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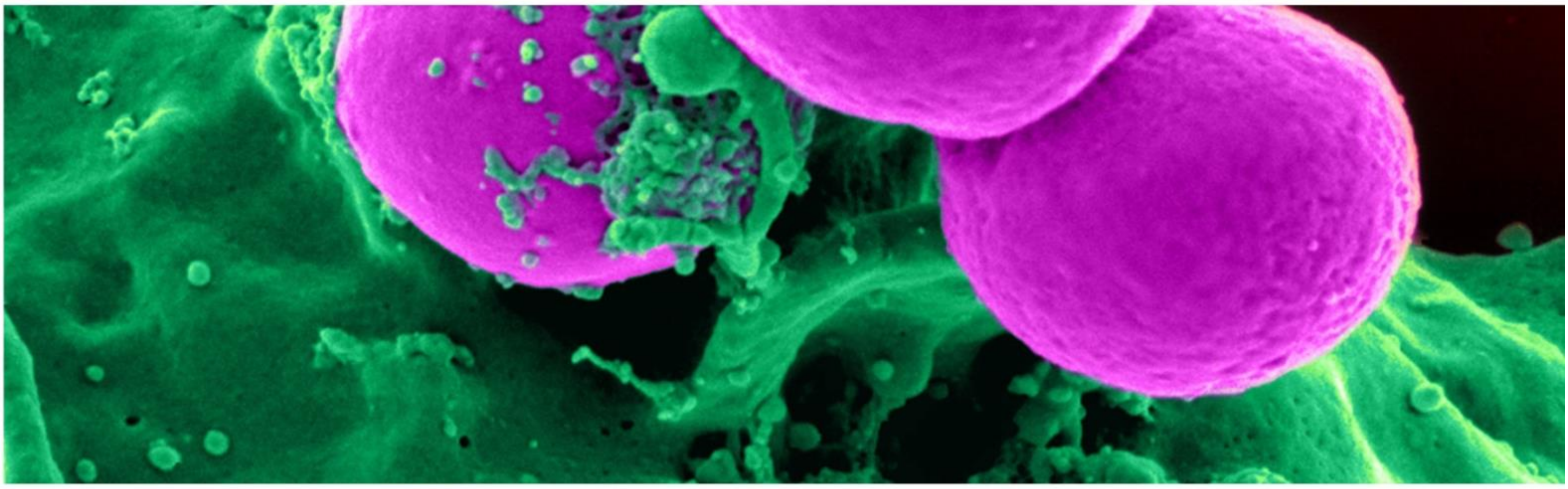

BIOLOGICAL WEALTH FOR ECONOMIC PROSPERITY

Biological Wealth for Economic Prosperity

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Preface

The biological wealth, planets' most valuable natural heritage is represented by the diversity of life forms that integrate species, ecosystems and dynamic ecological events. Bioresources; whether microorganisms, flora, fauna, ecosystems and landscapes provide goods and services to humans for all aspects of life, reflecting people's well-being and vitality of the earth. Biological resource-based industries such as food, brewing, pharmaceutical, paper and pulp etc. contribute immensely for economic growth. Modern technology and knowledge open-up an enormous potential for utilizing and redesigning bioresources into commercial products for sustainable global economic development. Sri Lanka is blessed with an immense biological wealth, exhibited by the rich biological diversity and adding economic value to its biological resources through responsible use is the key to economic development.

The Institute of Biology Sri Lanka (IOBSL), as the leading professional body for biologists in Sri Lanka, recognizes its task to contribute continuously to promote research, innovations, education and public awareness in utilizing bioresources sustainably. The thematic publication titled "Biological Wealth for Economic Prosperity" highlights recent discoveries and applications of biological resources for potential income generation that underpins substantial economic benefits.

The information provided in this book will be valuable to provide an inspiration for all those who are interested in exploring the enormous potential of biological wealth for economic development of the country.

Our sincere thanks go to the authors of the chapters, the reviewers, and the members of the council for their great support in producing this book.

