats reported with various surgical affections like abdominal hernia, overgrown and pointing horns, fracture of long bones, gangrenous mastitis, etc., at Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai, Thrissur of Kerala Veterinary and Animal Sciences University and selected for respective surgeries under general anaesthesia. The clinical status of the animals was assessed by recording heart rate, respiration rate, rectal temperature, electrocardiographic study and by conducting haematological and biochemical examinations.

Experimental design

The animals were randomly divided into groups viz., Group I and II, each group comprising six animals. All the animals fasted for 18 hours, and water was withheld for eight hours before the anaesthesia. The age in months and body weight in kilograms of selected animals were recorded. Detailed physical and clinical examination was performed, and the clinical status of the animals was assessed.

The animals of both groups were premedicated with two different combinations of drugs. Group I (X-B-TZ-Iso) received a combination of xylazine intravenously, at the dose rate of 0.02 mg/kg body weight and butorphanol at the dose rate of 0.05 mg/kg body weight intravenously as premedicants.

Group II (D-B-TZ-Iso) received a combination of dexmedetomidine intravenously, at the dose rate of 2.5 µg/kg body weight and butorphanol at the dose rate of 0.05 mg/kg body weight intravenously as premedicants. In all the animals, induction was done with tiletamine-zolazepam at the dose rate of 2.5mg/ kg body weight IV.

Upon achieving adequate muscle relaxation, the trachea was intubated with a cuffed endotracheal tube of suitable size and was connected to the small animal anaesthesia machine. For maintenance of anaesthesia, isoflurane was administered through an agent-specific vaporiser along with oxygen using a semi-closed rebreathing system. Oxygen 100 per cent along with isoflurane at three per cent was given with a fresh gas flow rate of 3 L/ min for the first three minutes to achieve saturation of the breathing circuit with isoflurane vapours

(Vishnuguruaran *et al.,* 2016). The fresh gas flow rate was then reduced and maintained between 1-2 L/min. The vaporiser setting was altered between zero and three percent during anaesthesia to maintain a uniform surgical plane of anaesthesia, observing the reflexes such as palpebral, swallowing and pedal reflexes and response to surgical stimulation.

Vital parameters like respiration rate, heart rate, SpO_2 and rectal temperature were monitored during the entire anaesthesia using multi-parameter monitor (Skanray True Scan S 400, patient Monitor, Skanray Technologies Ltd.). A single individual evaluated the quality and depth of anaesthesia through a blindfold study.

Scoring was done to assign numerical values starting from 1 to 4 (1-poor, 2-fair, 3-good, 4-excellent) for induction and maintenance quality (Bodh *et al.,* 2015). Qualitative and subjective effects (sedation, analgesia, muscle relaxation) of drugs were judged by observing the physical response of the anaesthetised goats to surgical stimulation during various surgeries. Weaning from the anaesthetic machine was done upon completion of the surgical procedure.

The endotracheal tube was removed on regaining the swallowing reflex. All the animals were administered normal saline in jugular vein throughout the surgery. The animals were monitored till complete recovery. The dial setting of the vaporiser was different in different animals at the end as per the requirement to maintain the surgical plane of anaesthesia. Goats were also observed for any difference in recovery.

Calculation of the amount of liquid isoflurane utilised

The changes in the fresh gas flow rate and vaporiser setting at various times were recorded. The total duration of anaesthesia in minutes was recorded from turning on to off the vaporiser. The data obtained were used for calculating the quantity of isoflurane consumed for the different anaesthetic combinations by following formula (Senthilkumar *et al.,* 2013).

Isoflurane vapour delivered (ml) = vaporiser setting (per cent) x fresh gas flow (litre per minute) x duration (minute) x 10.

The total isoflurane vapour delivered (ml) for the entire duration of anaesthesia was calculated by summing up the isoflurane vapour delivered for each fresh gas flow (FGF) and vaporiser settings employed.

Yadav *et al.* (2021) determined the isoflurane-sparing impact using the same formula used in the anaesthesia study conducted on buffaloes. To bring uniformity to data obtained for various cases in practical but theoretically the same time in various animals, the authors equated the total isoflurane vapour value to 400 kilograms of body weight. (average body of buffaloes) and 40-minute duration. This equation for standard weight and time duration aided in easy statistical comparison. The authors reported a method for calculating the amount of isoflurane vapour required for 400 kilograms and 40 minutes basis (ml) = Total isoflurane vapour delivered (ml) x 400 x 40 / body wt. (kg) x duration of maintenance (minutes).

In the present study, the isoflurane vapour value obtained was equated to 40 kg weight as 40 kg was the average weight of goats and 60 minutes duration,to ring uniformity in data and this set the basis for statistical comparison of percentage reduction in isoflurane utilised in Group I and II. The modified formula was as follows:

Isoflurane vapour delivered for 40 kilograms and 60 minutes basis (ml) = Total isoflurane vapour delivered (ml) x 40 x 60 / body wt. (kg) x duration of maintenance (minutes)

Table 1. Amount of isoflurane utilised (on 40 kg and 60-minute basis in ml) by individual goats of Group I and II during various surgical interventions

Isoflurane liquid utilised (ml)		
Animals	**Group I**	**Group II**
Animal A	13.41	9.79
Animal B	4.64	16.82
Animal C	6.65	4.26
Animal D	2.93	6.04
Animal E	5.13	4.19
Animal F	19.45	6.37
Mean ± S.E.	8.71 ± 2.61	7.92 ± 1.97
t-value (P-value)	0.241^{ns} (0.815)	

ns non-significant (P>0.05)

Table 2. Weight (kg) and duration of anaesthesia (min) of goats of Group I and Group II

Animal	Group I (X-B-TZ-Iso)		Group II (D-B-TZ-Iso)	
	Weight	**Duration of anaesthesia**	**Weight**	**Duration of anaesthesia**
1	16	48	28.2	32
2	40	65	12	68
3	28	42	33.1	32
4	54	44	25.5	29
5	37	76	36	98
6	15.5	24	30.5	32

The effect of ambient temperature and pressure on the calculated volume of liquid isoflurane was neutralized using Avogadro's principle as follows: Isoflurane vapour delivered for 40 kg and 60-minute basis (ml) x 181.4) x ambient temperature /273) x 760/ barometric pressure) The statistical analysis of data was done by one-way analysis of variance (ANOVA) and Demean's multiple range test (Duncan,1955)

Results and Discussion

The current study used a partial intravenous anaesthetic technique to administer anaesthesia. When an inhalational drug such as isoflurane is used as the sole agent, it is often not sufficient to abolish the desired autonomous and nociceptive responses to the surgical stimulus, potentially leading to inadequate peri and post-operative pain (Steffey and Mama, 2007). The use of inhaled anaesthetics along with intravenous medications (analgesics and/or sedatives) is known as partial intravenous anaesthesia (PIVA), which improves cardiopulmonary parameters by reducing the amount of anaesthesia needed by inhaled anaesthetic agents to prevent intraoperative awareness and consequently, lessens their dose-related cardiovascular depressing effects, (Doherty *et al.* (2006).

Since each anaesthetic has distinct pharmacodynamics and pharmacokinetics merits and demerits, it is wise to achieve surgical anaesthesia by combining a number of drugs (balanced anaesthesia), if necessary, to counteract any adverse effects of each agent. Lin (2014) reported that combining analgesic drugs with different pharmacological mechanisms may provide a higher degree of analgesia than each drug administered alone, which may significantly decrease hypnotic agents such as isoflurane.

In the present study, goats were premedicated with dexmedetomidine and xylazine along with butorphanol and anaesthesia was maintained with isoflurane. In veterinary practice, xylazine, an alpha-2 adrenergic receptor agonist, is the most often used sedative and a supplement to general anaesthesia, according to Ponser (2018). This drug produces its effects by binding to alpha-2 adrenergic receptors distributed centrally in the brain or supraspinal (for sedation and some antinociception) and in the dorsal horn of the spinal cord (for antinociception) as well as in the vessel vasculature (for vasoconstriction). Xylazine has a very effective muscle-relaxing action.

Dexmedetomidine is much more specific than xylazine for alpha-2 receptors than α_1 receptors. The pharmacologic effects of dexmedetomidine include depression of the central nervous system (sedation and anxiolysis), analgesia (somatic and visceral) and muscle relaxation (Plumb, 2008).

Butorphanol provides preemptive and postoperative analgesia in goats (Carroll and Hartsfield, 1996). Butorphanol is a partial agonist, antagonist, or k agonist, according to Depenbrock (2017).

Isoflurane is the preferred inhalant for comprising ruminants since it is not arrhythmogenic, relies less on metabolism for removal and has a quicker induction and recovery time (Carroll and Hartsfield, 1996). Isoflurane acts on the spinal cord to restrict movement in response to noxious stimuli. The dorsal horn, which regulates and conveys noxious sensations to other central nervous system locations, is a probable site of action (Jinks *et al.*, 1999).

The amount of isoflurane liquid in ml utilised (on a 40kg and 60-minute basis in ml) by the individual goat is shown in Table 1. It was observed that the volume of isoflurane utilised (mean ± S.E.) in Group I was 8.71 ± 2.61 ml, and in Group II was 7.92 ± 1.97 ml. There was a non-significant ($P>0.05$) difference between the amount of isoflurane utilised between the two groups, i.e., a 9.19% reduction in isoflurane was recorded in Group II, (Table 1). Sharma *et al.* (2014) performed studies on the inhalant-sparing effects in dogs. The authors found that the rapid biotransformation of xylazine, which had an elimination half-life of 30.1 minutes compared to dexmedetomidine, which had a half-life of 47 minutes, was the cause of the sparing effect of halothane in the dexmedetomidine administered group of dogs. When calves were premedicated with opioids such as butorphanol tartrate (0.02 mg/kg body weight) and buprenorphine hydrochloride (0.006 mg/kg body weight), respectively, Senthilkumar *et al.* (2013) obtained 18.7% and 14.63% reduction in MAC of isoflurane. In a study in cows, a continuous rate of lidocaine at a dose rate of 50 ml/kg/min significantly decreased the isoflurane requirement by 16.7% (Vesal *et al.*, 2011). The analgesic effects of butorphanol on the MAC of isoflurane, had been investigated in ruminants (Kumar *et al.*, 2013).

However, the reduction in the quantity of isoflurane in Group II was insignificant compared to goats of Group I, but its combination with opioids and alpha-2 agonists might have potentiated the sparing effect of isoflurane in goats. So, it is concluded that dexmedetomidine-butorphanol did not have a significant isoflurane-sparing effect than xylazine-butorphanol in goats but may be included in the balanced anaesthetic protocol for goats undergoing long-duration surgeries to reduce the quantity of isoflurane required for maintenance.

Conclusion

The inclusion of opioid analgesic (butorphanol) along with alpha-2 agonist (dexmedetomidine) reduced the quantity of isoflurane required for maintenance of anaesthesia in goats undergoing various surgeries induced with tiletamine-zolazepam.

The dexmedetomidine-butorphanol combination has a higher sparing effect (9.19%) on isoflurane than xylazine-butorphanol administration in goats.

Authors' declaration of interests: No conflicts of interest have been declared.

Source of funding: Ph. D. Research Fund by Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.

Acknowledgement

The authors are highly grateful to The Dean, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, for providing the necessary facilities and encouragement to conduct this study.

REFERENCES

Bodh, D., Singh, K., Mohindroo, J., Mahajan, S. K. and Saini, N. S. 2015. Sedative, analgesic and cardiopulmonary effects of midazolam-butorphanol premedication in water buffaloes (*Bubalus bubalis*). *Buffalo Bull.* **34**: 29-40.

Carroll, G. L. and Hartsfield, S. M. 1996. General anaesthetic techniques in ruminants *Vet. Clin. North Am. Food Anim. Pract.* 12(3):627-661.

Depenbrock, S. 2017. Ruminant field anesthesia and analgesia. *Penn Conference*. p:1-5. https://www.vet.upenn.edu/docs/default-source/penn-annual-conference/pac-2017-proceedings/pac2017-09-28/food-animal-track/ruminant-field-anesthesia-and-analgesia---dr depenbrock.pdf?sfvrsn=9b49e1ba_2.

Doherty, T. 2006. Effects of ketamine and magnesium on the minimum alveolar concentration of isoflurane in goats. *Am. J. Vet. Res.* **67**: 1962-1966.

Duncan, D. 1955. Multiple range and multiple F test. *Biometrics*.11:15-18.

Hikasa, Y., Saitob, K., Takaseb, K. and Ogasawara, S. 2000. Clinical, cardiopulmonary, haematological and serum biochemical effects of sevoflurane and isoflurane anesthesia in oxygen under spontaneous breathing in sheep. *Small Rumin. Res*. **36**: 241-249.

Jinks, S., Antognini, J. F., Carstens, E., Buzin, V. and Simons, C. 1999. Isoflurane can indirectly depress lumbar dorsal horn activity in the goat via action within the brain. *British. J. of Anaesth.* 82: 244-49.

Joubert, K. 1999. The hidden dangers of anaesthestic machines. *J.S. Afr. Vet. Assoc.* 70: 140-141.

Kumar, S. S., Rajendran, N., Dharmaceelan, S., Kathirvel, S., Subramanian, M. and Selvaraj, P. 2013. Effect of butorphanol and buprenorphine on inhalant sparing and gas concentrations during low flow isoflurane anesthesia in cattle. *Adv. Anim. Vet. Sci*. **1**: 29-32.

Lin, H. 2014. Inhalation anesthesia. In: *Farm Animal Anaesthesia: cattle, small ruminants camelids and pigs.* John Wiley and Sons: Ames, IA, USA pp. 95-110. https://doi.org/10.1002/9781118886700.ch5

Plumb, D. C. 2008. *Plumb's Veterinary Drug Handbook.* (8th Ed.). Blackwell Publishing Professional, Iowa, 1120p.

Poppel, N., Hopster, K., Geburek, F. and Kastner, S. 2015. Influence of ketamine or xylazine supplementation on isoflurane anaesthetized horses — A controlled clinical trial. *Vet Anaesth Analg.* 42 :30–38.

Senthilkumar, S., Rajendran, N., Dharmaceelan, S., Kathirvel, S., Subramanian, M. and Selvaraj, P. 2013. Fresh gas flowreduction and isoflurane sparing effect of butorphanol and buprenorphine during low flow isoflurane anesthetic in cattle. *Adv. Anim. Vet. Sci.* 1: 29-32.

Singh, G. D., Kinjavdekar, P., Amarpal, Aithal, H. P., Pawde, A. M. and Zama, M. M. 2013. Clinicophysiological and haemodynamic effects of fentanyl with xylazine, medetomidine and dexmedetomidine in isoflurane-anesthetized water buffaloes (*Bubalus bubalis*). *J. S. Afr. Vet. Assoc*. 84: 67-77.

Steffey, E.P. and Mama, K.R. 2007. Inhalation anaesthetics. In: Lumb and Jones' Veterinary Anaesthesia and Analgesia. (4th ed.) (eds. Tranquilli W.J., Thurmon J.C. and Grimm K.A.) Blackwell publishing Ames, Iowa, USA. pp. 355-393.

Tranquilli, W.J., Thurmon, J.C. and Grimm, K.A. 2007. Lumb and Jones' Veterinary anesthesia and analgesia. 4th ed., Blackwell Pulisher, pp.1112.

Vesal, N. Spadavecchia, C., Steiner, A., Kirscher, F. and Levionnois, O. L. (2011) Evaluation of the isoflurane- sparing effects of lidocaine infusion during umbilical surgery in calves. *Vet. Anaesth. Analg*. 38:451-460.

Vishnugurubaran, D. Kathirvel, S, Senthikumar, K., Balasundaram, K and Dharmaceelan, S. 2016. Isoflurane sparing effect of diazepam and midazolam to xylazine- ketamine induction and isoflurane maintenance in goats. *Indian j. Ani. Res.* 51:762-763.

Yadav, P., Chaudhari, R. N., Tiwari, D., Goyal, S., Arora, N., Sharma, S., Kumar, A. and Tayal, R. 2021. Evaluation of the isoflurane sparing effect of meloxicam in buffaloes undergoing diaphragmatic herniorrhaphy. *Haryana Vet.* 60: 115-118.

Received: 31st August 2023 Accepted: 20th September 2023

Journal of the Andaman Science Association Vol. 28(2):173-187 (2023)
ISSN 0970-4183, Printed in India

New hosts for the Andaman Baron butterfly, *Euthalia aconthea acontius* (Hewitson, 1874) (Lepidoptera: Nymphalidae) in an Island Ecosystem

Neelam Purti[1], [2] Lal Ji Singh[3*] and Arun K. Pandey[2]

[1]*Department of Environment and Forests, Manglutan Range, South Andaman Forest Division, Andaman & Nicobar Islands, India*

[2]*Department of Botany, Mansarovar Global University, Bhopal, Madhya Pradesh, India*

[3]*Botanical Survey of India, Andaman & Nicobar Regional Centre, Port Blair, Andaman & Nicobar Islands, India*

***Corresponding author's E-mail:** Lal Ji Singh (laljisingh1970@rediffmail.com), Botanical Survey of India, Andaman and Nicobar Regional Centre, Port Blair, Andaman and Nicobar Islands, India*

Abstract

Euthalia aconthea acontius (Hewitson, 1874) (Lepidoptera: Nymphalidae) has been reported as a pest of mango (*Mangifera* spp.) in the Andaman and Nicobar Islands, India for the first time. In the present communication, the impact of pest on host species is assessed. Larvae damage all above-ground plant parts especially the leaves, *Mangifera andamanica* King, *Mangifera griffithii* Hook. f. are recorded here for the first time as new host plants. *Mangifera andamanica* is endemic to the Andaman and Nicobar Islands and population of *M. griffithii* is very few and confined in distribution.

Key words: *Anacardiaceae, Euthalia, Lepidoptera, Mangifera, Nymphalidae*

Introduction

The study of life history traits (egg, larva and pupa stage) and fecundity is of fundamental importance for understanding on the biological and ecological parameters which are linked to extinction risk. Moreover, when a plant species becomes endangered due to damage by an invasive phytophagous insect, more knowledge about life history traits of all trophic levels may be important in sustainable conservation management. *Euthalia aconthea* (Lepidoptera: Nymphalidae) is a butterfly that has been reported as a pest of *Mangifera* species of the family Anacardiaceae.

Arthropod herbivores comprise one of threats to the native flora worldwide and in particular reveal a strong fecundity response to food choice. *E. aconthea* is recorded as a minor pest infesting *Mangifera* plants and is a serious threat to the mango populations (Tara & Gupta 2016). But nothing is known about Nymphalidae herbivory among the *Mangifera* species of Andaman and Nicobar Islands (ANI's). Recently Purti et al. (2022 & 2023) recorded cycads of the ANI's which Offered as larval food for Lycaenid species, *Luthrodes pandava* Horsfield. The present study report here for the first time that the *Mangifera* species of the ANI's are used as larval host plants. Impact of butterfly herbivory on *Mango* host species native to the ANI's was also assessed at natural population and *ex-situ* conservation site, Dhanikhari Experimental Garden-cum-Arboretum (DEGCA), Botanical Survey of India, Andaman and Nicobar Regional Centre. DEGCA is an excellent centre for collection of plant species and *ex-situ* as well as *in-situ* conservation. (Singh & Murugan 2014; Singh et al. 2014, 2021a, b)

Mango produces fruits with significant socioeconomic value. The first botanical description of *Mangifera* had been given by Linnaeus (Sp. Pl. 1: 200. 1753). Thereafter, it has been studied from time to time by various workers (Hooker 1886; Mukherjee 1953, 1985; Kostermans & Bompard 1993; Bompard & Schnell 1997; Dinesh et al. 2011, 2015; Kumar & Chakrabarty 2021). It is taxonomically complex genus and one of the most economically and culturally significant tropical fruits, particularly in Asia. Approximate 123 taxa have been recorded under the genus from the tropical and subtropical world (The Plant List 2013), however it is taxonomically complex at species level. It is very diverse in Southeast Asia, Indo-Malaysia, and the Solomon Islands. The genus *Mangifera* occurs throughout India including the ANI's. Biogeographically the ANI's lie in the proximity of Eastern coast of South Asia, Madagascar, Sri Lanka,

Thailand, Peninsular Malaysia, Myanmar, Sumatra and Java and widely recognized as one of the centers or hot spots of biodiversity. It is a rich and unique region in terms of plant diversity with a high number of endemics in India (Singh et al. 2014, 2020 a, b & 2021 a, b; Singh & Misra 2020; Singh & Ranjan 2021). In India, the species diversity confined to the ANI's and the genus is represented by five wild taxa viz., *Mangifera andamanica* King, *M. campto-sperma* Pierre, *M. griffithii* Hook., *M. indica* L., *M. nicobarica* Kostern. & *M. sylvatica* Roxb. and an introduced species, *M. indica* L. The distribution of *M. nicobarica* is confined to the Nicobar group of Islands whereas the *M. indica* is spread in all parts of the Islands due to its domestication and large consumption of fruits. *M. griffithii* has a small population that is restricted to Manipur Parvat National Park (previously known as Mount Harriet National Park). *M. andamanica, M. camptosperma*, and *M. sylvatica* populations are also sparsely distributed and can be found in the tropical evergreen forests of these Islands.

Mangifera species naturally occurring the ANI's are sparsely scattered and confined to a few localities, which face many significant threats such as anthropogenic pressures such as land transformation and shifting cultivation etc., leading to habitat loss. Furthermore, anthropogenic pressure and parasitization of Misletoes, as well as invasive arthropod herbivores also threaten local mango population. However, the ANI's rich and unique in terms of plant diversity in India with higher number of endemism (Singh et al. 2014; Singh et al. 2020a, b; Singh et al. 2021a, b; Singh & Misra 2020; Singh & Ranjan 2021) and constitute one of the hotspots of biodiversity with 572 Islands and islets, (N 6°45' to 13°41' E 92°12' to 93°57'). These Islands are situated in the tropical belt and frequently battered by tropical rains during both South-West monsoons (May to September) and North-East monsoon (October to December). The majority of the rainfall (76%) is received during South-West Monsoon, 22% during the North-East monsoon and the rest during summer season.

Except for few notable exceptions like *M. indica* (Young 1907; Bell 1909; Sevastopulo 1938; Pant & Chatterjee 1950; Wynter-Blyth 1957; Kunte 2000; Kalesh & Prakash 2007; Veenakumari et al. 2008; Robinson et al. 2010, 2023; Jayasinghe et al. 2014, 2021; Nitin et al. 2018; Nayanathara & Narayana 2020) to date, no lepidopteran has been identified as a pest of mango species other than *M. indica* from India. So far, this investigation aimed to record the mango host plants, detail the life history traits (morphology of the egg, larva and pupa stage) of an endemic lepidopteran, *Euthalia aconthea acontius* (Hewitson 1874) and assess its impact on *Mango* host species native to the ANI's. Although, more recently Purti et al. (2022, 2023) recorded cycads of the ANI's as larval host plants for one of the Lepidopteran butterfly, *Luthrodes pandava* Horsfield.

Materials and methods

The live plant and butterfly specimens were observed through ten field trips to localities where natural populations occur (Baratang, Chidiyatapu, Chouldhari, Karmatang, Khudirampur, Humpfrygunj, Jirkatang, Manipur Parvat National Park, Manjery, Rutland, Shoalbay, Wandoor) as well as *ex-situ Mangifera* conservation site, DEGCA throughout 2017–2022. Eggs of *E. a. acontius* were collected from the infested mango leaf, *M. andamanica* and *M. griffithii*. Field surveys were conducted to quantify the impact of *E. a. acontius* herbivory on field-planted *Mangifera* species at the DEGCA and naturally occurring plants (Fig. 1). GPS (Garmin Montana 680) coordinates of the localities of wild population were recorded. *E. a. acontius* eggs along with the host plants were placed in a separate sterile labeled plastic container. The droppings of the larva were cleaned periodically and fresh leaves of the host plants were replaced on daily basis. The larval stages of *E. a. acontius* were monitored daily, documented and photographed. The eggs of *E. a. acontius* laid on the leaves of the plant were observed and photographed under a stereomicroscope (Olympus SZ 61) at the ANRC, BSI.

Observations and Result (Fig. 1-3)

Based on field exploration conducted during the period 2016 to 2019, critical analysis of morphological characters and scrutiny of relevant literature and examination of the herbarium specimens kept in Indian herbaria (PBL) and digital herbaria (e-Floras 2008; WCSP 2012; The Plant

List 2013; GBIF 2020; JSTOR 2020 & The Herbarium Catalogue 2021), five wild and one cultivated species recorded in the islands as mentioned below.

Taxonomic Notes on host plants

Mangifera L. (Anacardiaceae) Sp. Pl.: 200 (1753)

Mangifera andamanica King. J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 65: 470 (1896)

Distinguishing features

Evergreen tree, rough bark. Leaves simple, alternate, obovate to oblanceolate, acuminate caudate base cuneate, entire, coriaceous, glabrous, shiny clustered at the tip of branchlets, petioles long. Flowers in terminal panicles, small yellowish green. Fruit a drupe, elliptic, yellowish orange on ripening. Mesocarp fleshy to fibrous. Seed ellipsoid reniform.

Distribution / Locality selected for *in-situ* Observations

Throughout Andaman Islands particularly South Andaman Islands, some Islands of Middle and North Andaman, (Fig. 1).

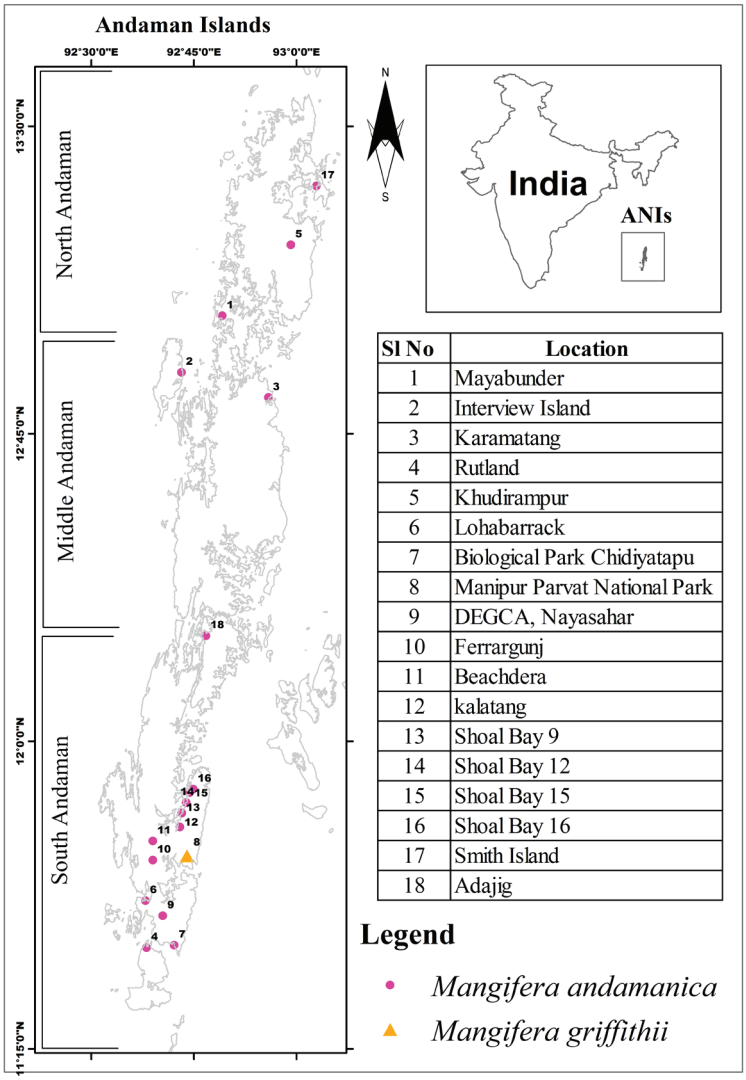


Fig. 1. Distribution and study localities of *Mangifera* species *used* as food by *Euthalia aconthea acontius*

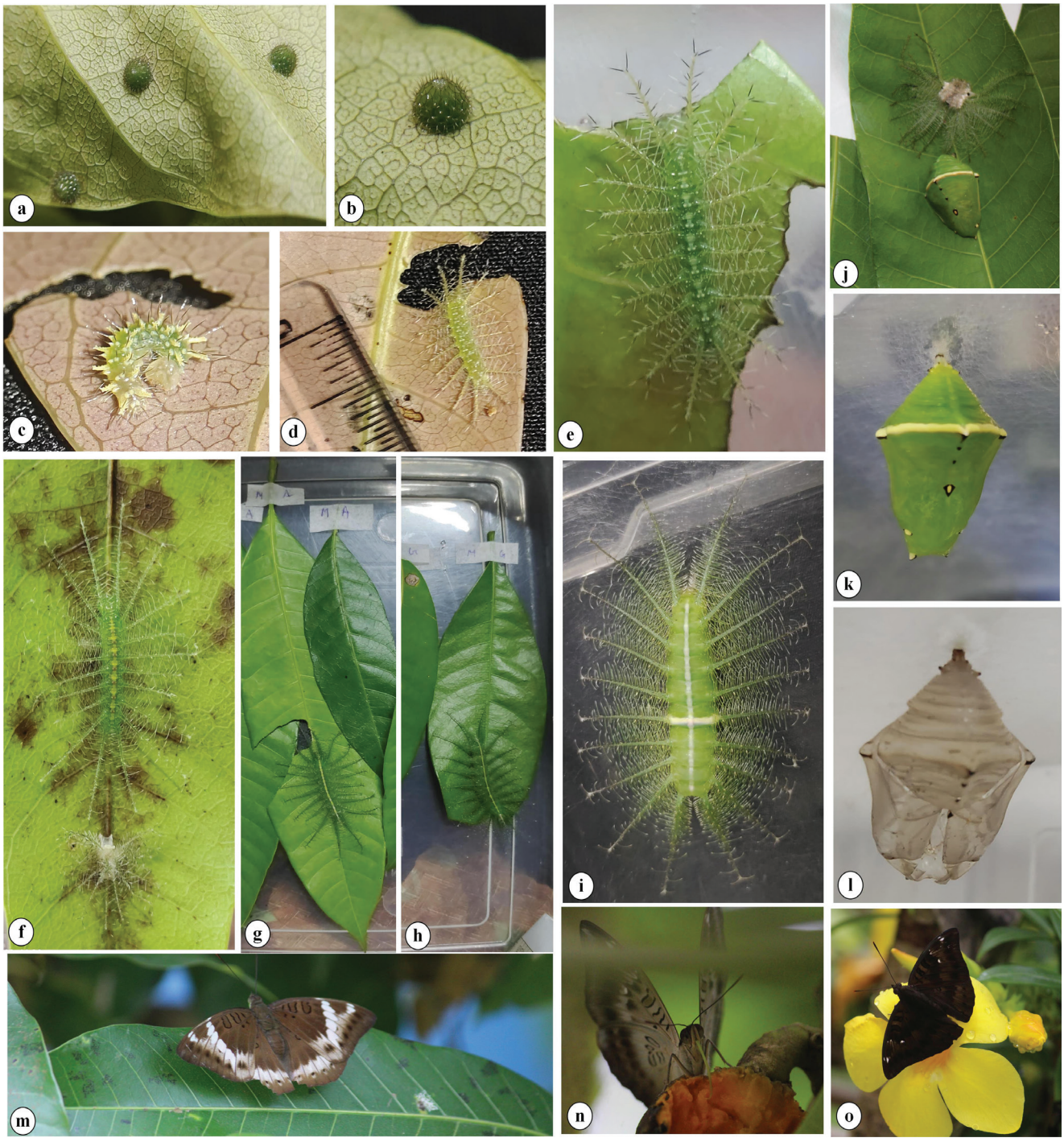


Fig. 2. Damage to *Mangifera leaves* caused by *Euthalia aconthea acontius*: a: Eggs on *Mangifera andamanica*; b: Close up view of egg; c: First Instarfeeding on *M. andamanica*; d: Second Instar on *M. andamanica*; e: Third Instarfeeding on *M. andamanica*; f: Fourth Instarfeeding on *M.griffithi*; g: Fifth Instar feeding on *Mangifera andamanica*; h: Fifth Instar on *M.griffithi*; i :Pre pupa; j: Pupa on *M. andamanica*; k: Pupa; l: Empty pupa; m: Female butterfly; n: Female butterfly feeding fruit juice of *Carica papaya*; o: Male butterfly seeking nectar on the flower of *Allamanda cathartica*

Habitat: Evergreen forests, Semi Evergreen forests

Conservation status

Near threatened. The populations of *M. andamanica* are sparsely scattered and confined to a few localities, which face many significant threats such as anthropogenic pressures such as land transformation and shifting cultivation etc., leading to habitat loss. Therefore, the species is assessed here as "Near threatened" based on the International Union for Conservation of Nature (IUCN) categories and criteria (IUCN, 2019).

Mangifera griffithii Hook. f. Linn. Soc. London 23: 168 (1860)

Distinguishing features

Evergreen tree. Leaves broadly elliptical to elliptical-oblong or obovate-oblong smooth leathery inflorescence in axillary or terminal panicles flowers small creamy white. Fruit yellowish to pink or rose-red, sometimes becoming purplish-black when ripe.

Distribution: India: Andaman & Nicobar Islands**,** Assam, Peninsula Thailand to W. Malesia.

Locality selected for *in-situ* Observations: Manipur Parvat National Park, South Andaman (Fig.1),

Habitat: Evergreen forests

Conservation status: Data deficient. Itis recorded only in the Manipur Parvat National Park South Andaman. Apart from this locality, there are no data on the population of the species available in other islands. Therefore, the new species is assessed here as "Data Deficient" (DD) based on the International Union for Conservation of Nature (IUCN) categories and criteria (IUCN 2019).

Note: In the ANI's geographical distribution recorded only from Mount Manipur Parvat National Park, South Andaman.

Except for preliminary account regarding host of *E. a. aconitus* there is no detailed information available in literature. The present investigation recorded here detailed account on host plant species along with the life history traits (egg, larva and pupa stage). The study began during a visit to the Forest nursery Chouldhari in the evening hours. Couples of Andaman baron butterflies were spotted involved in mating ritual. After the copulation the female was found to oviposit eggs on the *Mangifera andamanica* leaves. The eggs along with the leaf were collected and placed in a sterile plastic container.

The young leaves laden with the eggs were carefully dislodged from the tree and placed in an aseptic plastic labelled container to document the life cycle. Because, the literature suggests that the life cycle of this endemic butterfly of Andaman Islands were not reported till date. The leaves laden with the eggs were thoroughly examined and extruded for potential predators like spiders, fungus, etc.

The first instar was fed with young and mature leaves of *M. andamanica* and *M. griffithii* brought from Mt Harriet (Manipur Parvat National Park). It was observed that the first instar did not feed on the mature leaves of the aforementioned new larval host plants. This may be due to the inability of the larva to devour the mature leaves. However, the young leaves which were raised as seedlings in the park area were readily consumed by the larva.

As the larva progressed into further instar stages it was seen that that it consumed matures leaves too. The larvae consumed the mature leaves of the *M. andamanica* plant. Whereas in case of *M. griffithii* when the leaves were fed the larva did not consume the leaves may due to hard and leathery nature of the leaves. Whereas when the mature leaves of nursery raised plants of the same where fed, they were easily consumed by the larvae. Also, it was noticed that that there was a complete change in the morphology and texture of the sole standing tree of *M. griffithii* and the nursery raised plants in the Manipur Parvat National Park.

Also it has been observed that the larvae feed on the young and mature leaves leaving behind the hard costa of the leaves. In some leaves where the costa is soft, the whole leaf has been consumed by the butterfly larvae.

Notes on pest of *Mangifera* spp.

***Euthalia aconthea acontius* (Hewitson, 1874) (Lepidoptera: Nymphalidae)**

Euthalia aconthea acontius (Lepidoptera: Nymphalidae) is an endemic sub species and commonly called as Andaman Baron which was described by Hewitson (1874) from group of Andaman Islands. Although, Holloway (1989) stated that it remains considerably requires intensive field surveys that will contribute to the knowledge of the host plants of the butterflies naturally occurring in these islands. Andaman Baron, *E. a. acontius* has been reported as pest of common mango species *M. indica* (Veenakumari et al. 2008; Varshney & Smetacek 2015). The present study first time observed it's herbivory on native species of mango. It was also observed that the adult butterfly visited many other plants *viz*.; *Allamanda cathartica* L. (Apocynaceae), *Carica papaya* L. (Caricaceae), *Cycas zeylanica* (J. Schust.) A. Lindstrom & K.D. Hill (Cycadaceae), *Polyalthia longifolia* (Sonn.) Thwaites_(Annonaceae) for various purposes like protection from fatal attacks by predators, feeding of nectars, fruit juices, mating etc. (Fig.2 n, o, Fig. 3 c, d)

Morphology and Description: Male and female butterfly (Fig.2-4).

E. a. acontius, unlike other members of the order Lepidoptera, goes through four stages of development: egg, larva, pupa, and adult. The larval stage lasts the longest, followed by the pupa and incubation stages.

It glides and flies with stiff wings beats. The male has a dark brown upper side with a broad obscure post-discal band on both wings. Several small whitish spots can be found along the inner edge of this band on the forewing. When viewed in sidelight, the wings typically have a dark purplish tinge. The female is larger and pale bluff brown, with larger and more defined white spots on its forewing. The wings are paler on the underside and have a submarginal series of black spots on both the fore- and hindwings. Lime green is the colour of the proboscis.

Mating behavior and Oviposition behavior

Andaman Baron, *E. a. acontius* couples were found copulating during the dusk (around 1745 hrs) under *M. indica* at Manglutan forest range office dated 5th May 2022. The copulating act lasted for 60-70 minutes. During the course of mating these couples flew towards a wall and resting in the same compromised position. At this point these mating couples were captured in a sterile plastic contained without disturbing them and released into the cage with few leaves of *M. indica*.

Three single eggs were laid singly on the underside of the *M. indica*. Due to the oviposting pressure the female generally not biased to lay her eggs on the *M. indica* leaf. Under captive observation leaves of *M. indica* of different ages including the dry leaf as well was kept in the cage. The gravid female chooses to oviposit over a mature leaf (fig 2a). On 6th May 2022 leaves of *M. andamanica* and *M. griffithii* brought from Manipur Parvat national Park (Mt. Harriet) and placed in the contained after labelling it.

Egg stage (Plate 1)

Gravid females lay eggs singly on the surface (under or upper side) of mango leaves. Each egg is hemispherical in shape and has a base diameter of 1.5-1.8 mm. The egg's surface is covered in large hexagonal depressions with hair-like projections emerging from adjoining corners. When the egg is freshly laid, it is light green in colour, and as it matures, it becomes darker in colour. The egg is1.6-2.0mmlong with an average length of 4.86 ± 0.78 mm and 2 - 2.5 mm wide with an average width of 2.75 ± 0.25mm. Mature eggs turn a dark green colour, and the incubation period lasts 4 to 5 days on average. Young larvae begin hatching from incubated eggs on its host plant. First instar larvae wander around after hatching and begin feeding on the upper surfaces of leaves, forming small perforations.

Larval Stages

Five larval stages were recorded during the observation.

First Instar (Plate 1)

After 4-5 days, the first instar emerges and consumes the eggshell as its first meal. The caterpillar has a pale yellowish-brown head capsule and is dark greenish in colour. The body is made up of ten pairs of long, yellowish, fleshy dorsolatero protuberances. The body produces green setae. The first instar measured 4.5 - 7 mm in length with an average of 5.75 ± 0.93mm and 1.5-2 mm in width with an average of 1.75 ± 0.25mm (Table 1). The first instar lasted 2.5-5 days on average, with a maximum of 3.60.96 days. Larvae that have just hatched feed on the upper surfaces of leaves, causing small perforations.

Second instar (Plate 1)

The second instar larva is greenish in color. All the ten pairs of short protuberances have lengthened considerably. As the larva grows, white patches appear between all ten pairs of protuberances. Later on these patches become conjoined, forming a continuous dorsal band (which helps the larvae to blend in the surrounding when it rests on the midrib of a leaf). The second instar measured 10.5 - 14.5 mm in length with an average of 12.6 ± 1.67mm and width of 2.5 - 3 mm with an average of 2.75 ± 0.25mm (Table 1). Second instar lasted for 2.5-4 days with an average of 3.2 ± 0.570 days. Larvae feed by defoliating the leaves along their margins.

Third instar (Plate 1)

The larva of the second instar is greenish in colour. All ten pairs of short protuberances have grown significantly longer. White patches appear between all ten pairs of protuberances as the larva grows. Later on, these patches fuse to form a continuous dorsal band (which helps the larvae to blend in the surrounding when it rests on the midrib of a leaf). The second instar was 10.5 - 14.5 mm long with an average of 12.6 ± 1.67mm and 2.5- 3.0 mm wide with an average of 2.75 ± 0.25mm (Table 1). The third instar lasted 2.5-4 days on average, with a maximum of 3.2 ± 0.570 days. Larvae feed by defoliating the leaves' margins.

Fourth instar (Plate 1)

The fourth instar caterpillar looks similar to the third instar. The embedded purplish spots in the dorsal band are now easier to spot. The fourth instar was 2 3- 25 mm long, with an average of 23.8 ± 0.85 mm, and 3.5 - 4 mm wide, with an average of 4.75 ± 0.25 mm (Table 1). The fourth instar lasted 6 - 7 days on average, with a total of 6.56 ± 0.42 days.

Fifth instar (Plate 1)

The general appearance of the final instar larvae was similar to that of the fourth instar, with the exception of differences in body length and width, which measured 29-33 mm with an average of 31 ± 1.5mm and width of 4.5 - 5mm with an average of 5.75 ± 0.25mm (Table 1). The duration of the fifth instar ranged from 10.0 days to 12.0 days, with an average of 11.0 ± 0.79 days. Aside from its larger size, another noticeable change is the embedded purplish spots in the dorsal band. In this final instar, the spots have grown larger and more noticeable. The average larval duration ranged between 24.0 - 32.0 days, with an average of 27.8 ± 3.34 days.

Pupa (Plate 1)

Fully fed and matured larvae stopped feeding, and their bodies began to shrink and thicken in size. It then spins silken threads around its body and remains in this pre-pupal stage for approximately 1.25 ± 0.25 days. It then looks for a location on the underside of a leaf. There, it spins a large number of silk threads into a silk mound, to which its posterior claspers are attached. Later on, the pre-pupa hangs from this anchor point with its head down. The dorsal band has completely whitened by this point. A short transverse pale yellowish band appears on the dorsum around mid-body after some time. Pupae are almost naked, reddish brown, with only a few strands of silken threads loosely attached to their bodies. The pupa measured 18 - 20 mm in length with an average of 19.0 ± 0.79mm and 8-9mm in width with an average of 8.43

± 0.42mm. The pupal period ranged between 7.5 and 8 days, with an average of 7.75 ± 0.25 days.

Adult (Plate 1)

The adult has a brownish colour with hints of olive. Dark brown is found on the antennae, head, thorax, and abdomen. Lime green is the colour of the proboscis. The tip of the antenna is ochraceous. The forewing has two transverse black lines at the base, a black loop in the middle and one beyond the apex of the cell with dark brown centres, followed by an angulated discaldark brown band bordered outwardly by a series of five white spots; two preapical white spots beyond and a broad, somewhat diffuse, subterminal black band broadening over the apex and angulated inwards in interspace. The hind wing is also dark brown, with two crescent-shaped dark brown loop-like marks in cell, as well as a discal series of dark brown, elongate, outwardly acute, inwardly diffuse, somewhat hastate spots, followed by a sub-terminal series of small spots of the same colour. The underside is an ochraceous brown colour. The wings are paler on the underside and have a submarginal series of black spots on both the fore- and hindwings. The underside of the forewing has five transverse slender black lines that run across the cell. The wing span ranges from 68 to 79 mm.

E. a acontius is an important leaf destructive pest of *Mangifera* spp. Naturally occurring in the ANI's. Besides its earlier recorded host species, *M. indica* two endemic host species, *M. andamanica* and *M. griffithii* are added as a first record from these islands. It was also observed most of the times that by the end of 5th instar before entering into pupa stage the larvae leaves the mother host plants (Mango species) and pupates in the adjacent plants. In the present investigation it was observed that *Polyalthia longifolia* (Sonn.) Thwaites and *Cycas zeylanica* (J. Schust.) A. Lindstrom & K.D. Hill served to prevent fatal attacks by red ants other predators. Among these two adjacent plants C. *zeylanica* was better for the larvae for providing protection.

The *E. a. acontius* is has also variety of predatory birds like Common Myna, Hill Myna, Magpie Robin, Red whiskered Bulbul. It has also been observed that the host plant is mostly affected before the onset of phonological events (flowering, fruiting and leaf fall). As during this time the larvae would be safer from pollinators. *E. a. acontius* larvae feeds on the leaves of the plant. It has been observed that the larvae choose the leaf for its consumption according to its size. At the initial stage it feeds on young leaves later on its further growth it feeds on the semi mature and mature leaves too. The larvae start feeding the leaves from tip of the leaf and gradually to the petiole base and thus at last defoliates the leaf. The larvae choose the leaf according to its body size so that its complete body rest on the leaf and no bristles are hanging out of the leaf lamina. The larvae well blend with the colour of the leaf with its camouflaging property to hide from its predators. The larvae mostly rest and align itself with the mid rib of its body with that of the leaf. The larvae mostly align and camouflage on the upper surface of the leaf.

It has also been observed that the eggs are laid by the butterfly before the flowering and fruiting season. Since during the flowering season the inflorescence are being visited by many pollinators such as birds, wasps, bees etc.

During the study it was also observed that, adult butterfly *E. a. aconitus* visited on the other host plants such as papaya species for dietary and other essential events like feeding of nectars, fruit juices and mating. *E. a. acontius* fed on the various fruits like mango and papaya.

Various Herbivorous butterfly larvae have *been* recorded as pest of *Mangifera* spp. Worldwide (Table 1). Although, present study revealed that nothing is known about the larval food of Andaman Baron, *E. a. acontius* in great details. The following are the list of butterflies recorded which utilizes the *M. indica* plant as their larval host.

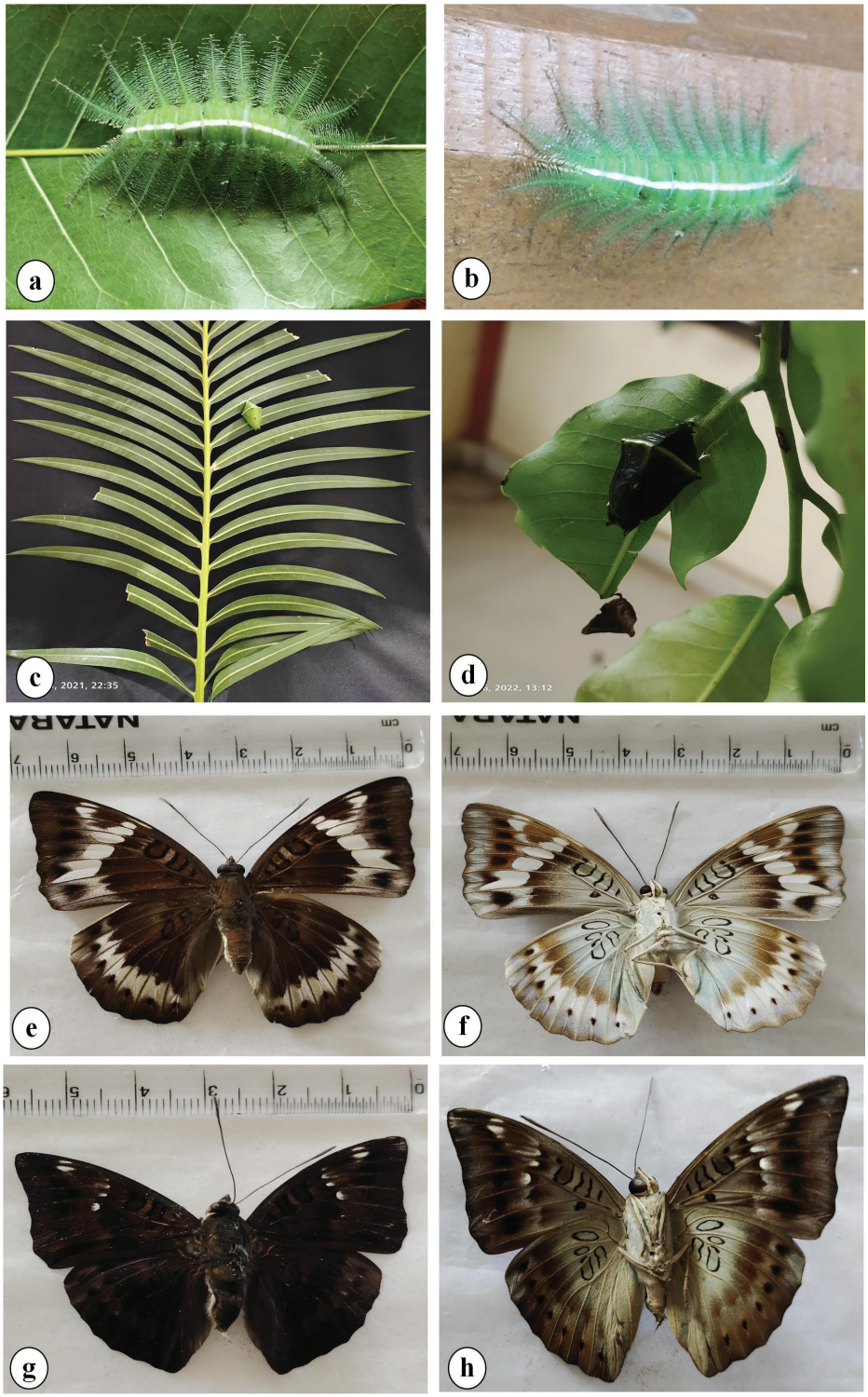


Fig. 3: *Mangifera* and other plant species used by *Euthalia aconthea acontius* for fulfill of various biological needs a: Pre pupa stage on *M. andamanica* leaf**; b:** Pre pupa stage on the door away from the host plant; c: Pupa on *Cycas zelanica* leaves; d: Parasitised pupa on *Polyalthia longifolia*; e: Female upper side; f: Female underside; g: Male upper side; h: Male under side;

Table 1: *Mangifera* spp. that were recorded as larval host plant for butterflies