Dr. R. Wimalasekera
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22nd September, 2023

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CHAPTER 01

Mangrove ecosystems for sustainable economic development

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Abstract

Mangroves are a unique, productive, and biodiverse ecosystem located in the inter-tidal zones of tropical and subtropical countries. With an estimated total of over 19,000 ha, they are found in all major climatic zones in Sri Lanka. There is an overall high value and many direct and indirect uses linked to mangroves under provisioning, regulating, supporting and cultural services. Direct uses include fishery and aquaculture, supply of timber, food, and fuel. Most of these are related to the traditional uses of mangroves and many are important to the local economy, as they support local livelihoods.

Moreover, mangroves afford other diverse economic prospects. The unique mangrove ecosystem provides an opportunity for bioprospecting for compounds such as drug lead molecules, antioxidants, and nutraceuticals. Biodiversity and recreational value can play an important role in developing both local and international ecotourism. These activities, if properly carried out, provide a continuous and stable income for local communities while supporting mangrove conservation.

Mangroves are regarded as a nature-based solution for climate change mitigation and adaptation. They are beneficial for facing climate change related events such as sea level rise and storm surges. Mangroves are blue carbon ecosystems and sequester carbon in large amounts. This provides the potential to trade carbon credits in the voluntary carbon market.

In an environment where sustainability and economic development are often viewed as mutually exclusive, mangroves provide an opportunity to unite the two, with nationally accrued economic and environmental benefits.

Keywords: *Bioprospecting, Blue carbon, Ecotourism, Mangroves, Sustainable economic development*

Introduction

Mangroves are a unique, highly productive, and biodiverse ecosystem found in tropical and subtropical regions. The term mangrove is concurrently used for both the ecosystem as well as for the specialised group of halophytic plants that occur in this ecosystem. Located along estuaries, riverbanks and lagoons, these shrubs and trees often experience periodic inundation during high tides. Globally, mangroves show diversity based on the climate, water salinity, topography and soil characteristics of their location.

In 2020, the global extent of mangroves was estimated at 147,359 km^2 with more than half of it located in the Asia Pacific region (UNEP, 2023). Asian mangroves show exceptionally high biodiversity with, over 50 different species including several endemics. High rainfall, significant riverine inputs and favourable coastal features have led to biodiverse and well-structured mangrove forests, particularly in South Asian and South East Asian countries (FAO, 2007).

Sri Lanka, an island nation, has many of the favourable characteristics mentioned above and boasts a mangrove forest cover estimated at 19,758 ha (Premakantha et al., 2021). Mangrove vegetation is present across wet, dry, arid, and intermediate climatic zones in Sri Lanka with a concentration in the southern, north-western, north-eastern and eastern parts of the country. *Avicennia marina, Lumnitzera racemosa, Rhizophora mucronata* and *Excoecaria agallocha* are regarded as some of the dominant mangrove species in Sri Lanka (Premakantha et al., 2022).

The mangrove ecosystem provides several ecosystem services in their localities. Thus, there is an overall high value and many direct and indirect uses linked to mangroves under provisioning, regulating, supporting and cultural services. According to Giesen et al. (2006), more than 70% of mangrove species in South East Asia have a known use, with many having multiple uses. Out of these, over 40% of all species are useful as medicines and over 20% are used as food-stuffs such as vegetables, fruits, and spices. Direct uses of mangroves include fishery and aquaculture, along with the supply of timber, tannin, food, fuel and herbal medicine. Most of these are related to the traditional uses of mangroves and many play a role in the local economy by supporting livelihoods.