S. No.	Mangifera species	Butterfly	Family	Location where reported	Record of references
1	*Mangifera andamanica* King	*Euthalia aconthea acontius* (Hewitson, 1874)	Nymphalidae	Andaman Islands	Present Study
2	*Mangifera griffithii* Hook. f.	*Euthalia aconthea acontius* (Hewitson, 1874)	Nymphalidae	Andaman Islands	Present Study
3	*Mangifera indica* L.	*Anthene emolus* (Godart, 1823)	Lycaenidae	India, Thailand	Robinson et al. 2023
4	*Mangifera indica* L.	*Anthene liodes* (Hewitson, 1874)	Lycaenidae	Southern Africa	Robinson et al. 2023
5	*Mangifera indica* L.	*Arhopala pseudocentaurus* (Doubleday, 1847)	Lycaenidae	Thailand	Robinson et al. 2023
6	*Mangifera indica* L.	*Callophrys herodotus* (Fabricius, 1793)	Lycaenidae	Neotropical	Robinson et al. 2023
7	*Mangifera indica* L.	*Calycopis cecrops* (Fabricius, 1793)	Lycaenidae	USA	Robinson et al. 2023
8	*Mangifera indica* L.	*Cheritra freja* (Fabricius 1793)	Lycaenidae	Laos	Robinson et al. 2023
9	*Mangifera indica* L.	*Chilades lajus* (Stoll, 1780)	Lycaenidae	India	Robinson et al. 2023
10	*Mangifera indica* L.	*Cyanophrys herodotus* (Fabricius, 1793)	Lycaenidae	Brazil	Robinson et al. 2023
11	*Mangifera indica* L.	*Euthalia aconthea* (Cramer, 1777)	Nymphalidae	Brunei India Sabah Thailand West Malaysia	Robinson et al. 2023; Kunte 2000
12	*Mangifera indica* L.	*Euthalia aconthea acontius* (Hewitson, 1874)	Nymphalidae	Andaman Islands	Robinson et al. 2023 &Veenakumari et al. 2008
13	*Mangifera indica* L.	*Euthalia aconthea garuda* (Moore, 1858)	Nymphalidae	India West Malaysia	Robinson et al. 2023
14	*Mangifera indica* L.	*Euthalia aconthea gurda* (Fruhstorfer 1906)	Nymphalidae	West Malaysia Sabah	Robinson et al. 2023
15	*Mangifera indica* L.	*Euthalia adonia* (Cramer, 1780)	Nymphalidae	West Malaysia	Robinson et al. 2023
16	*Mangifera indica* L.	*Euthalia alpheda* (Godart, 1824)	Nymphalidae	Philippines WestMalaysia	Robinson et al. 2023
17	*Mangifera indica* L.	*Euthalia anosia* (Moore, 1857)	Nymphalidae	India West Malaysia	Robinson et al. 2023
18	*Mangifera indica* L.	*Euthalia phemius* (Doubleday, 1848)	Nymphalidae	Hong Kong Oriental	Robinson et al. 2023
19	*Mangifera indica* L.	*Euthalia vasanta* (Moore, 1859)	Nymphalidae	India	Robinson et al. 2023
20	*Mangifera indica* L.	*Historis acheronta cadmus* (Cramer, 1775)	Nymphalidae	Puerto Rico	Robinson et al. 2023
21	*Mangifera indica* L.	*Horaga albimacula* (Wood-Mason & de Nicéville, 1881)	Lycaenidae	Laos	Robinson et al. 2023
22	*Mangifera indica* L.	*Phalanta phalantha* (Drury, 1773)	Nymphalidae	Thailand	Robinson et al. 2023

23	*Mangifera indica* L.	*Pseudo lycaena marsyas* (Linnaeus, 1758)	Lycaenidae	Brazil	Robinson et al. 2023
24	*Mangifera indica* L.	*Rapala iabus* (Fabricius, 1787)	Lycaenidae	Pakistan	Robinson et al. 2023
25	*Mangifera indica* L.	*Rapala manea (*Hewitson, 1863)	Lycaenidae	India	Robinson et al. 2023
26	*Mangifera indica* L.	*Rapala pheretima* (Hewitson, 1863)	Lycaenidae	West Malaysia Thailand	Robinson et al. 2023
27	*Mangifera indica* L.	*Rathinda amor* (Fabricius, 1775)	Lycaenidae	India	Robinson et al. 2023
28	*Mangifera indica* L.	*Spalgis epeus* (Westwood, 1851).	Lycaenidae	India	Robinson et al .2023
29	*Mangifera indica* L.	*Spalgis epius epius* (Westwood, 1851)	Lycaenidae	India	Nitin et al. 2018
30	*Mangifera indica* L.	*Tmolusechion echiolus* (Draudt, 1920)	Lycaenidae	Brazil	Robinson et al. 2023
31	*Mangifera indica* L.	*Euthalia aconthea vasanta* (Moore, 1858)	Nymphalidae	Sri Lanka	Jayasinghe et al. 2014
32	*Mangifera indica* L.	*Anthene emolus emolus* (Godart, 1824)	Lycaenidae	India	Nitin et al. 2018
33	*Mangifera indica* L.	*Rapala manea schistacea* (Moore, 1879)	Lycaenidae	India	Nitin et al. 2018
34	*Mangifera indica* L.	*Horaga onyx cingalensis* Moore, 1884)	Lycaenidae	India	Nitin et al. 2018
35	*Mangifera indica* L.	*Horaga viola* (Moore, 1882)	Lycaenidae	India	Nitin et al. 2018
36	*Mangifera indica* L.	*Rathinda amor* (Fabricius, 1775)	Lycaenidae	Sri Lanka	Jayasinghe et al. 2021
37	*Mangifera indica* L.	*Rathinda amor* (Fabricius, 1775)	Lycaenidae	India	Kalesh& Prakash 2007
38	*Mangifera indica* L.	*Euthalia aconthea meridionalis* (Fruhstorfer, 1913)	Nymphalidae	India	Young 1907 Bell 1909a Sevastopulo 1938 Pant & Chatterjee 1950 Wynter-Blyth 1957 Kunte 2000 Nitin et al. 2018
39	*Mangifera indica* L.	*Symphaedra nais* (Forster, 1771)	Nymphalidae	India	Young, 1907
40	*Mangifera pentandra* Hook.f.	*Euthalia alpheda* (Godart, 1824)	Nymphalidae	Thailand	Robinson et al. 2023
41	*Mangifera* **(Species name not available in literature)**	*Anthene lycaenina lycaenina* (R. Felder, 1868)	Lycaenidae	India	Nayanathara & Narayana, 2020
42	*Mangifera* **(Species name not available in literature)**	*Anthene liodes* (Hewitson, 1874)	Lycaenidae	East Africa	Robinson et al. 2023
43	*Mangifera* **(Species name not available in literature)**	*Euthalia alpheda* (Godart, 1824)	Nymphalidae	Indonesia	Robinson et al. 2023

Discussion

E. a. aconitus is an endemic sub species recorded here as pest of *Mangifera* species naturally occurring in ANI along with its impact on host plants and fruit feeding behavior. On the basis of observations made during present study, the choice of the host individuals corresponds with the natural habitat of the butterfly. The larva of *E. a. aconitus* feeds on the leaves of preferred host however the adult butterfly visited many other plants *viz.; Allamanda cathartica*, *Carica papaya*, *Cycas zeylanica*, *Polyalthia longifolia* for fulfill of various biological needs of the life. Among these plants, the genus *Cycas* also appeared as most susceptible larval host plant for butterflies in ANI. Numerous cycad species (*Cycas dharmrajii* L.J. Singh, *C. pschannae* R.C. Srivast. & L.J. Singh, *C. revoluta* Thunb. and *C. zeylanica* (J. Schust.) A. Lindstr. & K.D. Hill) offered as larval food influences fecundity of *Luthrodes pandava* Horsfield (Lepidoptera: Lycaenidae) in the islands ecosystem (Purti et al 2022, 2023). *L. pandava* also acts as a pest for endemic as well as introduced *Cycad* species in other parts of the world (Marler & Muniappan 2006; Marler et al 2012a,b, 2017; Purti et al 2022, 2023).

Except for few notable exceptions very little information has been recorded on the fruit feeding behavior of adult butterflies, it is also recorded here *in E. a. aconitus* for the first time from ANI's India. Fruit feeding behavior of adult butterflies evolved several times in nymphalid butterflies probably as an escape route from periods of low flower abundance (Hall & Willmott 2000; Shihan 2016; Krenn et al. 2001).

Under *ex-situ* observation it is found that the caterpillars of this pest their feeding preference with common *Mangifera* species, *M. indica*, and followed by *M. andamanica* and *M. griffithii. M. indica* is distributed throughout the islands, however, two host species *M. andamanica* and *M. griffithii* recorded here as a new hosts which are sparsely distributed and confined only in few localities. The IUCN status of *M. andamanica* and *M. griffithii* are near threatened and data deficient respectively. The host plant, *M. andamanica* is encountered only in reserve forest and protected areas of ANIs that are undisturbed habitat. While another host plant *M. griffithii* is found in Manipur Parvat National Park (Mt. Harriet).

Conclusions

The present study has revealed that the *Mangifera* spp. served as preferred host for both larva and adult form of the Andaman Baron butterfly, *Euthalia aconthea acontius* (Hewitson 1874) (Lepidoptera: Nymphalidae). Two endemic *Mangifera* spp. *viz. M. andamanica* and *M. griffithii* have been reports here for the first time as larval host plants from the ANI's, India. In the ANI's, it observed that only a single lepidopteran sub species, *E. a. acontius* larvae feeds on Mango host plants. It observed that *E. a. acontius* larvae acts as pests of both endemic and common *Mangifera* species in the ANI's. Due to natural calamities and anthropogenic pressures Mangifera species are under threatened condition. Besides distribution of endemic Mangifera species is sparsely confined only in few localities. In terms of declining populations, the present study revealed that in the near future this pest may assume more serious and destructive position specifically the endemic taxa. Although presently it is not reported as a serious pest of *Mangifera*.

Acknowledgements

Authors are grateful to the Director, Botanical Survey of India, Kolkata for constant support and facilities. We are also thankful to the Department of Environment and Forests, Andaman and Nicobar Islands, for necessary permission and logistic support in conducting field studies and to scientists and staff of Botanical Survey of India, teaching faculties of Department of Botany, Mansarovar Global University, who have always shown readiness for help. The authors are thankful to Dr. Debasis Bhattacharya, Editor in-Chief, Journal of Andaman Science Association, and anonymous reviewers for critical comments and suggestions that helped to improve the manuscript.

References

Bell, T.R. (1909a). The common butterflies of the plains of India (including those met with the hill stations of the Bombay Presidency). *Journal of the Bombay Natural History Society*, 19: 16–58.

Bompard, J.M. & Schnell, R.J. (1997). Taxonomy and Systematics. *In*: Litz, R.E., ed. The Mango: Botany, Production and Uses, CAB Intl., Wallingford, 21-47.

Dinesh, M.R., Hemanth, K.N., Vasabthaiah, K.N., Ravishankar, D., Thangadurai, P., Narayanswamy, Ali, Q., Kambiranda, D. & Basha, S.M. (2011). *Mangifera* in Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits (Ed: C. Koel). 61-74, https://dx.doi.org/10.1007/978-3-642-20447-0_4.

Dinesh, M.R., Ravishankar, K.V., Nischita, P., Sandya, B.S., Padmakar, B., Ganeshan, S., Chithiraichelvan, R. & Sharma, T.V.R.S. (2015). Exploration, Characterization and Phylogenetic Studies in Wild *Mangifera indica* Relatives. *American Journal of Plant Sciences*, 6, 2151-2160. http://dx.doi.org/10.4236 /ajps. 2015.613217.

e-Floras (2008). Missouri Botanical Garden, St. Louis, MO & Harvard Univ. Herbaria, Cambridge, MA, <http:// www.efloras.org>, accessed 9 September 2021.

GBIF (2020). GBIF backbone taxonomy.<https://www.gbif.org/species/>, accessed 13 January 2020.

Hall, J.P.W. & Willmott, K.R. (2000). Patterns of feeding behavior in adult male riodinid butterflies and their relationship to morphology and ecology. *Biological Journal of the Linnean Society,* 69: 1–23.

Hewitson, W.C. (1874). List of 49 butterflies collected by Capt. Wimberley in the Andaman Islands. *Annals and Magazine of Natural History* 14(4): 356–358.

Hooker, J.D. (1886). In The Flora of British India, Vol.1, L. Reeve & Co, London, pp. 7–44.

IUCN (International Union for Conservation of Nature). (2019). *Ochotona iliensis* (spatial data). *The IUCN Red List of Threatened Species*. *Version 20222*. https://www. iucnredlist.org. Accessed on 09 December 2022.

Jayasinghe, H.D., Rajapakshe S.S. & de Alvis, C. (2014). A compilation and analysis of food plants utilization of Sri Lankan butterfly larvae (Papilionoidea). *Taprobanica* Vol. 06, No. 02: pp. 110–131, pls. 12, 13.

Jayasinghe, H.D., Rajapakshe, S.S. & Ranasinghe, T. (2021). New additions to the larval food plants of Sri Lankan butterflies (Insecta: Lepidoptera: Papilionoidea). *Journal of Threatened Taxa,* 13(2):17731-17740.https://doi.org/10.11609/jot.6875.13.2.17731-17740

JSTOR (2020). Global Plants. JSTOR, Ithaka, <https://plants.jstor.org/>, accessed 28 July 2020.

Kalesh, S. & Prakash, S.K., (2007). Additions to the larval host plants of butterflies of the Western Ghats, Kerala, South India (Rhopalocera, Lepidoptera): Part 1. *Journal of Bombay Natural History Society*.104: 235-238.

Kostermans, A.J.G.H. & Bompard, J.M. (1993). The Mangoes: Their Botany, Nomenclature, Horticulture and Utilization. Academic Press, Waltham.

Krenn, H.W., Zulka, K.P. & Gatschnegg, T. (2001). Proboscis morphology and food preferences in nymphalid butterflies (Lepidoptera: Nymphalidae). *Journal of Zoology,* 254(1): 17–26.

Kumar, A. & Chakrabarty, T. (2021). Typification of two names in Mangifera (Anacardiaceae). *Journal of the Indian Association of Angiosperm Taxonomy*, 92-95, https://dx.doi.org/ 10.22244/rheedea.2021.31.02.11.

Kunte, K. (2000). Butterflies of Peninsular India. Universities Press (Hyderabad). *Indian Academy of Sciences* (Bangalore), 254 pp.

Marler, T.E. & Muniappan, R. (2006). Pests of *Cycas micronesica* leaf, stem, and male reproductive tissues with notes on current threat status. *Micronesica,* 39: 1–9.

Marler, T. E., Lindstrom, A. J. & Terry, L. I.(2012a). *Chilades pandava* damage among 85 *Cycas* species in a common garden setting. *Hort. Science,* 47(12): 1832–1836.

Marler, T.E., Lindstrom, A. J. & Terry, L. I. (2012b). Information-based or resource-based systems may mediate *Cycas* herbivore interactions. *Plant Signaling & Behavior*, 7: 760–762.

Marler, T.E., Lindstrom. A. J. & Marler, P. N. (2017). Diversity in *Cycas* (Cycadales: Cycadaceae) Species Offered as Larval Food Influences Fecundity of *Chilades pandava* (Lepidoptera: Lycaenidae) Adults. *International Journal of Insect Science,* 9: 1–6.

Mukherjee, S.K. (1953). Origin, Distribution and Phylogenetic Affinity of the Species of Mangifera L. *Journal of the Linnean Society of London*, Botany, 55, 65-83. http://dx. doi.org/10.1111/j.1095-8339.1953. tb00004.x.

Mukherjee, S.K. (1985). Systematic and Ecogeographic Studies on Crop Genepool: Mangifera L. International Board for Plant Genetic Resources, Rome, 86.

Nayanathara, J. & Narayana, R. (2020). Mango: A new host plant for the lycaeinid *Anthene lycaenina lycaenina* (R. Felder, 1868) ENTOMON 45(3): 237-238 Short Communication No. ent. 45310.

Nitin, R., Balakrishnan, V.C., Churi, P.V., Kalesh, S., Prakash,S. & Kunte, K. (2018). Larval host plants of the butterflies of the Western Ghats, India. *Journal of Threatened Taxa*, 10(4):11495–11550; http:// doi.org/ 10.11609/jott.3104.10.4.11495-11550.

Pant, G.D. & Chatterjee, N.C. (1950). A list of described immature stages of Indian *Lepidoptera, Part I: Rhopalocera*. *Indian Forest Records*. (New Series Entomology) 7:213–255.

Purti, N., Singh, L.J & Pandey, A. K. (2022). New hosts for the cycad blue butterfly, *Luthrodes pandava,* Horsfield (Lepidoptera: Lycaenidae) in an Island ecosystem *Feddes Repertorium.* 133(3): 234-243.

Purti, N.; Singh, L.J. & Pandey, A.K. (2023). Life history traits of cycad blue butterfly, *Luthrodes pandava,*Horsfield (Lepidoptera: Lycaenidae) on cycad hosts in an island ecosystem. *Feddes Repertorium* https://doi.org/10.10 02/fedr.202300001,

Robinson, G.S., Ackery, P.R., Kitching, I.J., Beccaloni, G.W. & Hernández, L.M. (2010). HOSTS-A Database of the World's Lepidopteran Host Plants. *Natural History Museum*, London. http://www.nhm.ac.uk/ hosts.

Robinson, G.S., Ackery, P.R., Kitching, I., Beccaloni, G.W. & Hernández, L.M. (2023). HOSTS (from HOSTS - a Database of the World's Lepidopteran Hostplants) [Data set resource]. Natural History Museum. https:// data.nhm.ac.uk/dataset/hosts/ resource /877f387a-36a3-486c-a0c1-b8d5fb 69f85a

Sevastopulo, D.G. (1938). The early stages of Indian Lepidoptera. *Journal of the Bombay Natural History Society*, 40: 391–408.

Shihan, T.R. (2016). An observation on the fruit feeding behavior of butterflies in some areas of Bangladesh. *Journal of Threatened Taxa,* 8(12):9479–9485; http:// dx.doi.org/ 10.11609/jott.2519. 8.12.9479-9485

Singh, L.J. & Murugan, C. (2014). Seed plant species diversity and conservation in Dhanikhari Experimental Garden cum Arboretum in Andaman and Nicobar Islands. *In* : Nehera, S; Gothwal, R.K. & Ghosh, P.(eds.) *Biodiversity in India : Assessment , scope and conservation.* Lambert Academic Publishing Heinrich-Booking-str. Saarbruken, Germany. 253-280.

Singh, L. J., Murugan,C. & Singh, P. (2014). Plant Genetic Diversity of Endemic Species in the Andaman and Nicobar Islands. *In*: *Nat. Conf. On Islands Biodiversity, U. P. State Biodiversity Board, Lucknow* 49-57.

Singh, L.J. & Misra, D.R. **(**2020): Reappraisal of the genus *Cycas* L. (Cycadaceae) in Andaman and Nicobar Islands, India. *–Indian Journal of Forestry*. 43(1):46-57.

Singh, L. J., Ekka, G.A., Sanjay Mishra, S., Vivek, C.P., Shankar, V.S. Naik, M.C. & Saleem F. (2020a): Habitat status of *Musa paramjitiana* L.J. Singh (Musaceae): a critically endangered, endemic species in Andaman and Nicobar Islands, India. *Pleione* 14(1):121 - 127.

Singh, L. J., Dwivedi, M.D., Kasana, S., Naik, M.C., Ekka, G.A. & Pandey, A.K. (2020b). Molecular systematics of the genus Musa L. (Zingiberales: Musaceae) in Andaman and Nicobar Islands. *Biologia* https://doi. org/ 10.2478/s11756-020-00552-5.

Singh, L. J. & Ranjan, V. (2021). *New Vistas in Indian Flora*. Vol. 1 & 2: Bishen Singh Mahendra Pal Singh, Dehra Dun, Uttarakhand, India, pp. 417& 819.

Singh, L. J., Ekka, G.A., C.P. Vivek & Misra, D.R. (2021a). Gymnosperms of the Andaman and Nicobar Islands: An Overview (In:eds. L.J. Singh & V. Ranjan, *New Vistas in Indian Flora*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1: 265-278.

Singh, L. J., Ranjan V., Sinha, B.K., Mishra, S., Purohit, C.S. Vivek C.P., Naik, M. C. and Ekka, G.A. (2021b). An Overview of Phytodiversity of the Andaman and Nicobar Islands, India. (*In*: eds. L.J. Singh & V. Ranjan, *New Vistas in Indian Flora*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 2: 381- 399.

Tara, J.S. & Gupta, M. (2016). First record of *Euthalia aconthea* (Lepidoptera: Nymphalidae), an important pest on Mango (*Mangifera indica*) from Jammu region. *International Journal of Entomology Research* 1(1): 01-03.

The Herbarium Catalogue (2021). R. Bot. Gard. Kew, <www.kew.org/herbcat>, accessed 9 September 2021.

The Plant List (2013). Ver. 1.1. <www. theplantlist.org/>, accessed 1 January 2013.

Varshney, R.K. & Smetacek, P. (eds.) (2015). A Synoptic Catalogue of the Butterflies of India. Butterfly Research Centre, Bhimtal & Indinov Publishing, New Delhi, pp.261.

Veenakumari, K., Mohanraj, P., Srivastava, R.C. and Jayakumar, V. (2008). Butterflies of Andaman and Nicobar Islands. *Central Agricultural Research Institute*, Port Blair, Andaman and Nicobar Islands, India, pp. 186.

WCSP (2012). World checklist of selected plant families. Facilitated by the R. Bot. Gard.Kew, <http://wcsp. science. kew.org/>, accessed 3 April 2017.

Wynter-Blyth, M.A. (1957). Butterflies of the Indian region. Oxford. *Bombay Natural History Society*, Bombay, 523pp

Young, L.C.H. (1907). The common butterflies of the plains of India. *Journal of the Bombay Natural History Society* 17: 921–927.

Received: 02nd November 2023 Accepted: 30th November 2023

Journal of the Andaman Science Association Vol. 28(2):188-192 (2023)
ISSN 0970-4183, Printed in India

Teramnus P. Browne (Papilionadeae- Leguminosae) - A new addition to flora of Andaman and Nicobar Islands, India.

Fouziya Saleem[1, 2], Lal Ji Singh[1]*, and Arun K. Pandey[2]
[1]*Botanical Survey of India, Andaman and Nicobar Regional Centre, Port Blair, Andaman and Nicobar Islands, India.*
[2]*Department of Botany, Mansarovar Global University, Sehore-466111, India.*
***Corresponding author's E-mail:** Lal Ji Singh (laljisingh1970@rediffmail.com), Botanical Survey of India, Andaman and Nicobar Regional Centre, Port Blair, Andaman and Nicobar Islands, India.*

Abstract

The genus *Teramnus* P. Browne is reported and described here as new record with single species *Teramnus mollis* Benth., for the first time from Andaman and Nicobar Islands, India.

Key words: *Leguminosae, papilionadeae, taxonomy, Teramnus*

Introduction

Teramnus P. Browne is the genus of the agriculturally important legumes family *Fabaceae* (Leguminosae) and distributed in tropical and subtropical regions with eight species. In India, the genus *Teramnus* is found throughout from Himalayan southwards and represented by only four species *viz, Teramnus flexilis* Benth., *Teramnus mollis* Benth., *Teramnus labialis* (L.f.) Spreng. and *Teramnus repens* (Taub.) Baker f. subsp. *gracilis* (Chiov.) Verdc. (Sanjappa 1992, 2020).

During botanical exploration in the Andaman group of islands, we encountered *T. mollis* and recorded and described here as an addition to the flora of Andaman and Nicobar Islands (ANI) with a generic record for the first time. ANI are a group of 572 Islands and islets and well recognized centre of hot spots of bio diversity with higher number of endemism (Singh et al., 2014, 2021a, b; Singh & Misra 2020; Singh & Ranjan 2021; Singh 2021, 2023; Sivaramakrishna et al., 2021) where the family *Fabaceae* has received little attention from taxonomists. Although Legumes of ANI are recorded time to time by various authors (Vasudeva Rao., 1986; Sanjappa, 1992; Lakshminarasimhan & Rao., 1996; Hajra et al., 1999; Pandey & Diwakar, 2008; Singh & Murugan, 2014; Murugan et al.,2016; Naik et al., 2020; Naik & Singh 2020, Singh et al., 2021, Sanjappa, 2020). Recent floristic explorations have resulted in discoveries of novelties in legumes (Saleem et al., 2023a, b).

The legumes collections from ANI were examined and found that found only one specimen deposited at the Herbaria of BSI (PBL) which was collected by *M.Venkat Ramana & Johny Kumar Tagore* (0055) in 2011 and identified only at generic level. This specimen is matches with our present collection. After critical examination, this have been identified as *Teramnus mollis* Benth., which is reported here as new generic record with single species. Currently *T. mollis* is the only species representing the genus *Teramnus* P. Browne from the ANI.

Material and methods

To verify the identity of the specimens critical analysis of morphological characters was carried out by comparing our collections with the herbarium specimens from Indian herbaria (CAL, PBL) and online taxonomic databases and digital herbaria (https://ivh.bsi.gov.in/,e-Floras2008; WCSP 2012; The Plant List 2013; POWO 2019; GBIF 2020; JSTOR 2020; The Herbarium Catalogue 2021) relevant taxonomic literature was also consulted. Herbarium specimens of the species are deposited in National Repository Herbarium (PBL) of Botanical Survey of India, Andaman and Nicobar Regional Centre. Field photographs of the species are provided for easy identification.

Result

Taxonomic Treatment

Teramnus mollis Benth., J. Linn. Soc. Bot. 8: 265. 1865. Baker in Hook. F., Fl. Brit. India 2:185.1876.