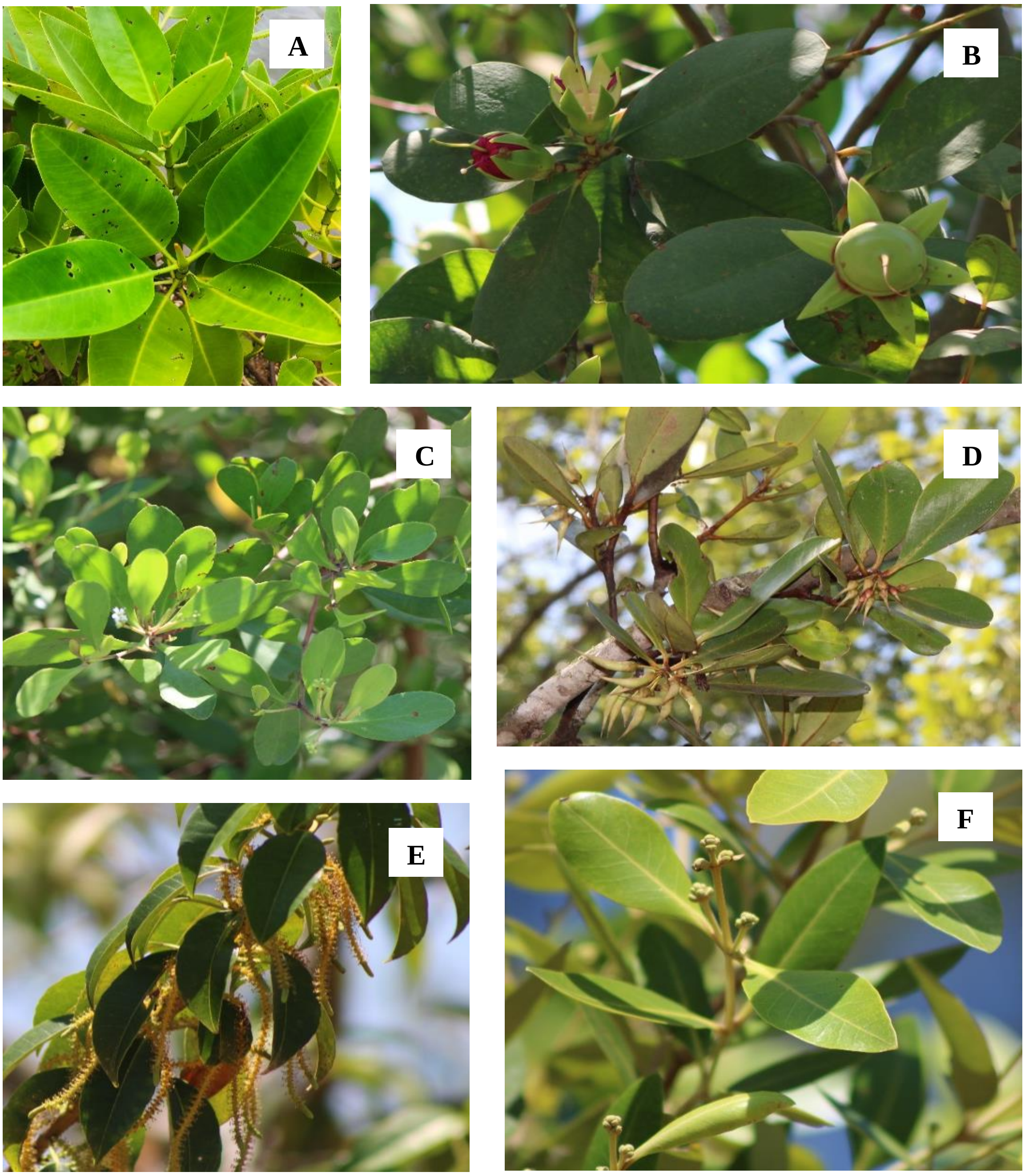

Figure 1: A few common mangrove plant species found in Sri Lanka
A-*Rhizophora mucronata,* ***B***-*Sonneratia caseolaris,* ***C***-*Lumnitzera racemosa,* ***D***-*Aegiceras corniculatum*, **E**-*Excoecaria agallocha* **F**-*Avicennia marina*
(Photo credit: Ravishani Ranathunga)

Some of the indirect uses or functions of mangroves include providing shoreline protection, supporting food webs, flood control, water filtration, and assisting in land accretion. They also provide a habitat for many faunal species which includes crustaceans, birds, molluscs, mammals, insects, and fish.