Perennial climber, trailing, branchlets terete, with white ferruginous hairs. Leaves trifoliolate, 10 x 6 cm, petioles 3cm long, glabrous; leaflets 3,ovate, acuminate at apex, rounded at base, entire on margin, glabrous on both surfaces. Inflorescences raceme; Flowers 1cm long, pedicellate, pedicels 4 mm long, hairy; bracts 5mm, lanceolate, acuminate at apex, hairy. Bracteole 3mm long, lanceolate, acuminate at apex, hairy; Calyx 1cm long, with adpressed hairs; calyx tube 8mm long, calyx teeth 2 mm, lanceolate, equal. Corolla white with purple tinge; standards 8x3mm, obovate, emarginate at apex, glabrous, wing petals 7x2 mm, oblong, truncate at base; keel petals 5x2 mm, slightly falcate. Stamens 10, diadelphous (9+1); staminal tube 6 mm, anthers uniform, 2mm long. Pod 7cm, linear, with adpressed hairs, beaked, beak 1 mm long, 8-10 seed per pod. Seeds almost cylindrical, covered with adpressed hairs, 4mm long and 3mm diam, blackish brown, aril slightly developed.

Flowering & fruiting: November-February.

Habitat and Ecology: Edges of Evergreen forest with moist soil. It grows in association with *Dysolobium pilosum* (J.G.Klein ex Willd.) Maréchal, *Chromolaena odorata* (L.) R.M. King & H.Rob. and *Mesosphaerum suaveolens* (L.) Kuntze.

Distribution: India: Assam, Manipur, West Bengal; **ANI:** Andaman Islands (Present work); **World:** Bangladesh, Bhutan, Burma and Thailand.

Conservation status

Taxon is currently known from a few localities of North and Middle Andaman. Distribution in two different localities indicates that it is quite probable that the species also found in other Islands. Due to lack of data on population distribution, the conservation status of *T. mollis* Benth. is assessed here as 'Data Deficient' (DD) category according to the IUCN criteria (IUCN 2020) for ANI.

Specimens examined: INDIA, Andaman & Nicobar Islands, Middle Andaman, Kalara Junction, 12°51'26"N, 92° 51'40"E. Alt. 53, 13.12.2021, *Fouziya Saleem* 33418; North Andamans, Kalara Junction, 13°11'07"N, 92°55'45"E. Alt. 74m, 19.12.2021, *Fouziya Saleem* 33431; North Andaman, Kalighat, 18.02.2011, *M. Venkat Ramana & Johny Kumar Tagore* 0055.

Discussion

ANI is one of the richest and unique phytogeographical region in India with higher number of endemism (Singh et al. 2014, 2021a, b; Singh & Ranjan 2021) whereas Leguminosae need to be explored in more depth to understand the diversity (Saleem et al., 2023a, b). During the plant explorations carried out in Andaman group of Islands, authors spotted the leguminous genus *Teramnus* P. Browne and described here as new generic record for the flora of ANI with single species *Teramnus mollis* Benth.

Acknowledgements

Authors are grateful to the Director, Botanical Survey of India, Kolkata for constant support and facilities. We are also thankful to the Department of Environment and Forests, Andaman and Nicobar Islands, for necessary permission and logistic support for conducting field studies and to scientists and staff of Botanical Survey of India, teaching faculties of Department of Botany, Mansarovar Global University, who have always shown readiness for help. The authors are thankful to Dr. Debasis Bhattacharya, Editor in-Chief, Journal of Andaman Science Association, and anonymous reviewers for critical comments and suggestions that helped to improve the manuscript.

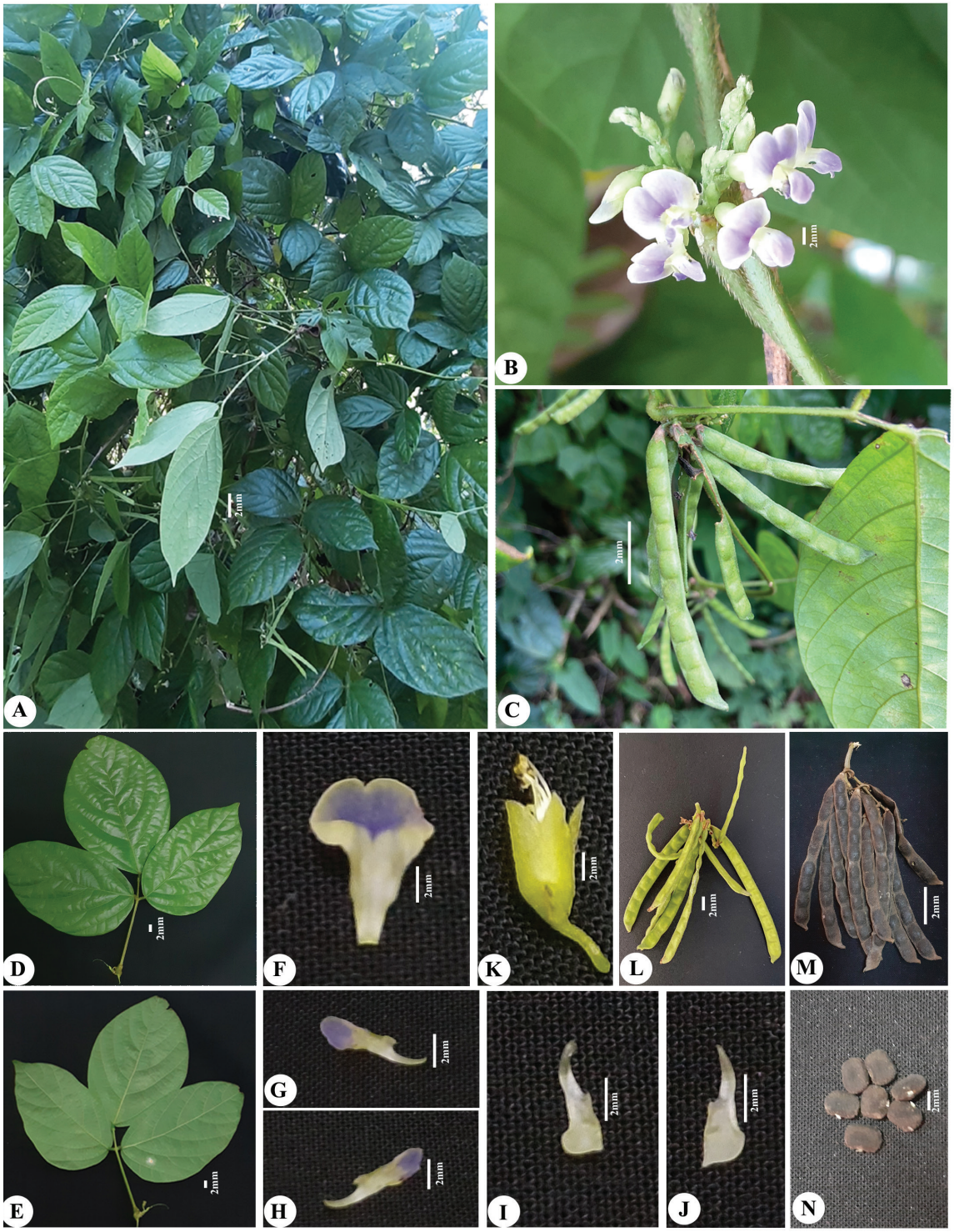


Fig.1: Teramnus mollis A: Habit, B: Flower; C: Fruit; D: Leaf (dorsal), E: Leaf (ventral),F:Standard petals, G-H: Wing petals, I-J: Keel petals, K: Calyx with Stamens, L-M: Pods, N: Seeds.

References

eFloras (2008). Missouri Botanical Garden, St. Louis, MO & Harvard Univ. Herbaria, Cambridge, MA, <http://www.efloras.org>, accessed 9 September 2021.

GBIF (2020). GBIF backbone taxonomy. <https://www.gbif.org/species/>, accessed 13 January 2020.

IUCN (2020). The IUCN Red List of Threatened Species, version 2020–2. IUCN Red List Unit, Cambridge U.K. Available from: https://www. iucnredlist.org (accessed 9 July 2020).

JSTOR (2020). Global plants. JSTOR, Ithaka,<https://plants.jstor.org/>, accessed 28 July 2020.

Hajra, P.K., Rao, P.S.N. & Mudgal, V. (1999). Flora of Andaman-Nicobar Islands (Ranunculaceae–Combretaceae) Botanical Survey of India, Calcutta, 1, pp. 1-487.

Lakshminarasimhan, P. & Rao, P.S.N. (1996). A supplementary list of Angiosperms recorded from Andaman and Nicobar Islands. *Journal of Economic and Taxonomic Botany* 20:175-185.

Murugan, C., Prabhu, S., Sathiyaseelan, R. & Pandey, R. P. (2016). A Checklist of Plants of Andaman and Nicobar Islands.ENVIS Centre on Floral Diversity. Botanical Survey of India, Kolkata. http://bsienvis.nic.in/ Database/ Checklist-of-Andaman-NicobarIslands244 27. aspx.

Naik, M.C. & Singh, L.J. (2020). Two legume species additions to the flora of Andaman & Nicobar Islands, India. *Abrahamia* 5(1): 1–4.

Naik, M.C., Singh, L.J. & Ganeshaiah, K.N. (2020). Floristic diversity and analysis of South Andaman Islands (South Andaman District), Andaman & Nicobar Islands, India. *Species,* 21(68): 343– 409.

Pandey, R.P. & Diwakar, P.G. (2008). An integrated checklist of plants in Andaman & Nicobar Islands, India. *Journal of Economic and Taxonomic Botany,* 32: 403–500.

POWO (2019). Plants of the World Online. Facilitated by the R. Bot. Gard. Kew, <www.plantsoftheworldonline.org/>, accessed 1 May 2021.

Rao, M.K.V. (1986). A preliminary report on the angiosperms of Andaman and Nicobar Islands. *Journal of Economic and Taxonomic Botany* 8: 107–184.

Saleem, F., Singh, L.J. & Pandey, A.K. (2023a) Two additions to the Legumes Flora of Andaman and Nicobar Islands, India. *Journal of the Andaman Science Association* 28(1):40-46.

Saleem, F., Singh, L.J., Subramaniam, S., Pandey, A.K. (2023b). *Crotolaria nicobarica* (Fabaceae): a new species from Andaman and Nicobar Islands, India. Doi: 10.1111/njb.04077.

Sanjappa, M. (1992). Legumes of India. Bishen Singh Mahendra Pal Singh, Dehra Dun.

Sanjappa, M. (2020). Fabaceae (=Leguminosae, nom alt.). In: Ashiho Asosii Mao and Sudhansu Sekhar dash (Eds.), Flowering Plants of India, An Annotated Checklist (Dicotyledons). 1:300-446.

Singh, L.J. (2021). *Septemeranthus* (Loranthaceae), a new monotypic genus from the Andaman and Nicobar Islands, India and its relationship with allied genera. *Feddes Repertorium* 132:193–203.

Singh, L.J. (2023). *Dendrophthoe longensis* L.J. Singh, A new species of Dendrophthoe (Loranthaceae) from Andaman and Nicobar Islands India. *Feddes Repertorium* 134(1):54–65.

Singh, L.J., Misra, D.R. (2020). Reappraisal of the genus Cycas L. (Cycadaceae) in Andaman and Nicobar Islands, India. *Indian Journal of Forestry*. 43(1):46-57.

Singh, L.J. & Murugan, C. (2014). Seed plant species diversity and conservation in Dhanikhari Experimental Garden cum Arboretum in Andaman and Nicobar Islands. *In*: Nehera, S; Gothwal, R.K. & Ghosh, p.(eds.) *Biodiversity in India : Assessment, scope and conservation.* Lambert Academic Publishing Heinrich- Booking- str. Saarbruken, Germany. 253-280.

Singh, L.J.; Murugan, C. & Singh, P. (2014). Plant Genetic Diversity of Endemic Species in the Andaman and Nicobar Islands. – *In: National Conference on Islands*

Biodiversity, U.P. State Biodiversity Board, Lucknow 49–57.

Singh, L.J. & Ranjan, V. (2021). *New Vistas in Indian Flora*. vol. 1 & 2:Bishen Singh Mahendra Pal Singh, Dehra Dun, Uttarakhand, India, pp. 417& 819.

Singh, L.J., Ranjan V., Sinha, B.K., Mishra, S., Purohit, C.S., Vivek C.P., Naik, M.C. & Ekka, G.A. (2021). An Overview of Phytodiversity of the Andaman and Nicobar Islands, India. (In:eds. L.J. Singh & V. Ranjan, *New Vistas in Indian Flora*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 2: 381- 399.

Singh, L.J., Ekka, G.A., Vivek, C.P., Misra, D.R. (2021a). Gymnosperms of the Andaman and Nicobar Islands: An Overview (*In*: eds. L.J. Singh & V. Ranjan, *New Vistas in Indian Flora*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1: 265-278.

Singh, L.J., Ranjan V., Sinha, B.K., Mishra, S., Purohit, C.S., Vivek C.P., Naik, M.C., Ekka, G.A. (2021b). An Overview of Phytodiversity of the Andaman and Nicobar Islands, India. (*In*: eds. L.J. Singh & V. Ranjan, *New Vistas in Indian Flora*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 2: 381- 399.

Sivaramakrishna P., Yughandhar P. & Singh L.J. (2021) *Crotalaria lamelliformis* (Fabaceae: Crotalarieae), a new species from Eastern Ghats of Andhra Pradesh, Peninsular India. *Phytotaxa* 490(1):71-81.

The Herbarium Catalogue (2021). R. Bot. Gard. Kew, <www.kew.org/herbcat>, accessed 9 September 2021.

The Plant List (2013). Version 1.1. Published on the Internet: http://www.theplantlist.org/ (accessed 28.12.2018).

WCSP (2012). World checklist of selected plant families. Facilitated by the R. Bot. Gard. Kew, <http://wcsp. science. kew.org/>, accessed 3 April 2017.

Received: 02nd November 2023 Accepted: 15th November 2023

Journal of the Andaman Science Association Vol. 28(2):193-197 (2023)
ISSN 0970-4183, Printed in India

Study on length-weight relationship and condition factor for few freshwater fish species from Southern India

N. Daniel
Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India
***Corresponding author's E-mail:** danielfnft@gmail.com*

Abstract

The present study aimed to estimate the length-weight relationship (LWR) and condition factor for three freshwater fish species: *Puntius vittatus* Day, 1865, *Aplocheilus lineatus* (Valenciennes, 1846), and *Pseudosphromenus cupanus* (Cuvier, 1831) which had not been previously documented in FishBase. The study was conducted by collecting of fish specimens weekly from the tributaries of Pechiparai reservoir located in Kanyakumari District of Tamil Nadu, India, between January 2021 to January 2022. The results indicated that the estimated mean allometric coefficient values (b value) ranged from 2.51 to 2.74, while the mean intercept values (a value) ranged from 0.016 to 0.019. Additionally, the mean condition factor (K) ranged from 0.9 to 1.61. It was observed that the b values for all three freshwater fish species were below 3, indicating negative allometric growth. However, these values fell within the recommended range of 2.5-3.5 for healthy fish. Furthermore, K values ranged from 0.9 to 1.61, which also fell within the recommended range for healthy fish. The findings of this study provide valuable information on the basic biology of these fish species, which can contribute to the conservation and management of fishery resources in the Southern Indian regions of Kanyakumari District, Tamil Nadu, India.

Key words: Cyprinidae, aplocheilidae, osphronemidae, fish sampling, length and weight, condition factor, pechiparai reservoir.

Introduction

Fisheries are an essential aspect of many countries' economies and play a crucial role in providing food and income for millions of people worldwide. India is home to a wide variety of freshwater fish species, many of which are economically important and play a significant role in the food security of local communities. However, the sustainability of these fisheries is threatened by overfishing, pollution, habitat destruction, and other factors. One of the fundamental aspects of fisheries management is to understand the basic biology of the fish species, such as their growth, reproduction, and mortality rates. The length-weight relationship (LWR) and condition factor (K) are two essential parameters that provide useful information on the growth and overall health of fish populations (Sekitar *et al*., 2015; Jisr *et al*., 2018; Pouladi *et al*., 2020; Das *et al*., 2021; Kumar *et al*., 2023). The LWR provides a simple method of estimating the weight of fish from their length and is widely used in fisheries science to evaluate growth and size at age (Stanislas *et al*., 2023). The K factor, on the other hand, is a measure of fish plumpness or condition and can indicate the overall health and wellbeing of fish populations (Arafat *et al*., 2022)

Recently, there has been increasing interest in studying the LWR and K factor of many fish species across the world, including India (Ali *et al*., 2014; Ferdaushy *et al*., 2015; Seiyaboh *et al*., 2016; Jisr *et al*., 2018; Batubara *et al*., 2019; Pouladi *et al*., 2020; Das *et al*., 2021; Daniel *et al*., 2021; Çiçek *et al*., 2022; Kumar *et al*., 2023). Despite this, many freshwater fish species lack such information, especially in Southern India. To address this gap, this study aims to estimate the length-weight relationship and condition factor of three freshwater fish species (*P. vittatus*, *A. lineatus*, and *P. cupanus*) which have been scarcely studied and remain unreported in FishBase.

MATERIALS AND METHODS

During the period from January 2021 to January 2022, fish specimens from four sites [(8°09'08.7''N 77°29'48.9''E); (8°09'09.9''N 77°28'30.1''E); (8°08'42.8''N 77°27'22.4''E); (8°08'28.3''N

77°26'53.3''E)] in Kanyakumari, South India were collected for the purpose of studying the length-weight relationship (LWR) and condition factor of three freshwater fish species. The fish specimens were captured using a hand net with a mesh size of 2mm and brought to the laboratory alive for further analysis. Standard literature was used to confirm the species identity, and measurements of their total length (TL) and weight (W) were taken with an accuracy of 0.1 cm and 0.01 g, respectively. The LWR was calculated using the Lecren formula $W= a L^b$, where W is the total weight (g), L is the total length (cm), and a and b are the intercept and slope of the regression curve (Lecren, 1951). To establish the LWR, outliers were excluded by plotting them on a graph (Froese, 2006). The Fulton condition factor (K) was determined using the standard equation $K = W/L^3 \times 100$, where W is the body weight in grams, L is the total length in centimeters, and the factor 100 is used to bring K close to unity (Okgerman, 2004). Statistical analysis was performed using Excel 2010.

RESULTS

In the present study, we established the length-weight relationships for three freshwater fish species (*P. vittatus*, *A. lineatus*, and *P. cupanus*) which is not previously available for these species which are represented in Table 1. The total length of the specimens ranged from 1.4-7 cm and total weight varied from 0.05-2.69 g. The estimated mean 'b' values ranged from 2.51-2.74, and mean 'a' values ranged from 0.016-0.019. Additionally, the results showed a reliable co-efficient determination (r^2) ranging between 0.973-0.988, indicating the data's reliability. The study also reports the Fulton condition factor (K) values, ranging from 0.9-1.61, for these three fish species.

DISCUSSION

The estimation of fish length and weight provides valuable information on their growth patterns in relation to their size. The allometric coefficient (b value) is a useful parameter in this regard, with an optimal range typically falling between 2.5 and 3.5 for most fish species (Froese, 2006). A 'b' value of 3 indicates isometric growth, while 'b' values less than 3 suggest negatively allometric growth and 'b' values greater than 3 indicate positively allometric growth (Rahman *et al*., 2021). In this study, we estimated the 'b' value for three freshwater fish species (See Table 1). *P. vittatus* exhibited an estimated 'b' value ranging from 2.69-2.79 with a mean 'b' value of 2.74. *A. lineatus* had an estimated 'b' value ranging from 2.45-2.56 with a mean 'b' value of 2.51. Similarly, *P. cupanus* exhibited an estimated 'b' value ranging from 2.54-2.64 with a mean 'b' value of 2.59.

All three freshwater fish species examined in this study exhibited b values less than 3, indicating negative allometric growth (see Table 1). Nonetheless, these values fall within the recommended range of 2.5-3.5 for fish species suggested by Froese (2006). *P. vittatus*, also known as the Greenstripe barb, belongs to the Cyprinidae family and is predominantly found in inland waters of India, Pakistan, and Sri Lanka. In our study, the estimated 'b' value for *P. vittatus* was 2.74. This value is higher than that reported for *Puntius ticto* (1.8032) from the Aasan River in Uttarakhand, India (Bahuguna *et al*., 2021), but slightly lower than the 'b' value of *Puntius conchonius* (2.94) from Dal Lake in Kashmir, India, as documented by Shafi *et al*. (2012). Furthermore, the 'b' value of *Puntius sophore* (3.142) from the Ganga River in Bihar, India, as reported by Ahirwal *et al*. (2022) is higher than that of *P. vittatus*. *A. lineatus*, known as the Striped panchax, is a colorful killifish variety belonging to the Aplocheilidae family, mostly inhabiting streams, rivers, swamps, and paddy fields in both freshwater and brackishwater environments. The estimated 'b' value for *A. lineatus* in this study was 2.51. However, no length and weight relationship data were available for comparison with congeners. *P. cupanus*, also referred to as the Spiketail paradisefish, is a colorful freshwater fish belonging to the Osphronemidae family, commonly found in shallow bodies of water characterized by slow-moving or stagnant conditions, such as ditches and paddy fields. Again, no length and weight relationship data were available for comparison with congeners. The negative allometric growth observed in these fish suggests that they tend to become slightly slimmer as their length increases, as noted by Jobling (2002). The variation in b value could be attributed to a combination of factors such as the

number of species examined, the environment in which they reside, fullness index, maturity stages of gonads, sex, and differences in length range of the specimens (Froese, 2006; Li *et al*., 2014; Sharma *et al*., 2016). Thus, future studies are warranted to determine the exact cause of the low b values observed in these fishes.

Table 1: Estimated length-weight relationship parameters and condition factor for three freshwater fish species

Family	Species	N	TL range (cm)	W range (g)	a (±95% Cl)	b (±95% Cl)	r^2	Condition factor (K)
Cyprinidae	*Puntius vittatus* Day, 1865	150	1.4-4.7	0.05-1.47	0.017 (0.016-0.018)	2.74 (2.69-2.79)	0.988	1.61
Aplocheilidae	*Aplocheilus lineatus* (Valenciennes, 1846)	230	1.6-7	0.08-2.69	0.016 (0.015-0.017)	2.51 (2.45-2.56)	0.973	1.36
Osphronemidae	*Pseudosphromenus cupanus* (Cuvier, 1831)	270	1.7-5.5	0.05-1.31	0.019 (0.018-0.020)	2.59 (2.54-2.64)	0.974	0.9

Note: N, sample size; TL range, total length range of a fish; W range, total weight range of a fish; a, intercept value; b, allometric coefficient value; Cl, confidence limits; r^2, coefficient of determination.

The Fulton condition factor (K) is a widely used parameter in fisheries and aquaculture to evaluate the overall health and condition of fish (Seher & Suleyman, 2012). It is calculated by dividing a fish's weight by its length cubed, and provides an indication of the fish's nutritional status and growth potential. In the present study, the K values determined for *P. vittatus*, *A. lineatus*, and *P. cupanus* were 1.61, 1.36, and 0.9, respectively. Typically, fish weight increases as length increases, and low K values suggest inadequate nutrition for proper growth, whereas high K values indicate that the fish are receiving sufficient food in their habitat (Perry *et al*., 1996). In the present study, K values observed ranged from 0.9 to 1.61, falling within the recommended range for healthy fish. Notably, the estimated K value for *P. vittatus* (1.61) exceeded the value reported for *Puntius sophore* (1.06 for males and 1.22 for females) in the Ganga River, Bihar, India (Ahirwal *et al*., 2022). However, it was lower than the K value of *Puntius ticto* (0.719) from the Aasan River in Uttarakhand, India (Bahuguna *et al*., 2021), and slightly lower than the K value of *Puntius conchonius* (ranging from 1.74 to 2.10) in Dal Lake, Kashmir, India (Shafi *et al*., 2012). K value of ≥ 1 suggests that the fish are receiving adequate nutrition and living in favorable environmental conditions (Ujjania *et al*., 2012). It is important to note that several factors can influence K values, including feeding intensity, food availability, fish size, age, sex, season, maturation stage, gut fullness, muscle development, fat reserves, and life history (Bagenal and Tesch, 1978; Ujjania *et al*., 2012; Gupta and Banerjee, 2015). Therefore, it is crucial to consider these factors when interpreting K values in fish.

CONCLUSION

In conclusion, this study has provided preliminary data on the length-weight relationship and condition factor of three freshwater fish species (*P. vittatus*, *A. lineatus*, and *P. cupanus*) from the tributaries of Pechiparai reservoir, located in the Southern Western Ghats region of Kanyakumari District, Tamil Nadu, India. The data generated in this study serve as baseline data for future efforts in managing and conserving the fishery resources of these three species in their natural habitats.

ACKNOWLEDGEMENTS

Author would like to express their gratitude to the Vice-Chancellor of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India, for providing the necessary infrastructure and the freedom to carry out this study. The authors extend their special thanks to Mr. T. Salomon, Mr. A. Sunil Kumar, and Mr. S. Angel Santo Rayen, Kanyakumari Parakkai Centre for Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, for their valuable help in collecting fish specimens used in this study.

REFERENCES

Ahirwal, S., Singh, J., Kumar, T., Bharti, V., Sarma, K. and Narayan, D., 2022. Biometric evaluation of Gangetic pool barb *Puntius sophore* (Hamilton, 1822) from the River Ganga, Bihar, India. *Indian J. Fish*, *69*(4): 52-58.

Ali, S., Barat, A., Kumar, P., Sati, J., Kumar, R. and Haldar, R.S., 2014. Study of length-weight relationship and condition factor for the golden mahseer, *Tor puttiora* from Himalayan rivers from India. *Journal of Environmental Biology*, *35*(1): 225-228.

Arafat, M.Y. and Bakhtiyar, Y., 2022. Length-weight relationship, growth pattern and condition factor of four indigenous cypriniform Schizothorax species from Vishav Stream of Kashmir Himalaya, India. *Journal of Fisheries*, *10*(1): 101202-101202.

Bahuguna, P.A.N.K.A.J., Selakoti, Ankita., Rayal, Rajesh. and Joshi, H.K., 2021. Length-weight relationships and relative condition factor of *Puntius ticto* in the Aasan River, Uttarakhand, India. *Uttar Pradesh Journal of Zoology*, *42*(14): 77-83.

Batubara, A.S., Muchlisin, Z.A., Efizon, D., Elvyra, R. and Irham, M., 2019. Length-weight relationships and condition factors of the naleh fish, *Barbonymus gonionotus* (Pisces, Cyprinidae) harvested from Nagan Raya Waters, Indonesia. *Vestnik Zoologii*, *53*(1): 75-82.

Çiçek, E., Seçer, B., Sungur, S., Öztürk, S. and Bahçeci, H., 2022. Length-weight relationships and condition factors of 28 fish species belonging to Leuciscidae (Cypriniformes) from Turkey. *Journal of Applied Ichthyology*, *38*(3): 64-367.

Daniel, N., Nirmal, T., Praveenraj, J. and Stephen Sampath kumar, J., 2021. Length weight relationships of three freshwater fishes from Kanyakumari, southern Western Ghats, Tamil Nadu, India. Journal of Indian Fisheries Association, 48: 69-72, 2021.

Das, S.K., Tou, W.X., Noor, N.M., De, M. and Samat, A., 2021. Length-weight relationship, condition factor, and age estimation of commercially important trawl species from Mersing coastal waters, Johor, Malaysia. *Sains Malaysiana*, *50*(1): 1-7.

Dirican, S. and Çilek, S., 2012. Condition factors of seven Cyprinid fish species from Çamligöze dam lake on central Anatolia, Turkey. *African Journal of Agricultural Research*, *7*(31): 4460-4464.

Ferdaushy, M.H. and Alam, M.M., 2015. Length–length and length-weight relationships and condition factor of nine freshwater fish species of Nageshwari, Bangladesh. *International Journal of Aquatic Biology*, *3*(3): 149-154.

Froese, R., 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. *Journal of applied ichthyology*, *22*(4): 241-253.

Gupta, S. and Banerjee, S., 2015. Length-weight relationship of Mystus tengara (Ham.-Buch., 1822), a freshwater catfish of Indian subcontinent. *International Journal of Aquatic Biology*, *3*(2): 114-118.

Jisr, N., Younes, G., Sukhn, C. and El-Dakdouki, M.H., 2018. Length-weight relationships and relative condition factor of fish inhabiting the marine area of the Eastern Mediterranean city, Tripoli-Lebanon. *The Egyptian Journal of Aquatic Research*, *44*(4): 299-305.

Kumar, G., Kashyap, A. and Serajuddin, M., 2023. Length-weight relationships and condition factor of Asian sheat catfish, *Wallago attu* (Bloch & Schneider, 1801) inhabiting different rivers of India. *Journal of Fisheries*, *11*(1): 111203-111203.

Le Cren, E.D., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *The Journal of Animal Ecology*: 201-219.

Li, Q., Xu, X.L. and Huang, J.R., 2014. Length–weight relationships of 16 fish species from the Liuxihe national aquatic germplasm resources conservation area, Guangdong, China. *Journal of Applied Ichthyology*, *30*(2): 434-435.

Okgerman, H., 2004. Seasonal variations in the length-weight relationship and condition factor of rudd (*Scardinius erythrophthalmus L.*) in Sapanca Lake. International journal of zoology reasech, 1 (1): 155-166.

Perry, R.I., Hargreaves, N.B., Waddell, B.J. and Mackas, D.L., 1996. Spatial variations in feeding and condition of juvenile pink and chum salmon off Vancouver Island, British Columbia. *Fisheries Oceanography*, *5*(2): 73-88.

Pouladi, M., Paighambari, S.Y., Millar, R.B. and Babanezhad, M., 2020. Length-weight relationships and condition factor of five marine fish species from Bushehr Province, Persian Gulf, Iran. *Thalassas: An International Journal of Marine Sciences*, *36*: 457-461.

Rahman, A., Hossain, Y., Hasan, R., Mawa, Z., Tanjin, S., Sarker, B.K. and Islam, A., 2021. Length weight relationships and form factor of 8 marine fishes from the Bay of Bengal. *Thalassas: An International Journal of Marine Sciences*, *37*(2): 891-895.

Seiyaboh, E.I., Harry, G.A. and Izah, S.C., 2016. Length-weight relationship and condition factor of five fish species from River Brass, Niger Delta. *Biotechnological Research*, *2*(4): 187-192.

Sekitar, P.K.A., Hamid, M., Mansor, M.A.S.H.H.O.R. and Nor, S.A.M., 2015. Length-weight relationship and condition factor of fish populations in Temengor Reservoir: Indication of environmental health. *Sains Malaysiana*, *44*(1): 61-66.

Shafi, S. and Yousuf, A.R., 2012. Length-weight relationship and condition factor in *Puntius conchonius* (Hamilton, 1822) from Dal Lake, Kashmir. *International Journal of Scientific and Research Publications*, *2*(3), p.1.

Sharma, N.K., Singh, R., Gupta, M., Pandey, N.N., Tiwari, V.K., Singh, R. and Akhtar, M.S., 2016. Length–weight relationships of four freshwater cyprinid species from a tributary of Ganga River Basin in North India. *Journal of Applied Ichthyology*, *32*(3): 497-498.

Stanislas, S.P., Rachad, S.I., Hamidou, A., Nambil, A.K. and Alphonse, A., 2023. Assessment of size structures, length-weight models and condition factors of Eleotridae (Pisces: Perciformes: Gobiodei) from the coastal waters of Benin (West Africa). *International Journal of Forest, Animal and Fisheries Research*, *7*(1).

T.B. and Tesch F.W. (1978). Methods of Assessment of Fish Production in Fresh Waters. IBP Handbook No. 3, 3rd ed. Oxford Blackwell Scientific Publication, London. pp. 101-136.

Ujjania, N.C., Kohli, M.P.S. and Sharma, L.L., 2012. Length-weight relationship and condition factors of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) in Mahi Bajaj Sagar, India. *Research Journal of Biology*, *2*(1): 30-36.

Received: 02nd July 2023 Accepted: 12th October 2023

Journal of the Andaman Science Association Vol. 28(2):198-202 (2023)
ISSN 0970-4183, Printed in India

Effects of Feeding Different Levels of Rice Distillers Dried Grains with Soluble on Growth Performance and Carcass Characteristics of Japanese quail *(Coturnix coturnix japonica)*

C. Pandian[1]*, A. Ashok[2], S. Ezhil Valavan[3] and R. Richard churchil[2]
[1] *Veterinary University Training and Research Centre, TANUVAS, Vellore-632 009*
[2] *Department of Poultry Science, Madras Veterinary College, TANUVAS, Chennai-600 007*
[3] *Poultry Research Station, TANUVAS, Chennai-600 051*
***Corresponding author's E-mail:** chinnaduraipandian75@gmail.com*

Abstract

The present study was carried out to determine the effects of incorporation of Rice Distillers Dried Grains with Solubles (DDGS) on growth performance in Japanese quail. A total of 300 Nandanam quail-3 day old chicks were weighed individually, then randomly assigned to three treatment groups of 100 chicks each. They were 25 chicks per replicate and four replicate per treatment group. Experimental diets were prepared with incorporation of Rice DDGS at 0% (T_1: control), 5% (T_2) and 10% (T_3) levels by marginal adjustment of other feed ingredients. The birds were housed in cages during the experiment period of 0-5 weeks. The parameters such as weekly body weight and body weight gain, feed consumption, feed conversion ratio (FCR), liveability and carcass characteristics were recorded. There was a significant difference ($P \leq 0.01$) in body weight and body weight gain was observed during 1st week to 5th week of age between treatment groups. The 5th week body weight in T_1, T_2 and T_3 group were 192.41, 195.23 and 204.06 g respectively. Similarly, the 5th week cumulative feed consumption (T_1-535.21, T_2-532.63 and T_3-527.97 g) and FCR (T_1-2.78, T_2-2.72 and T_3-2.58 g) showed significant difference ($P \leq 0.05$) among the treatment groups. The per cent livability showed no significant difference between treatment groups. The pre slaughter live weight showed significant difference ($P \leq 0.01$) among treatment groups; however the other carcass characteristics such as eviscerated weight and ready to cook yield showed no significant difference among treatment groups. The above study concluded that Rice DDGS can be incorporated at 10 % level in Japanese quail diet to improve body weight, body weight gain and FCR.

Key words: *Growth performance, japanese quail, rice DDGS.*

Introduction

Feed is the single largest expense in poultry production, accounting up to 70 % of total cost (Filgueira *et al*., 2014). Availability of soya and corn for feed could be a major challenge for the poultry industry (Mark and Vijay, 2016). Soybean meal is the major protein ingredient utilized in poultry diet. Due to scarcity of soybean, there is a need to utilize locally available alternate protein ingredients. The ingredient that gained importance in recent times is Distiller's Dried Grain with Solubles (DDGS). The DDGS is co-product of the ethanol industry produced during the dry milling process. Availability of DDGS is increasing as ethanol is used as biofuel. The DDGS contain all the nutrients from grain in a concentrated form except starch, which has been utilized in the fermentation process during ethanol production. Rice DDGS also have yeast enzyme which increases the level of production (Gupta *et al*., 2017). Rao *et al.* (2016) and Gupta (2016) found diets with 10% Rice DDGS was safe for broilers and layers respectively. Most of the researches are limited to feeding value of corn, wheat, sorghum, barley based DDGS with very scanty reports on Rice DDGS in Japanese quails. Hence, the present study was conducted to assess the efficiency of Rice DDGS by incorporating in the diets of Japanese quails.

Material and Methods

The study was conducted up to 5 weeks to evaluate the effects of incorporation of Rice Distillers Dried Grains with Solubles (DDGS) on growth performance in Nandanam quail 3 strain at Poultry Research Station, TANUVAS, Chennai in the year 2022. Nandanam quail

3 is a dual type Japanese quail strain developed by Tamil Nadu Veterinary and Animal Sciences University, Chennai -51 during the year 2004. Totally 300 Nandanam quail 3 day old chicks were weighed individually, then randomly assigned to three treatment groups of 100 chicks each. They were 25 chicks per replicate and four replicate per treatment group. The chicks in each replicate were housed in colony cages with standard floor space. Experimental diets were prepared with incorporation of Rice DDGS at 0 % (T_1: control), 5 % (T_2) and 10 % (T_3) levels by marginal adjustment of other feed ingredients. Rice DDGS were analyzed for proximate principles, amino acid profile and mycotoxin besides calcium, phosphorus, salt and gross energy by calculation. The value were presented as (% dry matter) mean ± S. E, moisture content 17.14 ± 0.62, crude protein 43.57 ± 1.04, crude fiber 2.85 ± 0.53, ether extract 4.69 ± 0.50, total ash 4.51 ± 0.60, calcium 0.21 ± 0.01, phosphorus 0.75 ± 0.05, salt 0.35 ± 0.05 and gross energy kcal/kg 4091 ± 26.46. An Iso nitrogenous and iso caloric experimental feeds were prepared and fed *adlibitum* under standard managemental conditions. The parameters such as weekly body weight and body weight gain, feed consumption, feed conversion ratio (FCR), livability and carcass characteristics were recorded. The data were analyzed as per standard statistical procedure described by Snedecor and Cochran (1994).

Results and Discussion

Body weight and body weight gain (g)

The effect of incorporation of Rice DDGS on mean body weight (g) of Nandanam quail 3 is presented in Table 1. The body weight (g) using graded level of Rice DDGS at 0 %, 5 % and 10 % level during 1st, 2nd, 3rd, 4th and 5th week of age showed significant ($P \leq 0.01$) difference. The 5th week body weight of Japanese quail fed with 0 %, 5 % and 10 % inclusion level were 192.41, 195.23 and 204.06 g respectively. Talsani *et al.* (2021) conducted an experiment in quails by feeding Rice DDGS from 0 to 20% levels and recorded improvement of body weights at 20 % Rice DDGS level (198.50 g) when compared with the control (185.75 g). Similarly, Karadagoglu *et al*. (2015) conducted an experiment in quails by feeding corn DDGS from 0 to 15 % levels and recorded improvement of body weight. In contrast, Dingore (2015) reported significantly lowest ($P<0.05$) body weight difference during finisher phase in case of broilers when fed with Rice DDGS.

Table 1. Effect of incorporation of Rice DDGS on body weight (Mean ± SE) of Nandanam quail-3 (n=100)

Week/Body weight	T1-Control	T2 (5 %)	T3 (10 %)	F-Value
Hatch weight	9.20±0.01	9.18±0.01	9.19±0.01	0.00^{NS}
1st week	31.63^{b}±0.74	32.51^{b}±0.65	34.48^{a}±0.76	8.78**
2nd week	62.57^{b}±1.47	66.54^{a}±1.58	67.06^{a}±1.70	7.06**
3rd week	102.46^{b}±2.24	104.21^{b}±2.84	115.48^{a}±2.65	44.14**
4th week	140.39^{b}±2.61	141.87^{b}±3.80	162.06^{a}±3.67	65.98**
5th week	192.41^{c}±3.84	195.23^{b}±4.21	204.06^{a}±4.20	78.21**

***Highly significant (P<0.01)*

In the present study, the cumulative body weight gain was (Table 2) significantly ($P<0.01$) higher at 10 % Rice DDGS inclusion level in comparison to control. The body weight gain between graded level of Rice DDGS at 0, 5, 10 % inclusion level during 5th week of age was 185.21, 186.05 and 194.87 (g) respectively. Similarly, Talsani *et al.* (2021) observed significantly higher body weight gain at 20 % Rice DDGS level (189.63 g) than control (176.95 g). El-Abd (2013) and Karadagoglu *et al.* (2015) reported significant improvement in body weight gain in quails by offering diets with corn DDGS. This improved body weight gain in quails fed diets incorporated with Rice DDGS could be related to more available protein or amino acid and concentrated nutrients of DDGS, which have come from grain (Babcock *et al*., 2008).

Table 2. Effect of incorporation of Rice DDGS on body weight gain (Mean ± SE) of Nandanam quail-3 (n=100)

Week/ Body weight gain	T1-Control	T2 (5 %)	T3 (10 %)	F-Value
1st week	22.43^{c}±0.23	23.33^{b}±0.21	25.29^{a}±0.20	27.24**
2rd week	53.37^{b}±0.42	57.36^{a}±0.35	57.87^{a}±0.31	24.51**
3th week	93.26^{c}±0.51	95.03^{b}±0.51	106.29^{a}±0.42	60.36**
4th week	131.19^{b}±0.74	132.69^{b}±0.52	152.87^{a}±0.67	77.23**
5th week	183.21^{c}±0.60	186.05^{b}±1.20	194.87^{a}±1.31	68.94**

***Highly significant (P<0.01)*

Feed consumption and feed conversion ratio (FCR)

Effect of incorporation of Rice DDGS on mean feed consumption (g/bird/week) of Nandanam quail 3 is presented in Table 3. There was a significant difference (P < 0.05) in feed consumption between treatment groups, until 5th week of age. The treatment group feed with 10 % inclusion level of Rice DDGS was consumed significantly (P ≤ 0.05) less feed (527.97 g) than 5 % (532.63 g) and 0 % (535.25 g) inclusion level during entire 5 weeks study periods.

Table 3. Effect of incorporation of Rice DDGS on weekly feed consumption (g) (Mean ± SE) of Nandanam quail-3 (n=100)

Week /Feed consumption	T1-Control	T2 (5 %)	T3 (10 %)	F-Value
1st week	26.10^{b}±1.29	26.22^{b}±1.54	25.13^{a}±1.12	4.12*
2rd week	66.20^{b}±1.38	65.11^{a}±1.67	65.01^{a}±1.75	3.58*
3th week	109.01^{b}±2.20	107.31^{a}±2.10	106.12^{a}±2.16	3.89*
4th week	142.32^{b}±2.51	142.74^{b}±2.89	141.28^{a}±2.58	4.18*
5th week	191.58^{b}±3.89	191.25^{b}±3.68	190.43^{a}±2.75	4.56*
Cumulative feed consumption (g)	535.21	532.63	527.97	

** Significant (P<0.05)*

The effect of incorporation of Rice DDGS on weekly feed conversion ratio (Mean±SE) of Nandanam quail 3 is presented in Fig.1. Similar to the feed consumption, the FCR also showed significant difference among the treatment group during the study (1st to 5th week). The cumulative FCR of 2.78, 2.72 and 2.58 was observed in 0 %, 5 % and 10 % Rice DDGS inclusion levels respectively, till the end of trail period. Similarly, Talsani *et al.* (2021) and Mikhail *et al.* (2013) recorded significantly (P<0.05) better FCR in quails at 20 % DDGS level. In contrast, Dinani *et al.* (2019) reported significantly (P>0.05) poor FCR in broilers when Rice DDGS was included at 15 % level in the diet.

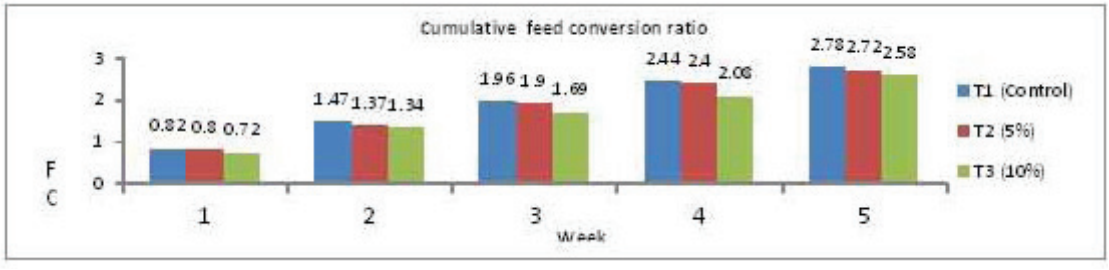


Fig.1. Effect of incorporation of Rice DDGS on weekly feed conversion ratio of Nandanam quail-3

Liveability (%)

The present study revealed that incorporation of 0, 5 and 10 % Rice DDGS in Japanese quail showed non-significant effect on per cent liveability (Fig 2). The mortality observed in the treatment group was non-specific.

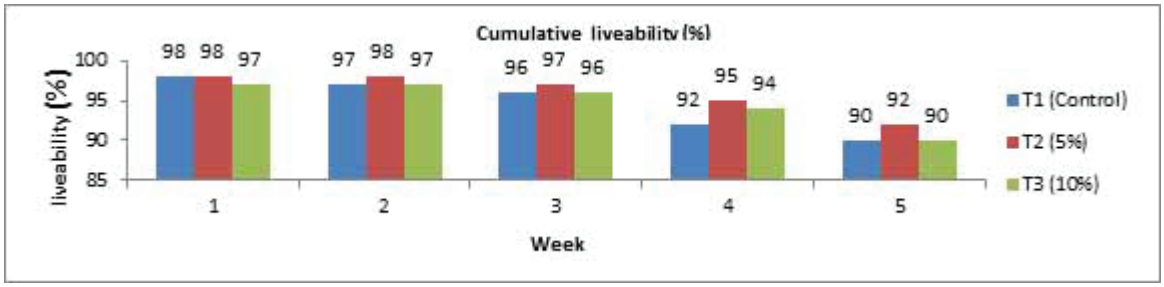


Fig.2. Effect of incorporation of Rice DDGS on liveability (%) of Nandanam quail-3

Carcass yields

The effect of incorporation of Rice DDGS on slaughter performance of Nandanam quail 3 is presented in Table 4. The pre slaughter live weight showed significant difference ($P \leq 0.01$) among treatments; however the other carcass characteristics such as eviscerated weight and ready to cook yield showed no significant difference among treatment and control groups.

Table 4. Effect of incorporation of Rice DDGS on carcass characteristics of Nandanam quail-3 (n=12)

Parameters	T1 (Control)	T2 (5 %)	T3 (10 %)	F-Value
Pre slaughter live weight (g)	192.41^{c}±3.84	195.23^{b}±4.21	204.06^{a}±4.20	78.21**
Eviscerated weight (g)	127.61±1.10	127.04±1.13	128.04±1.13	1.90^{NS}
Ready to cook yield %	64.47±1.10	64.59±1.10	64.72±1.10	0.54^{NS}

***Highly significant (P<0.01); NS – Non significant (P> 0.05).*

Conclusion

The above study concluded that, Rice DDGS can be incorporated at 10 % level in Japanese quail diet to improve body weight, body weight gain and FCR without any adverse effect on liveability and carcass characteristics.

References

Babcock, B.A., Hayes, D.J. & Lawrence, J.D. (2008). Using distillers grains in the US and international livestock and poultry industries. Midwest Agribusiness Trade Research and Information Center. PP: 7.

Dinani, O.P., Tyagi, P.K., Mandal, A.B., Tyagi, P.K. & Dutta, N. (2019). Evaluation of feeding value of Rice based distillers dried grains with Solubles (DDGS) for broiler chickens. Indian Journal of Animal Research, 53(7): 901-906.

Dingore, A.D. (2015). Effect of feeding different levels of Rice distillers dried grains with solubles (RDDGS) on performance of broilers. M.V.Sc. Thesis Maharashtra Animal and Fishery Sciences University, Nagpur.

El-Abd, N.M. (2013). Evaluation of using distillers dried grains with solubles (DDGS) in Japanese quail diets. World Appl. Sci. J., 22(1): 17-21.

Filgueira, T.M.B., Freitas, E.R., Quevedo Filho, I.B., Fernandes, D.R., Watanabe, P.H. & Oliveira, A.N. (2014). Corn replacement by broken Rice in meat-type quail diets. Br. Poult. Sci., 16: 345-350.

Gupta, S.L., Tyagi, Pramod K., Tyagi, Praveen K., Mandal, A.B., Kolluri, G., Mir, N.A. & Khan. A. (2017). The response of Rice based dry distiller's grains with soluble (DDGS) feeding on gastro intestinal microbiota and immunity in layer's diet. Indian J. Poult. Sci., 52(2): 133-137.

Gupta, S. (2016). Feeding value of Rice based dry distiller grains with soluble in white leghorn layers. Ph.D. Thesis Deemed University, IVRI, Izatnagar.

Karadagoglu, O., Ahin, T., Sari, M., Ogun, M. & Bingol, S.A. (2015). Effects of different levels of corn distillers dried grains with solubles on growth performance, carcass quality, some blood parameters and histologic structure of terminal ileum in quails. Rev. Med. Vet., 166(9-10): 253-258.

Mark, W. & Vijay, I. (2016). Poultry and Poultry Products Annual, GAIN Report Number: IN6151.

Mikhail, W.Z., Abd El-Samee, M.O., Shebl, M.A. & Abo-Atia, A.R. (2013). Using distillers dried grains with solubles (DDGS) supplemented with enzymes in quail diets. Egyptian Poultry Science Journal, 33(4): 805-823.

Rao, R.S.V., Raju, M.V.L.N., Prakash, B., Reddy, E.P.K. & Anusha, R. (2016). Effect of dietary supplementation of distillery dried grain soluble from Rice on performance of commercial broilers and white leghorn layers. J. Poult. Sci., 16: 342-351.

Snedecor, G.W. & Cochran, W.G. (1994). Statistical Methods. 9th ed. Oxford and IBH publishing Co., Calcutta.

Talsani, K.R , Naga Raja Kumari Kallam, Narendra Nath, D. & Srinivas Kumar D. (2021). Effect of incorporation of Rice based distiller's dried grain with soluble on growth performance and cost economics of Japanese quails. Indian. J. Poult. Sci., 56(2): 135−139.

Received: 15th October 2023 Accepted: 31st October 2023

Journal of the Andaman Science Association Vol. 28(2):203-209 (2023)
ISSN 0970-4183, Printed in India

Mutation Studies in Black Turmeric *(Curcuma caesia Roxb.)*

Bhoomika H. R [1]*, Anitta Benny [2] and Dushyanthakumar B.M.[3]
Department of Plantation, Spices, Medicinal and Aromatic Crops College of Horticulture,Mudigere,
Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences Shivamogga, Karnataka, India
***Corresponding author's E-mail:** bhoomi04@yahoo.co.in/bhoomikahr@uahs.edu.in*

Abstract

Black turmeric is an underground rhizomatous medicinal crop with bluish-black rhizomes. The rhizomes are employed in treating piles, bronchitis, asthma, impotency, cancer, epilepsy, fever and other health conditions especially by the tribes in North East India which is the home for this species. Due to over exploitation, it is listed under "Endangered species" category necessitating its conservation and cultivation. No named varieties are available as of now in black turmeric. Hence the present experiment was planned with the objective of locating useful genotypes by inducing variability through mutation treatments. The rhizomes were exposed to physical and chemical mutagens *viz.* gamma rays (10, 15, 20, 25, 30, 35 Gy), EMS (1, 1.25, 1.5 %) and colchicine (0.1, 0.2, 0.3 %). The experiment was laid out in RCBD design with thirteen treatments and two replications. The mutant populations were evaluated for growth, yield and quality parameters and were compared to untreated control. Among the different treatments, T13 (control) recorded maximum plant height (71.80cm), number of leaves (24.20), rhizome yield/plant (130.03g), number of primary (4.59) and secondary rhizome (11.64), oleoresin (6.8 %), crude fibre (4.69 %), curcumin (0.008 %) and essential oil (0.36 %) content. The mutagenic treatment resulted in reduction of growth and yield and altered the biochemical composition in the treated rhizomes.