Much of the above-described goods and services provided by mangroves are well known, with many of the goods being used by adjacent communities for centuries. However, in the present day there are several other spheres in which mangroves have been able to provide goods and services, many of which are built upon the age-old uses of these ecosystems and their constituents. In the present work these 'new' benefits of mangroves will be discussed under the potential for bioprospecting, climate change mitigation and adaptation, and ecotourism.

Bioprospecting for novel compounds from mangroves

Bioprospecting is a systematic and organized search for useful products derived from biological resources such as plants, microorganisms, and animals, that can be further developed for commercialization and to provide benefits to society (Oyemitan, 2017). These useful products include but are not limited to, economically valuable genes, bioactive compounds, novel food products and industrial raw materials.

Mangroves survive and thrive in anaerobic muddy soils under high salinity, tidal inundation, strong winds, high temperatures, and high sunlight (Perera and Amarasinghe, 2021). Generally, organisms living in extreme environments have adaptive mechanisms to survive the various stress conditions that they face. Therefore, living under heat stress, anaerobic conditions and physiological drought stress, many mangrove species synthesize specialised compounds that assist in their survival. This makes these species highly suitable candidates for bioprospecting. According to a review by Bibi et al. (2019), the most common phytochemical groups isolated from mangroves are terpenoids, tannins, steroids, alkaloids, flavonoids, saponins, and glycosides. Many mangrove species have been found to produce novel compounds with commercial potential as antimicrobial, antifungal, antioxidant and anticancer agents etc.

Bioprospecting for bioactive compounds in mangroves is well facilitated by their rich history of use in traditional medicine (Bandaranayake, 1998). This enables researchers to employ an ethnobotanical approach in bioprospecting where traditional knowledge assists in selecting potential plant species as well as the suitable plant part to use. Species such as *Bruguiera gymnorhiza, Rhizophora mucronata, Acanthus ilicifolius,* and

Heritiera fomes are widely used in the Asian region in traditional medicine and the pharmacological validity of some of their uses have been established by research as well (Bibi et al., 2019).

It should be noted that it is not only the mangrove plant species that can provide novel compounds of potential commercial value. Other constituents of the mangrove ecosystem, including microorganisms have yielded compounds of interest. For example, studies have reported on the pharmacological activity of compounds isolated from fungi that inhabit mangroves (manglicolous fungi) and lichens (Thatoi et al., 2013; Tatipamula et al., 2021).

Well managed bioprospecting can lead to development of new products, providing foreign income to biodiversity rich, economically challenged countries like Sri Lanka. It can also facilitate conservation due to the value provided to biodiversity and the possibility of reinvesting some of the dividends in conservation efforts. A systematic bioprospecting plan can provide both economic and social benefits to local communities, and thereby discourage them from resorting to unsustainable use of mangroves. Thus, well managed bioprospecting can lead to both short-term and long-term benefits in a sustainable manner both locally and nationally. However, mismanagement may lead to biopiracy of mangrove resources, where benefit sharing is unequitable or absent altogether. Therefore, drafting and implementation of relevant legislation is necessary to ensure that access to genetic resources, product development and benefit sharing occurs equitably.

Contribution of mangroves for climate change mitigation and adaptation

Article 1 of the United Nations Framework Convention on Climate Change defines climate change as, ‘a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods.’

Climate change mitigation deals with reducing or preventing greenhouse gas (GHG) emissions to the atmosphere and thereby stabilizing their levels. On the other hand, climate change adaptation is defined as actions taken to moderate, cope or take advantage of actual or anticipated changes in the climate (IPCC, 2007).

Mangroves play a vital role in mitigating and adapting to climate change at a global scale while providing economic benefits to local communities (UNEP, 2023). For this reason, they are considered as a nature-based solution (NbS) for climate change. Nature-based solutions are inspired and supported by nature, designed to address societal challenges that are cost-effective, simultaneously provide environmental, social and economic benefits, and help build resilience (European Commission, 2016). Like many developing nations, Sri Lanka is particularly vulnerable to the adverse effects of climate change and has limited financial resources to invest in adaptation measures. For example, mangroves can protect communities and landscapes against high energy waves like tsunamis and storm surges from extreme weather. This protection would be many times more cost-effective than hard engineering solutions. Therefore, nature-based solutions such as those provided by mangrove ecosystems are particularly suitable, as it is a cost-effective method to develop climate resilience while providing benefits to local stakeholders such as resource rights.