Key words: *Curcuma caesia, black, turmeric, mutation,*

Introduction

Black turmeric (*Curcuma caesia* Roxb.) is a medicinal plant belonging to family Zingiberaceae comprising of more than 1300 species of flowering plants. Ginger and turmeric are the most commercially exploited plants of in the family along with many other economically important species. Black turmeric is one such economically important species which is enlisted as endangered species by the central forest department of India due to overexploitation from the wild (Venugopal *et al*., 2017). The plant of black turmeric resembles that of turmeric but the leaves have purple streaks running along the midrib to the length of the lamina. Unlike the bright yellow rhizomes of turmeric the rhizomes are bluish-black inside and have a bitter taste (Plate 1). The rhizomes are credited with high medicinal value and are used in the treatment of piles, bronchitis, asthma, impotency, cancer, epilepsy and fever *etc*.

Due to the lack of seed set in the species, genetic improvement by other approaches such as hybridization is challenging. Mutation breeding has been proven to be an effective tool in the improvement of vegetatively propagated crops by generating variability in the existing population. So, inducing mutation and isolating desirable mutants are the means of crop improvement. Since no systematic attempts in crop improvement are done in black turmeric, an attempt was done to induce desirable mutants in the species.

Plant

Inflorescence

Rhizomes

Cut rhizomes

Plate 1. Plant morphology of black turmeric

Material and Methods

Physical mutagen gamma (10, 15, 20, 25, 30, 35 Gy) and chemical mutagens, EMS (1, 1.25, 1.5%) and colchicine (0.1, 0.2, 0.3%) were employed in the study. The rhizomes were cut into pieces of 15 to 20g having an active eye bud and were subjected to mutagenic treatments. Gamma irradiation was carried out using 60Co (Cobalt 60) as irradiation source in a Gamma Chamber. Rhizomes were dipped in EMS and colchicine solutions in a glass beaker according to treatments. Treated rhizomes were field planted at a spacing of 30 x 30 cm after having sprouted in a nursery protray. The experimental design was RCBD with two replications comprising of fifty plants each. Standard cultural practices were practiced as recommended for turmeric.

The observations were recorded for various morphological parameters such as plant height, number of tillers per clump, number of leaves per clump, chlorophyll mutants and morphological mutants. Chlorophyll mutants were observed 2 months after planting and it was classified according to Gustafsson (1940) as Xantha and Striata. Yield parameters like yield per plant, number of primary rhizomes, number of secondary rhizomes were recorded immediately after harvest. Quality parameters *viz*., curcumin content, oleoresin content, crude fibre and essential oil were also assesses from rhizome samples of each treatment.

The curcumin content was calculated using the method described by Manjunath *et al.* (1991). Soxhlet apparatus was used for estimating oleoresin content as per the AOAC (1980) method. Crude fibre content was

estimated as per the procedure suggested by Chopra and Kanwar (1976). Essential oil was extracted by hydro-distillation using Clevenger apparatus.

The recorded data was statistically analyzed to compare and find out the significant differences among the various growth, yield and quality parameters among different treatment group.

Results and discussions

The data pertaining to plant height, number of tillers per clump and number of leaves per clump are presented in table 1. Maximum plant height of 71.80 cm was observed in control (T13) and the minimum plant height (43.35 cm) was noticed in T12 (colchicine @ 0.3 %). In the present study, plant height recorded a gradual increase and then decreased with increase in the dose of gamma irradiation up to 35 Gy. In case of EMS and colchicine treatment, increase in the concentration resulted in reduction in plant height. Gamma rays might have negatively affected the apical meristems or caused the partial failure of the internodes to elongate resulting in reduction in the number of proliferating cells (Khalil *et al.*, 1986).

Table 1. Effect of different mutagens on growth parameters of black turmeric

Treatment	Plant height (cm)	No. of tillers/ clump	No. of leaves/ clump
T1 (Gamma @ 10 Gy)	60.98^{de}	4.60^{bcdef}	19.90^{bcde}
T2 (Gamma @15 Gy)	61.84^{de}	5.20^{abcd}	20.85^{bcde}
T3 (Gamma @ 20 Gy)	66.20^{b}	5.50^{ab}	22.65^{ab}
T4 (Gamma @ 25 Gy)	64.48^{bc}	5.40^{abc}	21.10 ^{abcd}
T5 (Gamma @ 30Gy)	59.93^{ef}	4.30^{cdefg}	18.90^{cdef}
T6 (Gamma @ 35 Gy)	58.20^{f}	4.10^{defg}	18.60^{def}
T7 (EMS @ 1%)	64.65^{bc}	5.30^{abc}	22.00^{abc}
T8 (EMS @ 1.25 %)	63.08^{cd}	5.00^{abcde}	19.75^{bcde}
T9 (EMS @ 1.5 %)	60.81^{def}	4.30^{cdefg}	17.70^{ef}
T10 (Colchicine @ 0.1%)	47.30^{g}	3.90^{efg}	16.25^{fg}
T11 (Colchicine @ 0.2%)	45.78^{g}	3.55^{fg}	14.15^{gh}
T12 (Colchicine @ 0.3%)	43.13^{h}	3.30^{g}	12.55^{h}
T13 (Untreated control)	71.80^{a}	5.85^{a}	24.20^{a}

Note: DMRT at 5% of level of significance to compare the pair of treatment means. Any two means having common letter, are not significantly different at 5% level of significance.

Similar results have been obtained with gamma treatments by Giridharan (1984) in ginger, Norfadzrin *et al.* (2007) in tomato and okra. Misra and Bajai (1983) reported decrease in plant height in gladiolus with EMS treatment and opined it might be due to physiological disturbance and reduction in cell division by suppressing the mitotic division and negative effect on auxin. Damon (1958) reported that colchicine treatment arrests metaphase cells in the shoot tip.

Significant differences were noted for tiller and leaf production. Maximum number of tillers per clump (5.85) and number of leaves per clump (25.30) were observed

in control (T_{13}). The lowest value was noticed in T_{12} (colchicine @ 0.3 %). Mutagens at higher doses having lethal effect might have caused inactivation or killing of growing points, thus, resulted in the reduction of tiller and leaf production.

Among different treatments, highest chlorophyll mutation frequency (13.30 %) was observed in T_4 (Gamma @ 25 Gy). Both xantha and striata were observed. Chlorophyll mutants were not recorded in T_7 (EMS @1%), T_8 (EMS @1.25%), T_{11} (Colchicine @ 0.2%) and T_{13} (untreated control). Dwarf mutants were observed in EMS @ 1.5% and Colchicine @ 0.2% (Table 2).

Table 2. Effect of mutagens on induction of chlorophyll and morphological mutants

Treatments	Xantha(%)	Striata(%)	Total chlorophyll mutation (%)	Split leaf type (%)	Dwarf plant type (%)
T_1 (Gamma @ 10 Gy)	4.00 (2.12)	2.67(1.78)	6.67(2.68)	0.00(0.71)	0.00(0.71)
T_2 (Gamma @15 Gy)	6.94 (2.73)	1.39(1.38)	8.33(2.97)	5.00(2.35)	0.00(0.71)
T_3 (Gamma @ 20 Gy)	6.67 (2.68)	0.00(0.71)	6.67(2.68)	3.33(1.96)	0.00(0.71)
T_4 (Gamma @ 25 Gy)	11.64(3.48)	1.67(1.47)	13.30(3.71)	6.67(2.68)	0.00(0.71)
T_5 (Gamma @ 30Gy)	10.00(3.24)	0.00(0.71)	10.00(3.24)	0.00(0.71)	0.00(0.71)
T_6 (Gamma @ 35 Gy)	8.33(2.97)	1.67(1.47)	10.00(3.24)	1.67(1.47)	0.00(0.71)
T_7 (EMS @ 1%)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
T_8 (EMS @ 1.25 %)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
T_9 (EMS @ 1.5 %)	3.33(1.96)	0.00(0.71)	3.33(1.96)	5.00(2.35)	1.67(1.47)
T_{10} (Colchicine @ 0.1%)	1.67(1.47)	0.00(0.71)	1.66(1.47)	0.00(0.71)	0.00(0.71)
T_{11} (Colchicine @ 0.2%)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	1.67(1.47)
T_{12} (Colchicine @ 0.3%)	0.00(0.71)	1.67(1.47)	1.66(1.47)	0.00(0.71)	0.00(0.71)
T_{13} (Untreated control)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)

Note: Figures in parenthesis are the values of square root transformation ($\sqrt{x}+0.5$) for zero observation

Significant effects of mutagenic treatment on black turmeric were observed on various yield parameters as presented in table 3. Maximum fresh rhizome yield (130.03 g/clump), number of primary rhizomes (4.59), number of secondary rhizomes (11.64/ clump) were observed in control (T_{13}). The lowest values were noticed in T_{12} (colchicine @ 0.3 %).

There was a reduction in yield with increasing concentration of EMS and colchicine. This could be related to the fact that mutagenic treatment at higher dose damaged or disrupted the physiology of the plant, affecting photosynthesis and respiration, resulting in poor plant growth in terms of plant height, number of leaves, leaf area and number of tillers. Similar line of work had been reported by Raju *et al.* (1980) in ginger, Gupta *et al.* (1982) in costus, Madhuri (2017) in ginger and Laxmi *et al.* (2019) in turmeric.

The maximum curcumin (0.008 %), oleoresin (6.80 %), crude fibre (4.69 %) and essential oil (0.36 %) contents were observed in control (T_{13}). The lowest value was noticed in colchicine @ 0.3% (Figures 1, 2 and 3).

Changes in the production of essential oil, oleoresin and crude fibre could be attributed to the promotive or inhibitory influence of mutagens at various dosages. These findings are in accordance with the reports of Gupta *et al.* (1982) in costus. The change in chemical constituents in response to mutagenic treatment is most likely owing to mutagenic sensitivity of these components at various doses.

Table 3. Effect of different mutagens on yield parameters of black turmeric

Treatments	Yield/ plant (g)	Number of primary Rhizomes/ clump	Number of secondary Rhizomes/clump
T1(Gamma@10Gy)	115.13^{cd}	3.59^{de}	9.10^{cd}
T2(Gamma@15Gy)	119.99^{bc}	3.91^{cd}	9.24^{c}
T3(Gamma@20Gy)	123.61^{b}	4.31^{ab}	10.84^{ab}
T4(Gamma@25Gy)	121.87^{b}	3.99^{bc}	9.44^{c}
T5(Gamma@30Gy)	110.96^{de}	3.59^{de}	8.44^{cde}
T6(Gamma@35Gy)	106.88^{e}	3.10^{fg}	7.44^{ef}
T7(EMS@ 1%)	122.09^{b}	3.90^{cde}	9.74^{bc}
T8(EMS @1.25 %)	114.87^{cd}	3.68^{cde}	9.14^{cd}
T9(EMS @1.5 %)	98.29^{f}	3.61^{cde}	7.84^{de}
T10(Colchicine@0.1%)	50.93^{g}	3.50^{ef}	6.48^{fg}
T11(Colchicine@0.2%)	47.05^{gh}	2.90^{g}	6.24^{fg}
T12(Colchicine@0.3%)	42.67^{h}	2.71^{g}	5.84^{g}
T13(Untreated control)	130.03^{a}	4.59^{a}	11.64^{a}

Note: DMRT at 5% of level of significance to compare the pair of treatment means. Any two means having common letter, are not significantly different at 5% level of significance.

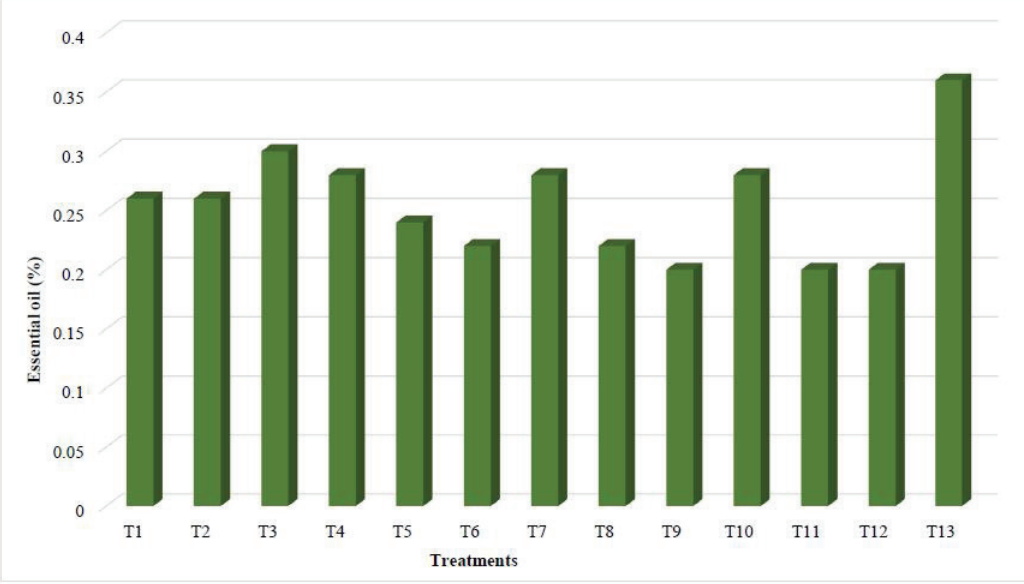


Figure1. Effect of different mutagenic treatments on curcumin content (%) in black turmeric

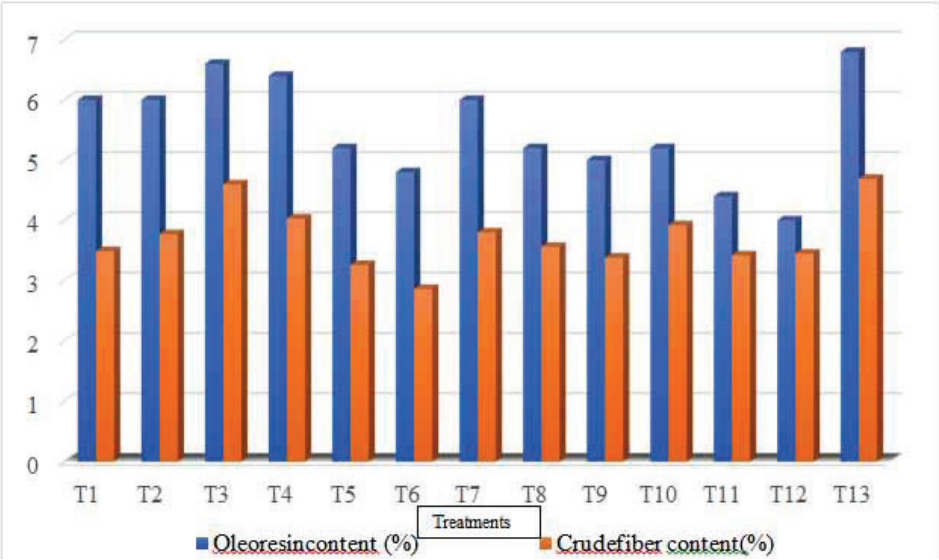


Figure 2. Effect of different mutagenic treatments on oleoresin (%) and crude fibre content

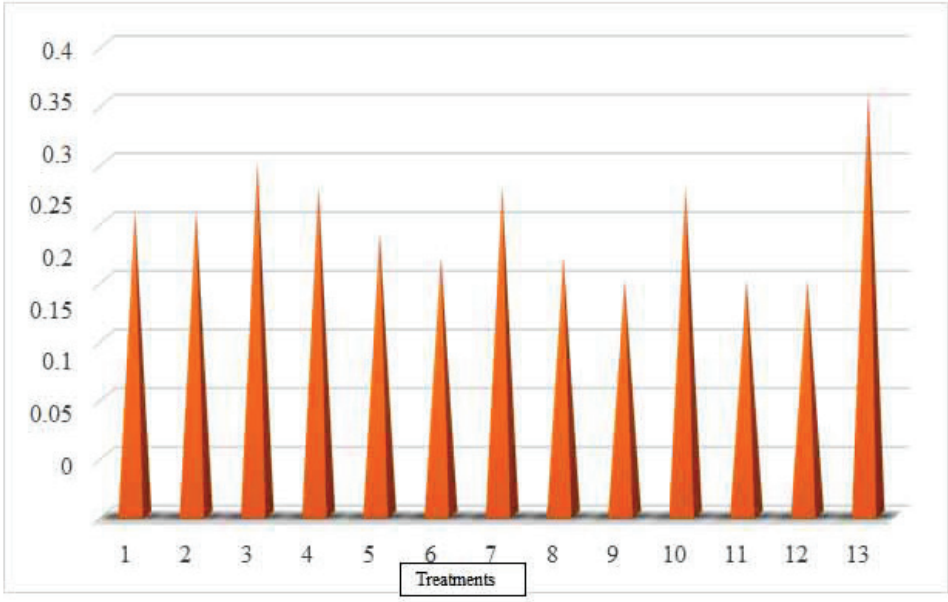


Figure 3. Effect of different mutagenic treatments on essential oil content (%)

Conclusion

It was evident from the study that mutagenic treatment resulted in creation of considerable degree of variability in the existing black turmeric population for plant growth, yield and secondary metabolite contents. The populations need to be further evaluated in ensuing generations for their consistency in performance for yield and quality attributes.

References

A.O.A.C., 1980, Official Method of Analysis, Volume 2. Washington: Association of Official Analytical Chemists.

Chopra, S.L. and Kanwar, J.S.1976. *.Analytical Agricultural Chem*istry, Kalyani publishers, Ludiana. pp.332-341.

Damon, E.J.1958. Investigations into the cytomorphogentic effects of colchicine on varieties of *Sorghum vulgare. Proc.Okta.Acatt.Sci*. **38:** 9-13.

Giridharan, M.P. 1984. Effect of gamma irradiation in ginger (*Zingiberofficinale*.Rosc.). *M.Sc.(Hort.) Thesis, Kerala Agric.Univ., Vellanikkara.*

Gupta, M.N., Lakshmi, V., Dixit, V.S. and Srivastava, S.N. 1982. Gamma ray induced variability in *Costus speciosus*. *Progressive Hortic*.**14**: 193-97.

Gustafsson, A.1940. The mutation system of the chlorophyll apparatus. *Acta Univ. Lund*. **36** (11): 1-40.

Khalil, S.J., Rehman, S., Afridi, K. and Jan, M.T. 1986. Damage induced by gamma irradiation in morphological and chemical characteristics of barley. *Sarhad J.Agric.* **29** (1): 45-54.

Laxmi, P.R., Kale, V.S., Nagre, P.K., Dala, L.S.R. and Potdukhe, N.R. 2019. Induction of mutations for morphological and yield parameters in ginger (*Zingiber officinale* Rosc.) during vM1 generation. *J. Pharmacogn. Phytochem.* **8** (6):1660-1663.

Madhuri, M.L. 2017. Induction of variations in turmeric through irradiation and chemical mutagen. *Ph.D. Thesis, Dr. Y. S. R. Hortic. Univ.*

Manjunath, M.M., Sattigeri, V.V. and Nagaraj, K.V. 1991. Curcumin in turmeric. *Spices India.***4** (3): 7-9.

Misra, R.L. and Bajpai, P.N. 1983. Mutational studies in gladioli (*Gladiolus* L.): Effect of physical and chemical mutagens sprouting and survival of corms. *Haryana J. Hortic. Sci*. **12** (1-2): 16.

Norfadzrin, F., Ahmed, O.H., Shaharudin, S. and Rahman, D.A. 2007. A preliminary study on gamma radio sensitivity of tomato (*Lycopersicon esculentum*) and okra (*Abelmoschus esculentus*). *Int. J. Agric. Res.* **2**(7): 620-625.

Raju, E.C., Patel, J.D., Shah, J.J. 1980. Effects of gamma irradiation in morphology of leaf and shoot apex of ginger, turmeric and mango ginger. *Proc. Indian Acad. Sci.* **89**: 173-178.

Venugopal, A., Rinu, K.A. and Joseph, D. 2017. Medicinal properties of black turmeric: a review. *Innoriginal Int.J.Sci*. **4**(3):1-4.

Received: 15th October 2023 Accepted: 01st November 2023

Journal of the Andaman Science Association Vol. 28(2):210-217 (2023)
ISSN 0970-4183, Printed in India

Matrix Metallo Proteinases modulates the semen quality profiles of Kangeyam bull

S. Anandhi[1], T. C. Balamurugan[1], R. Prakash Krupakaran[1], C. Senthamil Pandian[1] and P. Perumal[2]
[1]*TANUVAS-Veterinary College and Research Institute, Salem-636 112, Tamil Nadu, India*
[2]*ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India*
***Corresponding author's E-mail:** tcbalamurugan@gmail.com*

Abstract

A study was conducted to find out the existence of Matrix Metallo Proteinases (MMP) in the semen of native cattle of Tamil Nadu 'Kangeyam'. Sixteen (n=18) apparently healthy Kangeyam bulls of approximately 4 to 6 years of age with good body condition (score 5-6) were selected. Semen samples were collected from the experimental animals during early morning before concentrate feeding and routine semen quality parameters were analysed. Based on the semen characters, bulls were divided into two groups Group I Normospermic (n=9) and Group II Oligospermic (n=9). Semen samples were subjected to gelatin zymography. It was confirmed that the major bands were observed at 72 kDa of MMP-2 and 92 kDa of MMP-9 in all the experimental animals in both the groups. In normospermic groups, the major bands were observed at 72 kDa and 92 kDa and it represents the latent forms of MMP-2 and MMP-9, respectively. Further, in each group, two lytic bands were observed at 220 kDa and 135 kDa and it represents the proforms of MMP-9. The intensity of 72 kDa of MMP-2 was 2-3 times higher than the marker. In oligospermic groups, both latent (92 kDa) and active (87 kDa) forms of MMP-9 was observed. Further, the intensity of latent form of (92 kDa) MMP-9 was 1.5 times higher than that the normospermic groups. Similarly, in oligospermic groups, two more prominent bands were noticed at 220 kDa and 135 kDa revealed that they were the lytic bands of MMP-9 indicating the proenzymatic forms of MMP-9. It was concluded that the expression of latent and active forms of MMP-9 and latent form of MMP-2 were observed in all experimental animals. But the expression of MMP-9 was positively associated with low sperm count as it was strongly expressed in oligospermic groups whereas the expression of MMP-2 was positively associated with high sperm count as it was strongly expressed in normospermic groups. Further, more studies required to ascertain the enzymatic activity of MMP-2 and MMP-9 which may serve as an alternative biomarker in determining semen quality.

Key words: Matrix Metallo Proteinase; gelatin zymography; semen

Introduction

Matrix metalloproteinase (MMP) family consists of at least 20 structurally related zinc metallo endopeptidases capable of degrading the extracellular matrix components. These enzymes participate in embryonic development, morphogenesis, blastocyst implantation, angiogenesis and tissue resorption, and in diseases such as arthritis, cancer cell invasion and metastasis (Nagase, 1996). This group of enzymatic proteins is classified into collagenases, gelatinases, stromelysins, and membrane-type MMPs (Nagase and Woessner, 1999). MMPs have primary roles in cellular functions such as cell proliferation, apoptosis, migration, differentiation, and angiogenesis. They are also effective in physiological processes such as reproduction, fetal growth, tissue reconstruction, wound healing, and bone development (Klein and Bischoff, 2011). MMP-2 and MMP-9 (also known as gelatinases A and B) particularly degrade gelatin, elastin, as well as various types of collagens. MMPs are capable of degrading nearly all kinds of proteins of the extra cellular matrix (ECM). At the same time, MMPs are also involved in the processing of signaling proteins such as cytokines and chemokines, thereby, modulating their release and/or activity (Bonnans *et al.,* 2014).

Seminal plasma contains many proteinases originating either from testicular cells or from prostate and other accessory sex glands (Yin *et al.,* 1990). MMP-2 takes part in penetration of sperm into oocyte and functions as acrosin. Thus, probably this enzyme is located on the inner acrosomal membrane of sperm (Ferrer *et al*.,

2012). In male reproductive system, MMP-2 and MMP-9 are detected in seminal plasma. MMP-2 and MMP-9 are released from prostate and seminal vesicles; however, there is less information about the function of this enzyme in male reproduction compared to female reproductive system (Baumgart *et al.,* 2002). MMP-2 and MMP-9 help the movement of germ cells in spermatogenesis by digesting of extracellular matrix (Chen, 2011). MMP-2 and MMP-9 are involved in the breakdown of the oocyte membranes to allow sperm to enter the oocyte. MMPs along with other proteins in seminal plasma regulate spermatogenesis, sperm motility, antioxidant protection, and retain lipid stability in sperm membrane (Dietrich *et al.,* 2014).

Most of the studies were carried out in female animals and there were limited studies in male domestic animals. Several studies evaluated the association among alterations of genes and proteins of MMPs with sperm parameters, semen quality, and normospermic infertility (Baumgart *et al.,* 2002, Mohagheghi *et al.,* 2015, Kurzawski *et al.,* 2017, Mohagheghi *et al.,* 2018). Till date, the existence of gelatinase in native bulls of India was not carried out, and their relationship with semen parameters was not attempted. Kangeyam cattle is a recognized and registered breed of cattle (dual purpose breed for draught and milk) in the home tract of Erode and Coimbatore districts of Tamil Nadu. Hence, the present study was carried out to find out the expression of MMP-2 and MMP-9 in seminal plasma samples and to assess the association between MMP-2 and MMP-9 expression and semen quality parameters in native cattle breed of Tamil Nadu, Kangeyam.

Materials and Methods

The proposed study was conducted at the Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute (TANUVAS), Orathanadu, Tamil Nadu, India. The institute is located 30 meters above sea level at latitudes 10.6°N and 79.3°W.

Experimental animals

Eighteen (n=18) apparently healthy Kangeyam bulls of approximately 4 to 6 years of age with good body condition (score 5-6) were selected from the herd of organized farms, Salem, India. All these animals were fed with standard basal diet consisting of concentrate mixture and roughage (wheat straw) to meet their nutrient requirements according to NRC (1985) throughout the study period. All the animals were maintained under uniform feeding, housing and managemental conditions as per the management of farm. Further, all the bulls were vaccinated and feeding and watering were done as per the feeding standard of the farm.

Collection of semen

Semen samples were collected once a week from all the bulls for 6 weeks with use of standardised artificial vagina method. The collections were obtained by exposing the bulls to the oestrous cow. Immediately after collection, the tubes containing semen were placed in a small thermo-flask having water at 37 ˚C for further processing. The ejaculates were analysed and accepted for evaluation as tested for routine seminal parameters and accepted for evaluation after meeting the minimum standard protocol (MSP) standards like-wise concentration: >500 million/ mL; mass activity: >3+, individual motility: >70% and overall abnormality not more than 10%. A total of six ejaculates from each bull (18 x 6 = 108) were collected; after screening, 72 of 108 ejaculates were selected and assessed.

Semen analysis

The volume of ejaculated sperm was measured and recorded in milliliters (mL) straight from the graded sperm collection tube. A small drop of freshly collected semen was put on a clean, grease-free, pre-warmed glass slide at 37°C and viewed without a cover slip using a low power (100×) phase contrast microscope (Nikon, Eclipse 80i) to assess the mass activity of the semen sample. The appearance of waves and swirls was used to rate the mass activity on a scale of 0 to 5. The concentration of sperm ($\times 10^6$/mL) in neat sperm was determined using a haemocytometer and the red blood cell counting process. Special care was taken when diluting the sample with 1% formal saline, which was then cross-checked by diluting neat semen at a ratio of 1:200 with a micro pipette. The homogeneity of the sperm samples was ensured by hand

shaking the tube containing the sperm samples as well as charging the haemocytometer.

Seminal parameters, viz. sperm motility (Salisbury *et al.,* 1985), viability and total sperm morphological abnormalities by Eosin–Nigrosin staining (Agarwal *et al.,* 2016), acrosomal integrity by Giemsa staining (Watson, 1975) and plasma membrane integrity by hypo-osmotic swelling test (Jeyendran *et al.,* 1984) were determined with standard procedures.

Separation and preparation of seminal plasma

One mL of semen was centrifuged at 4000 rpm for 20 min at 4°C to separate sperm pellets and seminal plasma. Seminal plasma was stored at -80°C for further analysis. The supernatant containing seminal plasma proteins was separated for SDS- PAGE analysis.

Gelatin zymography

Seminal plasma samples were subjected to gelatin zymography by modified SDS-PAGE (modified from the method of Laemmi, 1970) as performed by Heussen and Dowdle (1980). In this method, gelatin (0.3%) was added as a copolymerization substrate to obtain (final concentration 0.15%) a separating gel (8%). The samples were electrophoresed at 100 V for 20 min. Renaturation was performed with 2.5% Triton X-100 for 3 h on a mechanical shaker with gentle shaking. The gel was then incubated in 10 mM $CaCl_2$, 0.15 M NaCl, and 50 mM Tris, pH 7.5, for 18 h at 37 °C. The gel was stained with 0.25% Coomassie brilliant blue for 2 h, followed by destaining with a colorless solution for 1 h. Finally, the gel was washed with distilled water.

Analyzing the results of gelatin zymogram

Human capillary blood gelatinase served as a standard marker for the evaluation of zymogram groups according to the protocol described by Makowski and Ramsby (1996). Blood was drawn from a capillary by pricking a fingertip and an accurate analytical weight was measured in a tarred polypropylene tube. Samples were then combined with a 20 × volume of Laemmli buffer and mixed thoroughly. These lots were stable for 3 months at -20°C.

Results and Discussion

The semen samples were subjected to gelatin zymography from both groups. All the semen samples were proteolytically active, since all completely degraded the gelatin. The major bands were observed in both groups at 72 kDa of MMP-2, 92 kDa, and 87 kDa of MMP-9. Both latent (92 kDa) and active (87 kDa) forms of MMP-9 and latent form of (72 kDa) MMP-2 was observed in both groups. Further, two more lytic bands were observed at 220 kDa, and 135 kDa indicate the proforms of MMP-9. The intensity of latent form of MMP-2 (72 kDa) was 2-3 times higher than that the latent form of 92 kDa of MMP-9 and four times higher than marker in all the experimental animals. However, the active form of (72 kDa) MMP-2 was not observed in both groups. It was clearly visible that the latent forms of MMP-9 and MMP-2 were dominant than the active forms. Various authors reported the existence of gelatinases in seminal plasma of human (Shimokawa *et al.,* 2002, Warnick *et al.,* 2015, Baumgart *et al.,* 2002, Kratz *et al.,* 2014) and canine (Saengsoi *et al.,* 2012). Similarly, in human seminal plasma, both latent and active forms of MMP-2 and MMP-9 were detected by Kratz *et al.* (2014) and Tentes *et al.* (2007) and concluded that the latent forms were the predominant ones. Gelatin zymogram reveals the existence of gelatinases in both the groups as shown in Fig. 1.

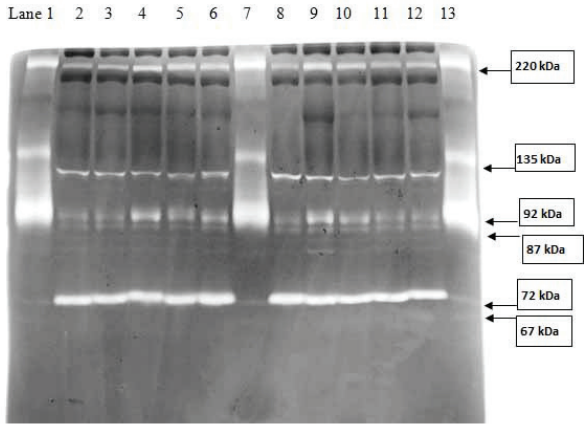


Fig.1: Gelatin zymography of Kangeyam bull Semen

Lane 1, 7 & 13 - Human capillary blood MMP marker

Lane 2, 3, 8, 11, 12 - Kangeyam Normospermic animals

Lane 4, 6, 9, 10 - Kangeyam Oligospermic animals

Lane	Group
1.	Human capillary blood MMP marker
2.	Normospermic
3.	Normospermic
4.	Oligospermic
5.	Normospermic
6.	Oligospermic
7.	Human capillary blood MMP marker
8.	Normospermic
9.	Oligospermic
10.	Oligospermic
11.	Normospermic
12.	Normospermic
13.	Human capillary blood MMP marker

In normospermic groups (G I: Lane 2, 3, 5, 8, 11, 12), the major bands were observed at 72 kDa and 92 kDa indicating the latent forms of MMP-2 and MMP-9, respectively. Further, one minor band was observed at 87 kDa indicating the active form of MMP-9 and two more lytic bands observed at 135 kDa, and 220 kDa indicating the proforms of MMP-9. The intensity of latent form of MMP-2 (72 kDa) was 2-3 times higher than marker (Lane 1, 7, 3). The intensity of latent form of (92 kDa) MMP-9 was lighter than the latent form of MMP-2 in all the experimental animals in this group. However, the active form of (67 kDa) MMP-2 is not observed in all the animals. The intensity of (72 kDa) MMP-2 was 1.5 times higher in normospermic group than oligospermic groups (G II: Lane 4, 6, 9, 10). Further, one faint band was observed at 80 kDa indicated the proform of MMP-2 in normospermic groups. It was obviously indicated that the expression of MMP-2 higher than MMP-9. It might be inferred that the expression of MMP-2 was associated with sperm concentration and characteristics. Our results were in agreement with the results of Baumgart *et al.* (2002), Shimokawa *et al.* (2002), Tentes *et al.* (2007), Saengsoi *et al.* (2012) and Warnick *et al.* (2015). Similar to these results, Warnick *et al.* (2015) observed that gelatinase

activities between normal and abnormal semen samples showed a significant and six-fold higher in proMMP-2 and MMP-2 activity in high than low sperm concentration samples ($p < 0.001$).

In oligospermic groups (G II: Lane 4, 6, 9, 10), the major bands observed at 92 kDa and 72 kDa indicating the latent forms of MMP-9 and MMP-2, respectively. Further, one minor band was observed at 87 kDa indicating the active form of MMP-9 and two more lytic bands observed at 135 kDa, and 220 kDa indicating the proforms of MMP-9. The intensity of latent form (92 kDa) of MMP-9 was two times higher than normospermic groups (G I: Lane 2, 3, 5, 8, 11, 12) but lower than the marker. As compared to normospermic groups, the oligospermic groups (G II: Lane 4, 6, 9, 10) showed thicker bands at 135 kDa and 220 kDa indicating the expression of proenzymatic forms of MMP-9 was very clear. The present study results were in agreement with results of various authors (Shimokawa *et al.,* 2002, Warnick *et al.,* 2015, Baumgart *et al.,* 2002). To concurrence with these results, in human subjects, Baumgart *et al.* (2002) observed that ProMMP-9 and MMP-9 levels were significantly elevated in samples with low sperm counts compared to those with high sperm density ($p < 0.001$). Further, High level of proMMP-2 and MMP-2 was associated with high sperm motility (≥70%, $p < 0.001$).

From these results, it was inferred that the expression of MMP-9 was higher in oligospermic groups. It might be associated MMP-9 expression increased with low sperm concentration. Similarly, in human seminal plasma Shimokawa *et al.* (2002) observed that three major bands of gelatinase activity at 72 kDa, 67 kDa and 52 kDa and minor bands at 92 kDa, 84 kDa and 45 kDa in gelatin zymography. These results indicate that two kinds of pro-form and active-form matrix metalloproteinases, MMP-2 and MMP-9, and their degradation products, are present in human seminal plasma. Further, these proteinases were all recognized by the polyclonal antibodies for MMP-2 or MMP-9. These activities were the metalloproteinases proMMP-9 (92kDa), proMMP-2 (72kDa) and MMP-2 (67kDa), and that their degradation products were present at lower molecular weights. Hence, the expression of MMP-9 was higher in low sperm concentration animals than high sperm concentration animals. To harmony with these results, Tentes *et al.* (2007) observed that ProMMP-9 levels were higher in semen samples with abnormally low concentration ($\leq 19\times10^6$/ml) compared with semen samples with concentration $\geq 50\times10^6$/ml.

Relationship of Semen characters with expression of MMP-9 and MMP-2

The basic semen characteristics of both groups were analyzed and presented in the Table 1. In both groups, the major bands at 72 kDa, 92 kDa, and 87 kDa and major bands at 220 kDa, and 130 kDa were observed. Both latent (92 kDa) and active (87 kDa) forms of MMP-9 and latent form of (72 kDa) MMP-2 were observed in both groups. But the active form of (72 kDa) MMP-2 was not observed in both groups. The expression of latent form of (72 kDa) MMP-2 was higher in normospermic groups (G I: Lane 2, 3, 5, 8, 11, 12) than the oligospermic groups (G II: Lane 4, 6, 9, 10). But in oligospermic groups, the latent and active forms of (92 kDa and 87 kDa) of MMP-9 were higher than the normospermic groups. It was clearly understood that in olgospermic groups, the expression of MMP-9 was higher is due to low sperm count in the respective group. Likewise, the expression of MMP-2 was higher in normospermic groups is due to high sperm count and sperm without abnormality. Though, the active form of MMP-2 was not present in this group, one minor band was observed at 80 kDa indicated the proform of MMP-2. Our results were in agreement with the results of Baumgart *et al.* (2002), Shimokawa *et al.* (2002), Tentes *et al.* (2007), Saengsoi *et al.* (2012) and Warnick *et al.* (2015). In human, MMP-2 concentration in seminal plasma was significantly associated with sperm count in a linear trend (Baumgart *et al.,* 2002). Similarly, Buchman-Shaked *et al.* (2002) conducted a study on human subjects and found that the major bands were observed at 92 kDa, 72 kDa, 62 kDa, and 28 kDa molecular-weight bands exhibiting gelatin-degrading activity in both normal and abnormal sperm samples. The 92 kDa, 72 kDa, and 62 kDa bands with gelatinolytic activity were consistent with pro-MMP-9, pro-MMP-2, and active MMP-2, respectively. Further, they concluded that a higher 28 kDa activity and a lower 92 kDa MMP activity in normal sperm samples relative to abnormal samples were detected.

Table 1. Descriptive statistics of basic semen parameters and MMPs in two group

Parameters	Group I Normospermic ($n=9$)	Group II Oligospermic ($n=9$)
Semen volume (ml)	4.15±0.57[a]	4.52±0.36[a]
Sperm concentration ($\times 10^6$/mL)	1058.85±4.87[a]	624.34±6.86[b]
Total motility (%)	76.54±0.87[a]	32.75±0.76[b]
Viability (%)	80.74±1.21[a]	38.98±0.65[b]
Total sperm abnormality (%)	5.72±0.32[a]	16.83±0.87[b]
Acrosomal Integrity (%)	86.46±0.78[a]	41.65±0.43[b]
Plasma membrane Integrity (%)	81.34±0.98[a]	39.12±0.67[b]

Values are presented as mean ± standard error.
*Values indicated are significantly different ($p<0.05$).

On contrary, Atabakhsh *et al.* (2018) observed in human subjects that MMP-2 and MMP-9 activities in seminal plasma have a positive effect on sperm count and motility. Further, direct correlation between activity of MMP-2 and MMP-9 in follicular fluid with oocyte quality and fertilization rate was reported. Similarly, in stallion, Kareskoski *et al.* (2021) observed that latent pro-MMP-2, active MMP-2 and total MMP-9 were present in all fractions of the stallion's ejaculate, with higher relative activity levels of the latent than active forms and the highest relative activity in the high fraction. Because these MMPs are associated with sperm concentration and total number of sperm, and they are emitted into the first sperm-rich fractions of the ejaculate, the glands contributing to these fractions are probably their main source.

To conclude, MMP-2 expression was higher in normospermic groups and MMP-9 expression was higher in oligospermic groups. To harmony with our results Kratz *et al.* (2014) observed that seminal MMP-9 expression was higher in childless men than in fertile subjects, whereas there were no significant differences in MMP-2 expression between the analysed seminal groups. Tentes *et al.* (2007) observed that MMP-2 and MMP-9 were both present in human semen, and low sperm concentration semen samples have higher MMP-2 and lower MMP-9. Semen samples with a normal sperm count, semen samples with a low sperm count ($\leq 19 \times 10^6$/ml) showed reduced sperm viability, a reduced percentage of Grade A sperm, a reduced percentage of morphologically-normal sperm, and lower proMMP-9 and MMP-9 but higher proMMP-2 and MMP-2 levels ($P < 0.05$). There were correlations between MMP-2 and MMP-9 expression and the percentage of Grade A sperm and morphologically-normal sperm ($P < 0.05$).

Conclusion

It was concluded that the expression of latent and active forms of MMP-9 and latent form of MMP-2 were observed in all the experimental animals. But the expression of MMP-9 was positively associated with low sperm count as it was strongly expressed in oligospermic groups. Whereas the expression of MMP-2 was positively associated with high sperm count as it was strongly expressed in normospermic groups. Further, more studies are required to ascertain the enzymatic activity of MMP-2 and MMP-9 which may serve as an alternative biomarker in determining semen quality.

References

Agarwal, A., Gupta, S. & Sharma, R. (2016). Eosin-Nigrosin staining procedure. In: Agarwal, A., Gupta, S. & Sharma, R. (Eds.), Andrological evaluation of male infertility. Springer. https://doi.org/10.1007/978-3-319-26797-5_8

Atabakhsh, M., Khodadadi, I., Amiri, I., Mahjub, H. & Tavilani, H. (2018). Activity of matrix metalloproteinase 2 and 9 in follicular fluid and seminal plasma and its relation to embryo quality and fertilization rate. J. Reprod. Infertil. 19(3):140-145.

Baumgart, E., Lenk, S.V., Loening, S. A. & Jung, K. (2002). Quantitative differences in matrix metalloproteinase (MMP)-2, but not in MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 or TIMP-2, in seminal plasma of normozoospermic and azoospermic patients. Hum. Reprod. 17(11): 2919–2923.

Buchman-Shaked, O. R., Kraiem, Z., Gonen, Y. & Goldman, S. (2002). Presence of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinase in human sperm. J. Androl. 23(5): 702–708.

Chen, H., Fok, K. L., Yu, S., Jiang, J., Chen, Z., Gui, Y., Cai, Z. & Chan, H. C. (2011). CD147 is required for matrix metalloproteinases-2 production and germ cell migration during spermatogenesis. Mol. Hum. Reprod. 17(7): 405–414.

Dietrich, M. A., Georg, J., Arnold, J. N., Thomas, F., Kathrin, O. & Andrzej, C. (2014). Characterization of carp seminal plasma proteome in relation to blood plasma. J. Proteomics. 98: 218-232.

Ferrer, M., Rodriguez, H., Zara, L., Yu, Y., Xu, W. & Oko, R. (2012). MMP-2 and acrosin are major proteinases associated with the inner acrosomal membrane and may cooperate in sperm penetration of the zona pellucida during fertilization. Cell Tissue Res. 349(3): 881–895.

Heussen, C. and Dowdle, E. B. (1980). Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. Anal. Biochem. 102(1):196-202.

Jeyendran, R. S., Vander Ven, H. H., Parez-Pelaez, M., Crabo, B. G. & Zaneveld, L. J. (1984). Development of an assay to assess the functional integrity of the human membrane and its relationship to other semen characteristics. J. Reprod. Fertil. 70: 219–228.

Kareskoski., M, Vakkamaki, J., Laukkanen, K., Palviainen, M., Johannisson, A. & Katila, T. (2021). Matrix metalloproteinase (MMP)-2, MMP-9, semen quality and sperm longevity in fractionated stallion semen. Theriogenology. 164: 93-99.

Kratz, E. M., Kałuza, A., Ferens-Sieczkowska, M., Olejnik, B., Fiutek, R., Zimmer, M. & Piwowar, A. (2016). Gelatinases and their tissue inhibitors are associated with oxidative stress: a potential set of markers connected with male infertility. Reprod. Fertil. Dev. 28(7): 1029–1037.

Kurzawski, M., Kaczmarek, M., Kłysz, M., Malinowski, D., Kazienko, A., Kurzawa, R. & Drozdzik, M. (2017). MMP 2, MMP 9 and TIMP 2 polymorphisms affect sperm parameters but not fertility in polish males. Andrologia. 49(5): 12654.

Laemmli, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature. 227: 680 – 685.

Makowski, G. S. & Ramsby, M. L. (1996). Calibrating gelatin zymograms with human gelatinase standards. Anal. Biochem. 236(2): 353-356.

Mohagheghi, A., Khodadadi, I., Karami, M., Amiri, I. & Tavilani, H. (2015). The impact of G1575A matrixmetalloprotease-2 gene polymorphism on male fertility. Avicenna. J. Med. Biochem. 3(1): 6–27826.

Mohagheghi, S., Khodadadi, I., Karami, M., Amiri, I. & Tavilani, H. (2018). Gene polymorphism of matrix metalloproteinase 9 in asthenozoospermic male subjects. Int. J. Fertil. Steril. 11(4): 247.

Mondal, S., Adhikari, N., Banerjee, S., Amin, S.A. & Jha, T. (2020). Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: a minireview. Eur. J. Med. Chem. 194: 112260.

Nagase, H. & Woessner, J. F. (1999). Matrix metalloproteinases. J. Biol. Chem. 274(31): 21491–21494.

Nagase, H. (1996) Matrix metalloproteinases. In Hooper, N.M. (ed.) Zinc Metalloproteases in Health and Disease. Taylor and Francis, London, pp. 153–204.

Saengsoi, W., Shia, W. Y., Shyu, C. L., Wu, J. T., Warinrak, C., Lee, W. M. & Cheng, F. P. (2011). Detection of matrix metalloproteinase (MMP)-2 and MMP-9 in canine seminal plasma. Anim. Reprod. Sci. 127(1–2): 114–119.

Salisbury, G.W., VanDemark, N.L. & Lodge, J. R. (1985). Physiology of reproduction and artificial insemination of cattle (2nd ed.). WH Freeman and Company, pp. 268–274.

Shimokawa, K. I., Katayama, M., Matsuda, Y., Takahashi, H., Hara, I. & Sato, H. (2003). Synergistic effects of TIMP2-418G/C and MMP9-1562C/T variants on the male infertility risk. Mol. Biol. Rep. 46(1): 861–866.

Shimokawa, K., Katayama, M., Matsuda, Y., Takahashi, H., Hara, I., Sato, H. & Kaneko S. (2002). Matrix metalloproteinase (MMP)-2 and MMP-9 activities in human seminal plasma. Mol. Hum. Reprod. 8: 32–36.

Tentes, I., Asimakopoulos, B., Mourvati, E., Diedrich, K., Al-Hasani, S. & Nikolettos, N. (2007). Matrix metalloproteinase (MMP)-2 and MMP-9 in seminal plasma. J. Assist. Reprod. Genet. 24(7): 278-281.

Warinrak, C., Wu, J. T., Hsu, W. L., Liao, J. W., Chang, S. C. & Cheng, F. P. (2015). Expression of matrix metalloproteinases (MMP-2, MMP-9) and their inhibitors (TIMP-1, TIMP-2) in canine testis, epididymis and semen. Reprod. Dom. Anim. 50(1): 48-57.

Watson, P. F. (1975). Use of Giemsa stain to detect change in acrosome of frozen ram spermatozoa. Vet. Rec. 97: 12–15.

Woessner, J. P. Jr. (1994). The family of matrix metalloproteinases. Ann. NY Acad. Sci. 732: 11–21.

Yin H Z, Vogel M M, Schneider M, Ercole C, Zhang G, Sinha A A and Wilson M J. (1990). Gelatinolytic proteinase activities in human seminal plasma. J. Reprod. Fertil. 88: 491–501.

Received: 31st August 2023 Accepted: 01st November 2023

Journal of the Andaman Science Association Vol. 28(2):218-224 (2023)
ISSN 0970-4183, Printed in India

Influence of sex and age on Serum Matrix Metalloproteinases expression in bovine species

T. C. Balamurugan[1], R. Prakash Krupakaran[1], S. Anandhi[1], C. Senthamil Pandian[1] and P. Perumal[2]
[1]*TANUVAS-Veterinary College and Research Institute, Salem-636 112, Tamil Nadu, India*
[2]*ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India*
***Corresponding author's E-mail:** tcbalamurugan@gmail.com*

Abstract

A comparative study was conducted to find out the existence of Matrix Metallo Proteinases (MMPs) in the serum of cattle breed of Tamil Nadu (Umblachery; n=24) and Jersey crossbred cattle (n=24). Umblachery breed is a recognized and registered breed of cattle in the home tract of Cauvery delta region of Tamil Nadu, India. Based on their age and sex, these experimental cattle were divided into four groups for each breed and each group consisted of six animals viz., Group I: Male (1 - 3 years), Group II: Male (4 - 6 years), Group III: Female (1 - 3 years) and Group IV: Female (4 - 6 years). Blood samples were collected in the morning prior to concentrate feeding. Blood samples were centrifuged and separated the serum. These serum samples were subjected to gelatin zymography. Major bands at 220, 135 and 92 kDa of MMP-9 and at 72 kDa of MMP-2 were observed in different experimental groups in both breeds. In Umblachery breed, two prominent bands were observed at 92 and 72 kDa and they represent the latent forms of MMP-9 and MMP-2, respectively. Further, in each experimental group, two lytic bands were observed at 220 and 135 kDa and they represent as the proforms of MMP-9. Intensity of latent form of MMP-2 (72 kDa) was 1.5 times higher in groups of aged as compared to younger cattle. Similarly, aged cattle had showed thicker bands at 135 and 220 kDa; indicating that the expression of pro-enzymatic forms of MMP-9 was clear and higher. In context of sex, intensity of latent form of MMP-2 (72 kDa) was 1.5 times higher in male than female cattle in Umblachery breed. On the other hand, intensity of latent form of MMP-9 was lower in male than the female cattle in Umblachery breed. Similarly, male cattle expressed two more prominent bands at 220 and 135 kDa; these are lytic band and pro-enzymatic form of MMP-9, respectively. The study concluded that MMPs were expressed in both cattle breeds. MMP-2 expression was increased and MMP-9 expression was decreased as age advanced in both breeds and both sexes. Similarly, MMP-2 expression was higher in male than female cattle whereas MMP-9 expression was higher in female than male cattle in both breeds. Therefore, results indicated that breed, age and sex modulate the expression of MMPs profiles in bovine species because MMPs are involved in extracellular matrix accumulation and are connected with concentric remodeling of tissues.