Mangroves take up 700 Tg C yr^{-1} through Gross Primary Production and respire 525 Tg C yr^{-1} (75%) back to the atmosphere as CO_2 (Alongi et al., 2014). Mangrove forests are regarded as a blue carbon ecosystem and are sizable sinks of GHGs due to its carbon sequestration ability. Blue carbon refers to organic carbon captured and stored by the oceans and coastal ecosystems, particularly vegetated coastal ecosystems (Macreadie et al., 2019).

It is estimated that the overall ecosystem carbon density of Sri Lankan mangrove vegetation (excluding soil) is 95.92 Mg/ha and that on average one hectare of mangrove vegetation can sequester around 351.7 Mg of atmospheric carbon dioxide in its biomass (Alawathugoda et al., 2022). However, there can be variation in localities based on the environmental setting, ecological profile as well as the type, scale and duration of human influence (UNEP, 2023).

The carbon sequestration ability of mangroves can be used for economic development through trading in voluntary carbon markets. In fact, there is greater interest by developed nations on offshore carbon off setting schemes as these countries seek to reduce emissions under the Paris Agreement. Currently tools are available for accounting and crediting carbon payments for coastal wetland conservation, restoration and creation, with some countries such as Kenya already using these frameworks (UNEP, 2023). However, certain technical and policy barriers remain and further development is required, especially regarding creating a suitable price for blue carbon in voluntary carbon markets.

Ecotourism in mangrove areas

Mangroves are highly biodiverse ecosystems. A global review of mangrove forest faunal species identified 853 vertebrate species including 790 birds, 40 mammal, 20 reptiles and three amphibian species (Luther and Greenberg 2009). For Sri Lankan mangroves, Arulnayagam et al. (2021) report 99 invertebrate species and 214 vertebrate species from a comprehensive review of the literature. In terms of mangrove flora, Sri Lanka has about 21 true mangrove species. The rich biodiversity, the associated water elements and overall scenic beauty showcase the potential of mangroves as a tourist attraction. However, the sensitivity of this ecosystem coupled with its immense value both locally and globally also makes it vulnerable to the negative aspects of tourism. Mass scale tourism can severely affect mangroves due to hotel developments, associated degradation of forested land, erosion, disturbance to fauna, dumping of waste matter etc.

In this background, ecotourism, may be the answer as it is viewed as a sustainable, economically sound, and local stakeholder friendly subset of tourism.

Ecotourism is defined as 'responsible travel to natural areas that conserves the environment, sustains the well-being of the local people, and involves interpretation and education' (TIES, 2015). Therefore, true ecotourism would lead to a sustainable economy and empowerment of local communities along with the protection of the environment.

Development of recreational water-based activities such as boating and kayaking could be encouraged along with trekking, bird watching or camping, as mangrove ecosystems are a unique nexus between land and water. However, due to the same reason, there may be a need to build certain visitor friendly facilities such as walk-ways etc particularly to facilitate trekking during the rainy periods. Education and research-oriented tourism could also be carried out at the mangrove sites. Well organised guided tours would also create environmental awareness and cultural understanding (Dahanayake et al., 2015). An entry payment to visit sites, which is managed at the regional government level would be useful to maintain visitor facilities as well as contribute to conservation efforts. As many of the activities may be of interest to both local and foreign tourists, a two-tier fee payment structure would be beneficial.

Figure 2: Mangrove vegetation at Rekawa, Sri Lanka
(Photo credit – Ravishani Ranathunga)

Ecotourism, if properly carried out, can provide a continuous and stable income to local communities. It would also support mangrove conservation, as locals would be convinced that in the long-term, the mangrove resource would be more valuable intact, than when short term extractions are done from the ecosystem (Salam et al., 2000). In addition, there are even suggestions to protect privately owned mangrove vegetation by encouraging landowners to utilize them for ecotourism activities (Premakantha et al., 2022). However, in order to maintain standards and the true spirit of ecotourism, it would be necessary to have strict regulations for the operators and visitors, along with a viable monitoring mechanism.

Conclusion

Local communities have used mangrove ecosystems and the resources therein for millennia. While many of the age-old goods and services provided by mangroves are important, it is prudent to look at new approaches that would enable conservation of this ecosystem while utilising it in a sustainable manner. Aspects discussed in this chapter such as bioprospecting for bioactive compounds from mangroves and ecotourism in mangrove areas are examples for this approach. However, for sustainable economic development to occur, it is vital that both activities are organized with the implementation of national legislation where appropriate. Further, part of the economic dividends of these activities should trickle back into the local community and the ecosystem itself, in order to facilitate conservation efforts.

Mangroves have been hailed as a nature-based solution for climate change, which as outlined above, is justifiable. It can favourably contribute to both adaptation and mitigation activities, having an impact both in the short and long term. Furthermore, it can be a cost-effective solution to achieve climate resilience for economically challenged, climate vulnerable, developing nations like Sri Lanka.

Thus, in an environment where sustainability and economic development are often viewed as mutually exclusive, mangroves provide an opportunity to unite the two, with nationally and locally accrued economic and environmental benefits.

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CHAPTER 02

Underutilized Marine Genetic Resources (MGR) to upscale the Sri Lankan economy: A biotechnological approach

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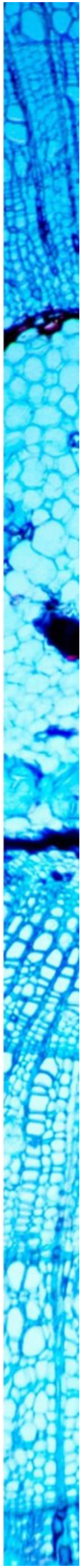

Abstract

Marine biotechnology involves the use of marine bio resources, either as the source or the target of biotechnological applications. Being an island, Sri Lanka is bestowed with an immense coastal and marine biodiversity, yet scarcely unexplored. Particularly, the Indian Ocean around the country is considered the richest biological asset, as it is associated with a comprehensive biological and chemical diversity, ensuing ample space to explore and investigate novel compounds.

Despite the global concern of utilising marine genetic resources for biotechnological applications, Sri Lanka is still at its infancy. Exploring ocean around Sri Lanka to utilize, to develop products which are beneficial for humans both economically and socially, is one of the important facts to be considered. The country has been facing its largest economical deficit since 2021/2022 and there is an urgent need of a promising and sustainable solution. These solutions should be novel initiatives, based on scientific research and investigation, comprehensive analysis and wise use of technology to uplift the nation from the current economic catastrophe. The proper utilization of marine resources would be one of the best and feasible approaches in this regard.

Major opportunities do exist to extend the use of ocean bio resources in local and foreign markets for industrial applications such as biopolymers, enzymes, pharmaceuticals, functional foods, cosmetics and many more. Most of the marine biopolymers can be successfully extracted, purified and developed into value added products. Similarly, marine invertebrate-secreted secondary metabolites can be developed to successful therapeutic entities. The legal aspects of sustainable utilization of marine resources and patenting the products or mechanisms associated with Sri Lankan Marine Genetic Resources (MGRs) should be prioritized. Those steps together with proper planning and sustainable use of the underutilized marine genetic resources will thus make the Sri Lanka economically strong and benefited for its people.