Key words: *Matrix Metallo proteinase, gelatin zymography, serum, cattle*

Introduction

Tissue matrix homeostasis is a complex process which is essential for normal growth, development and wound healing processes (Hingorani *et al*., 2018). MMPs are members of the metzincins family; a family of zinc-dependent proteases that degrade the components of the extracellular matrix (ECM) (Nagase and Woessner, 1999). Several types of proteinases are involved in the degradation of matrix proteins; however, MMPs, also known as matrixins, are the most important proteinases involved. Most connective tissue remodeling processes are accomplished by degradation of extracellular components by MMPs. These proteins are involved in the release and activation of growth factors and cytokines and to regulate the apoptosis in the human reproductive system (Riccioli *et al*., 2005). MMP-2 and MMP-9 are members of a family of more than 25 zinc-dependent endopeptidases that degrade or cleave many extracellular proteins including the extracellular matrix (ECM) components.

MMPs are classified as (i) gelatinases (ii) stromelysins (iii) collagenases and (iv) membrane-type (MT) MMPs (transmembrane enzymes that cleave ECM

components and activate other MMPs) (Nagase and Woessner, 1999). MMPs degrade various extracellular matrix components such as collagen, elastin, laminin, and proteoglycans (Visse and Nagase, 2003). Members of the MMP family are widely expressed in many reproductive processes including menstruation, ovulation and embryo implantation as well as the processes of uterine, mammary gland and prostate gland involution (Jezierska and Motyl, 2009). In many organs, MMP activity affects the cell behavior by altering the key functions such as proliferation, differentiation, migration and apoptosis (Jezierska and Motyl, 2009). Currently, more than 25 different types of MMPs have been identified in vertebrates and most of them are expressed and have similar functions in both humans and dogs (Aresu *et al.,* 2011).

Umblachery breed is a recognized and registered breed of cattle in the home tract of Cauvery delta region of Tamil Nadu, India. Expression of MMPs between men and women have been previously identified and discussed (Mattey *et al*., 2012). However, no study was conducted and no report was available in bovine species with different age groups and sex on expression of MMPs. Therefore, objective of the present study was to examine the expression pattern of MMPs (MMP-2 and MMP-9) in serum of Umblachery and Jersey crossbred cattle in different age groups of both sexes.

Materials and Methods

The present study was conducted at Department of Veterinary Physiology and Biochemistry, TANUVAS-Veterinary College and Research Institute, Orathanadu, Tanjore, Tamil Nadu, India. The institute is located 30 meters above mean sea level with a latitude of 10.6°N and 79.3°W.

Experimental animals

Experimental animals (n=48) were selected from an organized farm. Umblachery breed (n=24) and Jersey Crossbred (n=24) cattle were divided into four groups and each group consisted of six animals, viz., Group I: Male (1 -3 years), Group II: Male (4 - 6 years), Group III: Female (1 - 3 years) and Group IV: Female (4 - 6 years). All the animals were vaccinated and feeding and watering was followed as per the farm schedule.

Collection and evaluation of serum

Blood samples were collected from the experimental animals in blood clot activator in the morning prior to concentrate feeding. The blood samples were centrifuged at 3000 rpm for 15 min at 4°C and serum was separated and labelled. The protein content of the serum samples was estimated by the standard procedure of the Lowry method (Lowry *et al*., 1951) with use of a spectrophotometer (Thermoscientific, Germany). A standard curve was constructed using different concentrations of bovine serum albumin as a standard. Serum samples were stored at −80° C for further analysis.

Gelatin zymography

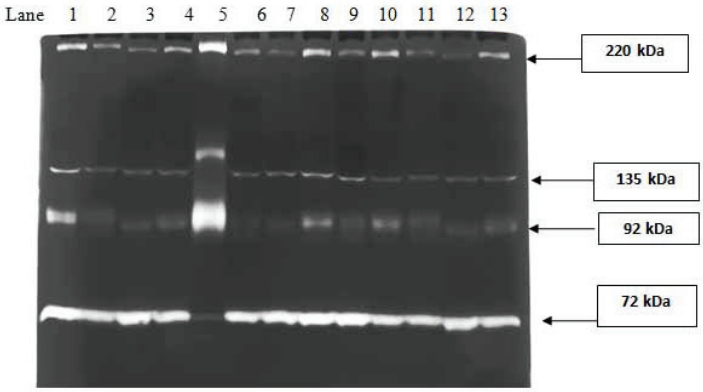
Serum samples were subjected to gelatin zymography by modified SDS-PAGE [a modified method of Laemmli (1970) as performed by Heussen and Dowdle (1980). In this method, gelatin (0.3%) was added as a copolymerization substrate to obtain (final concentration 0.15%) the resolving gel (8%). The samples were electrophoresed at 100 V for 20 min. Renaturation was performed with 2.5% Triton X-100 for 3 h on a mechanical shaker with gentle shaking. The gel was then incubated in 10 mM $CaCl_2$, 0.15 M NaCl and 50 mM Tris, pH 7.5, for 18 h at 37 °C. The gel was stained with 0.25% Coomassie brilliant blue for 2 h, followed by destaining with a destaining solution for 1 h. Finally, the gel was washed with distilled water.

Analyzing the results of gelatin zymogram

Human capillary blood gelatinase served as the standard marker for evaluating the zymogram bands, following the protocol outlined by Makowski and Ramsby (1996). By performing a finger stick, blood was obtained from a capillary and measured using a precise analytical balance in a tarred polypropylene tube. Afterward, the samples were combined with 20× volume of Laemmli buffer and thoroughly blended. These aliquots remained stable for a duration of 3 months at -20° C.

Results and Discussion

Serum samples of four groups for both breeds were subjected to gelatin zymography. Serum samples were proteolytically active as all completely degraded the gelatin. Major bands at 220, 135 and 92 kDa of MMP-9 and 72 kDa of MMP-2 were observed in all four experimental groups in both breeds. In Umblachery breed, two prominent bands at 92 kDa and 72 kDa were observed and they represent the latent forms of MMP-9 and MMP-2, respectively. Further, in each experimental group, two lytic bands were observed at 220, and 135 kDa and they represent as the proforms of MMP-9. However, the active form of MMP-9 and MMP-2 is not observed in serum sample of different experimental groups of Umblachery breed. On the other hand, latent forms of MMP-9 (92 kDa) and MMP-2 (72 kDa) were observed in different experimental groups of Jersey crossbred cattle. In Umblachery breed, the proforms of MMP-9 was observed at 220 and 135 kDa in different experimental groups. MMP-2 (72 kDa) was a prominent proteinase in the bovine species as compared to human marker (lane 5). The results of present study were in agreement with the results of Bannikov *et al*. (2011), Newby *et al*. (2014) and Krupakaran *et al*. (2016) in bovine species and Krupakaran *et al*. (2015) in bubaline species. Similarly, Krupakaran *et al*. (2016) reported that the latent form of MMP-2 (72 kDa) was more prominent as compared to that of MMP-9 monomer (92 kDa) in serum of Jersey crossbred bull (Fig. 1).



Lane	Breed	Sex	Group	Age (Years)	No. of animals
1.	Umblachery	Male	I	0-3	6
2.	Umblachery	Female	III	0-3	6
3.	Umblachery	Male	II	4-6	6
4.	Umblachery	Female	IV	4-6	6
5.	Human capillary blood MMP marker				
6.	Jersey Crossbred	Male	I	0-3	6
7.	Jersey Crossbred	Male	II	4-6	6
8.	Jersey Crossbred	Male	I	0-3	6
9.	Jersey Crossbred	male	II	4-6	6
10.	Jersey Crossbred	Female	III	0-3	6
11.	Jersey Crossbred	Female	III	0-3	6
12.	Jersey Crossbred	Female	IV	4-6	6
13.	Jersey Crossbred	Female	IV	4-6	6

Fig. 1. Comparative Gelatin Zymogram of Umblachery (n=24) and Jersey Crossbred (n=24) cattle of male and female

Effect of age on the expression of gelatinase activity

Gelatin zymogram of different age groups in both breeds were compared to find out the expression pattern of gelatinase with respect to age. In Umblachery breed, the intensity of latent form of MMP-2 (72 kDa) was 2-3 times higher than that of the latent form of MMP-9 (92 kDa) in different age groups. Similarly, expression intensity of latent form of MMP-2 (72 kDa) was 1.5 times higher in aged (G II: lane 3 & IV: lane 2) as compared to younger experimental cattle (G I: lane 1 & III: lane 4). Similarly, two more prominent bands at 220 and 135 kDa were observed in younger groups as they are lytic bands of MMP-9 which indicated that they are the pro-enzymatic forms of MMP-9.

In Jersey crossbred, intensity of the latent form of MMP-2 (72 kDa) was 2-3 times higher than the latent form of MMP-9 (92 kDa) in different age groups. Similarly, the intensity of the latent form of MMP-2 (72 kDa) was 1.5 times higher in aged groups (G II: lane 7, 9 & G IV: lane 12,13) than younger groups (G I lane 6,8 & III: lane 10,11). Similar to Umblachery breed, the younger groups (G I lane 6,8 & III: lane 10,11) exhibited thicker bands at 135 and 220 kDa indicating that they are the lytic bands of MMP-9 and they are the pro-enzymatic forms of MMP-9 was very prudent and clear.

Results of the present study were in agreement with the results obtained by Bonnema *et al*. (2007), McNulty *et al*. (2005) and Cancemi *et al*. (2020). Similar to the present study, the expression pattern of MMP-2 is age dependent as expression was increased as age advanced; higher expression was noticed in aged groups than younger groups. Similar result was obtained in human subjects that ageing process is associated with higher activities of MMP-2 (McNulty *et al*., 2005). Similarly, Bonnema *et al*. (2007) conducted a study on human subjects from age groups of 20 to 90 years and the result revealed that MMP-2 concentration was increased (from 1188 ± 99 to 1507 ± 76 ng/mL) as age increased. Further, Cancemi *et al*. (2020) reported that the serum activity of MMP-2 was higher in long-living individuals as compared to younger individuals. Yu *et al*. (2013) also reported that the activities of both MMP-2 and MMP-9 were higher in the tendons of aging than in younger rats.

In the present study, the expression of MMP-9 was decreased in aged groups than younger groups of same sex. This results clearly indicated that the concentration of MMP-9 was decreased as age advanced and in younger animals showed marked higher concentration. Similar to the results of the present study, Paczek *et al*. (2008) and Bonnema *et al*. (2007; from 29±7 to 8±2 ng/mL) reported that the concentration of active MMP-9 decreased as age advanced in human. Therefore, alteration in expression and concentration of MMPs is age-dependent as because MMPs are involved in extracellular matrix accumulation and are connected with concentric remodeling of tissues. Moreover, MMPs play a key role in regulating the matrix remodeling as they are responsible for the degradation of collagens and proteoglycans (Sharma and Maffulli, 2006).

Effect of sex on the expression of gelatinase activity

Gelatin zymogram of male and female cattle in both breeds were compared to find out the relationship between the MMPs expression and sex. In Umblachery breed, the intensity of latent form of MMP-2 (72 kDa) was 1.5 times higher in male (G II: lane 3; G I lane 1) as compared to female groups (G III: lane 2; G IV: lane 4). The intensity of latent form of MMP-9 was lower in male (G II: lane 3; G I lane 1) compared to female cattle (G III: lane 2; G IV: lane 4). The female cattle (G III: lane 2; G IV: lane 4) had thicker bands at 135 and 220 kDa as compared to male groups indicated that the expression of pro-enzymatic forms of MMP-9 was very clear and they were lytic bands of MMP-9.

In Jersey crossbred, the intensity of latent form of MMP-2 (72 kDa) was 1.5 times higher in female (III: lane 10,11; IV: lane 12,13) than in male (G I: lane 6,8 & G II: lane 7, 9) cattle. The intensity of latent form of MMP-9 was lower in male (G I: lane 6,8 & G II: lane 7, 9) than the female cattle groups (G III: lane 10,11; G IV: lane 12,13). The female cattle (G III: lane 10,11; G IV: lane 12,13) exhibited thicker bands at 135 and 220 kDa than the male cattle indicated that the expression of pro-enzymatic forms of MMP-9 was prudent and clear and they are the lytic bands of MMP-9.

The present study results were in agreement with the results obtained by Bonnema *et al.* (2007), Vanessa

A. Belo *et al.* (2009), Kusnierova *et al.* (2015), and Cancemi *et al.* (2020). Similar to the results of the present study, the expression of MMP-2 was higher in male groups compared to female cattle groups. On contrary, expression of MMP-9 was higher in female than in male groups. To agree with our results, a human study on MMPs revealed that Pro-MMP-2 activity was increased as age advanced in male gender; but not in female gender (Cancemi *et al.,* 2020). This is because the expression of MMPs is also dependent on the hormonal status in women (Berg *et al.,* 2014). Similar to the results of the present study, Kusnierova *et al.* (2015) reported that the plasma MMP-2 level was significantly correlated with age in human as lower concentration was detected in persons ≤ 49 years of age. However, the plasma MMP-3 was significantly associated with both age and gender as lower concentration was detected in persons of ≤ 47 years of age and among the women. Plasma MMP-9 level was not age dependent; it was associated with gender, showed lower concentration of MMP-9 in women (Kusnierova *et al.,* 2015). Thus, Kusnierova *et al.* (2015) concluded that MMP-2 and MMP-3 levels were found to be age dependent and MMP-3 and MMP-9 levels were gender dependent. Similarly, Bonnema *et al.* (2007) reported that women had non-significantly lower value (MMP-2: 1352 ± 58 Vs 1300 ± 49; MMP-9: 16.5 ± 6.3 Vs 19.4 ± 3.8; TIMP-1: 1058 ± 54 Vs 867 ± 37; TIMP-2: 43 ± 6 Vs 43 ± 5) as compared to men with respect to MMPs (ng/mL).

On the contrary to the present study results, Belo *et al.* (2009) reported that non-significant difference was observed between boys and girls on different MMPs (MMP-8, pro-MMP-9, MMP-9, MMP-2, TIMP-1, and TIMP-2) and the concentration of Pro-MMP-9 (A.U) in girls (0.99) than boys (0.76) but the concentration of MMP-2 was equal in both sexes (1.59). Further, Andreas Jonsson *et al.* (2016) reported that MMPs (MMP-1, -2, -7, -8, -9 and -13) had shown non-significant difference between plasma and serum samples in men and women. However, Sathyamoorthy *et al.* (2015) reported that MMP-1, MMP-3, MMP-8 and MMP-9 were higher in men than women infected with tuberculosis. Further, Sathyamoorthy *et al.* (2015) found that plasma MMP-8 concentration was 1.51-fold higher in men than women with tuberculosis and this difference was not due to greater disease severity in men. Men mount a greater and often more damaging inflammatory response to infection compared to women of reproductive age (Guerra-Silveira Abad-Franch, 2013). Higher MMP level alteration observed in male patients as compared with female patients (Yadav *et al.,* 2018). These results of the present study clearly indicated that the expression of MMP-9 is more in female than in the male animals. It might be due to female sex hormones are protective (Marriott and Huet-Hudson, 2006) and female neutrophils have been found to express decreased MMP-9 during the period of the menstrual cycle when oestrogen levels are higher (Smith *et al.,* 2007). Hence, it might be inferred that the levels of gelatinases expression may be correlated with individual physiological status within the sex and age groups.

Conclusion

It was concluded that expression of gelatinase activity was confirmed in both breeds of Umblachery and Jersey crossbred. Expression of MMP-2 was higher as age advanced and aged animals had higher MMP-2 than the younger animals. Expression of MMP-9 was lower as age advanced and aged animals had lower MMP-9 than the younger animals in male and female cattle. Similarly, the expression of MMP-2 was higher in male than the female animals whereas the expression of MMP-9 was higher in female than in male animals. Therefore, the present study results indicated that breed, age and sex modulate the expression of MMPs profiles in bovine species because MMPs are involved in extracellular matrix accumulation and are connected with concentric remodeling of tissues.

References

Aresu, L., Giantin, M., Morello, E., Vascellari, M., Castagnaro, M., Lopparelli, R., Zancanella, V., Granato, A., Garbisa, S., Arico, A., Bradaschia, A., Mutinelli, F. & Dacasto, M. (2011). Matrix metalloproteinases and their inhibitors in canine mammary tumors. BMC Vet. Res. 7: 33.

Bannikov, G. A., Hinds, C. A., Rajala-Schultz, P. J., Premanandan, C., Rings, D. M. & Lakritz, J. (2011). Serum haptoglobin-matrix metalloproteinase 9 (Hp-MMP 9) complex as a biomarker of systemic

inflammation in cattle. Vet. Immunol. Immunopathol. 139: 41–49.

Belo, V. A., Souza-Costa, D. C., Lana, C. M., Caputo, F. L., Marcaccini, A. M., Gerlach, R. F., Bastos, M. G. & Tanus-Santos, J. E. (2009). Assessment of matrix metalloproteinase (MMP)-2, MMP-8, MMP-9, and their inhibitors, the tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2 in obese children and adolescents. Clin. Biochem. 42**:** 984–990.

Berg, G., Schreier, L. & Miksztowicz, V. (2014) Circulating and adipose tissue matrix metalloproteinases in cardiometabolic risk environments: pathophysiological aspects. Hormone Mol. Biol. Clin. Invest. 17(2): 79–87.

Bonnema, D. D., Webb, C. S., Pennington, W. R., Stroud, R. E., Leonardi, A. E., Clark, L. L., McClure, C. D., Finklea, L., Spinale, F. G. & Zile, M. R. (2007). Effects of age on plasma matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs). J. Cardiac Fail. 13(7): 530-540.

Cancemi, P., Aiello, A., Accardi, G., Caldarella, R., Candore, G., Caruso, C., Ciaccio, M., Cristaldi, L., Di Gaudio, F., Siino, V. & Vasto, S. (2020). The Role of Matrix Metalloproteinases (MMP-2 and MMP-9) in Ageing and Longevity: Focus on Sicilian Long-Living Individuals (LLIs). Mediators of Inflammation. 5: 8635158.

Guerra-Silveira, F. & Abad-Franch, F. (2013). Sex bias in infectious disease epidemiology: patterns and processes. *PLoS ONE.* 8: e62390.

Heussen, C. & Dowdle, E. B. (1980). Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. Anal. Biochem. 102: 196–202.

Hingorani, D. V., Lippert, C. N., Crisp, J. L., Savariar, E. N., Hasselmann, J. P. C., Kuo, C., Nguyen, Q. T., Tsien, R. Y., Whitney, M. A. & Ellies, L. G. (2018). Impact of MMP-2 and MMP-9 enzyme activity on wound healing, tumor growth and RACPP cleavage. *PLoS ONE.* 13(9): 0198464.

Jezierska, A. T. & Motyl, T. (2009). Matrix metalloproteinase-2 involvement in breast cancer progression: a mini-review. *Medical Science Monitor:* Int. Med. J. Exp. Clin. Res. 15(2): R32–R40.

Jonsson, A., Hjalmarsson, C., Falk, P. & Ivarsson, M. L. (2016). Levels of matrix metalloproteinases differ in plasma and serum - aspects regarding analysis of biological markers in cancer. Br. J. Cancer. 115(6): 703-706.

Kusnierova, P., Vsiansky, F., Pleva, L., Plevova, P., Safarcik, K. & Svagera, Z. (2015). Reference intervals of plasma matrix metalloproteinases 2, 3, and 9 and serum asymmetric dimethylarginine levels. Scand. J. Clin. Lab. Invest. 75(6): 508-513.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with folin phenol reagent. J. Biol. Chem. 193**:** 265-275.

Makowski, G. S. & Ramsby, M. L. (1996). Calibrating gelatin zymograms with human gelatinase standards. Anal. Biochem. 236: 353–356.

Mattey, D. L., Nixon, N. B. & Dawes, P. T. (2012). Association of circulating levels of MMP-8 with mortality from respiratory disease in patients with rheumatoid arthritis. Arthritis Res. Therap. 14: R204.

McNulty, M., Spiers, P., McGovern, E. & Feely, J. (2005). Aging is associated with increased matrix metalloproteinase-2 activity in the human aorta. Am. J. Hypertension. 18(4 pt1): 504–509.

Nagase, H. & Woessner, J. F. (1999). Matrix metalloproteinases. J. Biol. Chem. 274**:** 21491–21494.

Newby, A. C. (2005). Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol. Rev. 85: 1– 31.

Paczek, L., Michalska, W. & Bartlomiejczyk, I. (2008). Trypsin, elastase, plasmin and MMP-9 activity in the serum during the human ageing process. Age and Ageing. 37(3): 318 –323.

Prakash Krupakaran, R., Balamurugan, T. C., Pandiyan, G. D. V., Arunkumar, S. & Perumal, P. (2015). Alterations in serum matrix metalloproteinases during different reproductive stages of Murrah buffaloes. Indian J. Anim. Sci. 85(5): 458-461.

Prakash Krupakaran, R., Balamurugan, T. C., Lakshmi, R. D., Sheeba, A. & Perumal, P. (2016). Comparative study on serum matrix metalloproteinases in various species of domestic animals. Indian J. Anim. Sci. **86**(5): 545-549.

Riccioli, A., Dal Secco, V., De Cesaris, P., Starace, D., Gandini, L., Lenzi, A., Dondero, F., Padula, F., Filippini, A. & Ziparo, E. (2005). Presence of membrane and soluble forms of Fas ligand and of matrilysin (MMP-7) activity in normal and abnormal human semen. Human Reprod. 20(10): 2814-2820.

Sathyamoorthy, T., Sandhu, G., Tezera, L. B., Thomas, R., Singhania, A., Woelk, C. H., Dimitrov, B. D., Agranoff, D., Evans, C. A., Friedland, J. S. & Elkington, P. T. (2015). Gender-dependent differences in plasma matrix metalloproteinase-8 elevated in pulmonary tuberculosis. PLoS One. 10(1): e0117605.

Sharma, P. & Maffulli, N. (2006). Biology of tendon injury: healing, modeling and remodeling. J. Musculoskelet. Neuronal. Interact. 6: 181-190.

Smith, J. M., Shen, Z., Wira, C. R., Fanger, M. W. & Shen, L. (2007). Effects of menstrual cycle status and gender on human neutrophil phenotype. Am. J. Reprod. Immunol. 58: 111–119.

Visse, R. & Nagase, H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Cir. Res. 92(8): 827–839.

Yadav, S. S., Singh, M. K., Dwivedi, P., Mandal, R. K., Usman, K., Khattri, S. & Pant, K. K. (2018). Significance of Impaired Serum Gelatinases Activities in Metabolic Syndrome. Toxicol. Int. 21(1): 107–111.

Yu, T. Y., Pang, J. H., Wu, K. P., Chen, M. J., Chen, C. H. & Tsai, W. C. (2013). Aging is associated with increased activities of matrix metalloproteinase-2 and -9 in tenocytes. BMC Musculoskelet. Disord. 14**:** 2.

Received: 01st November 2023 Accepted: 30th November 2023

Andaman Science Association (Regd. 544)

HQ C/o Central Agricultural Research Institute (CARI)
Garacharma, Port Blair -744 105, (Andaman and Nicobar Island)
Email : andamanscience84@gmail.com, asa_portblair@yahoo.com

MEMBERSHIP APPLICATION FORM

Affix passport size photograph

The General Secretary,
Andaman Science Association
HQ: C/o ICAR - Central Island Agricultural Research Institute
Port Blair -744101 (A & N Islands) India

Dear Sir,

I am sending herewith the sum of Rupees …… (Rs……) by cash/ B.D./ Cheque No…….. dated…… of ..... (Name of the Bank) being my subscription as a **life/ annual** member of the **Andaman Science Association** from the year ..... Please enrol my name as a member of the society.

Kindly acknowledge the receipt and membership.

Yours faithfully

Signature: _____

Full Name: _____

Date: ……..

(Please strike ✓ in the appropriate Box)

Individual Membership

Annual Membership ☐

Life Membership ☐

Institutional/ Organizational Membership

Ordinary Membership ☐

Corporate Membership ☐

Membership No.: ASA / 20___/

Personal Details:

(Please fill up the Form in BLOCK LETTERS)

Name in full : **Dr/ Shri/ Smt.** _____

Designation : _____

Field of specialization : _____

Address for communication : _____

_____

_____

Permanent Address : _____

_____

_____

Telephone : (+91) _____ Fax: (+91) _____

Mobile No. : (+91) _____ **e-mail** :_____

Subscription Rates

Individual Annual Members:	Indian Nationals	NRI & Foreign National
Admission fee	: Rs.100/-	USD 05/-
Annual Subscription	: Rs.800/-	USD 20/-
Individual Life Members	: Rs.4000/-	USD 100/-
Student Membership	: Rs.700 (Annual)	--
Institutions & Libraries Members:		
Annual Members	: Rs. 3000/- per year	--
Corporate/Institutional Membership	: Rs. 30000/- at a time	--

Note: Subscription should be sent by ***Cash/ /Bank Draft/ Multicity Cheques*** addressed to the **General Secretary, Andaman Science Association, Post ICAR-Central Island Agricultural Research Institute, Port Blair – 744 101 (A & N Islands)** in the form of Bank Drafts/ Multicity Cheques should be drawn on the S.B.I., Garacharma Branch in favour of "Andaman Science Association" Multicity Cheques will be accepted only.