Keywords: *Marine biotechnology, Marine drugs, Marine genetic resources, Marine resources, Pharmaceuticals*

Prologue to marine biotechnology

Coastal countries are benefited with a vast array of resources from the ocean. With the discovery of two molecules, *Ara A* and *Ara C* from the Caribbean marine sponge species, *Cryptotheca crypta* (Schwartsmann et al., 2001), a new arena was opened to the relationship between the ocean and human. This novel relationship, which we now called "Marine Biotechnology" is resulted with the advancement of science and technology, targeting marine organisms for a new purpose rather than obtaining food and other physical resources. Marine biotechnology identifies the diverse and unique properties of marine derived compounds produced by marine organisms due to the various challenges they face throughout the evolution. These challenges include drastic changes in the physical, chemical, and hydrological parameters such as temperature, light intensity, salinity, and pressure, in the surrounding water and biological threats such as predatory pressure, fouling organisms and many more.

Marine compounds mostly contain unique chemical structures, which make them extraordinary and specific with respect to their terrestrial counterparts. These biomolecules such as secondary metabolites, enzymes, and biopolymers are not a sudden or fortuitous process. Marine organisms secrete secondary metabolites for the sake of survival of their own (Mayer et al., 2010). Due to this reason, most marine-derived compounds are profoundly toxic and produced at very high concentrations to repel fouling organisms and predators. Interestingly, these high toxic compounds are also capable of employing bioactivities, which can be useful in various disciplines including medicine (Newman and Cragg, 2004). Other than bioactive secondary metabolites, various macromolecules such as proteins, fatty acids, polysaccharides too, are produced by marine organisms as primary products. These are highly used in various industrial applications.

Although most countries have put the initial step in exploring the biotechnological potential of marine resources, Sri Lanka still is at its infancy with respect to the use of marine resources for bioprospecting. At the outset, this chapter focuses on various types of marine genetic resources and the areas of their potential applications to support the economy of the country. The latter part of the chapter discusses the challenges of using marine genetic resources in a sustainable manner.

Sri Lanka as a treasure of Marine Genetic Resources (MGR)

Being an island in the Indian Ocean, Sri Lanka is situated in the south of the Indian sub-continent between 5°55' and 9°51' N latitude, and 79°41' and 81°53' E longitude north of the equator. The country hosts nearly 65,000 km^2 of land area with a coastline of about 1, 585 km, with beaches of 300 km (Lowry and Wickremaratne, 1989; Olsen 1992; Rajasooriya and White, 1995). The continental shelf is about 31,000 sq km and the width ranges from 9 to 45 km with an average depth of 66 m (Cooray, 1994). Despite the small land size, Sri Lanka is globally recognized as one of the richest biological hot spots in the Indian Ocean, undoubtedly due to topographic and climatic heterogeneity as well as its coastal influence (Gunatilleke et al., 2008). There is a major contribution of marine ecosystems to biodiversity as they are enriched with various ecosystems such as coral reefs, sea grass beds, sand dunes etc (Gunatilleke et al., 2008). The underlying reason for the massive biodiversity in marine organisms is the genetic diversity, associated with the long evolutionary history, ecosystems diversity and many more.

When the genetic diversity is explored for its true potential, it can be called as a resource. Thus, Marine Genetic Resource (MGR) can be broadly defined as genetic material, i.e DNA or RNA of actual or potential value (Harden-Davies, 2018). It may include individual cells, strains, stocks, and communities of organisms of actual/potential value for food and agriculture or any other human needs such as health.

The true potential of biotechnological applications can be specifically coined as bioprospecting, which refers to the systematic approach to exploring novel biological products and activities with biotechnological applications (Harden-Davies, 2018). The process starts with the comprehensive literature survey, collection of biomaterials, storing and screening for bioactivities and apply the usage. This process is continued with the product up scaling and value addition. Various areas of marine biotechnology are targeted to reach the goal of sustainable use of marine resources to provide goods and service to human needs.

As the world population is burgeoning at a rapid rate, the demand for food and other health facilities are projected to grow. With the predicted value of the world population of 9 billion people in 2050 (Ball, 2014), the consumption of food and other basic needs are predicted to intensify laterally. Although benefits arise from the rapid growth of aquaculture, there are other problems such as environmental pollution, biodiversity degradation etc. associated with the rapid development of agriculture and aquaculture which cannot be ignored. Sustainable and environmental-friendly approach in development of blue biotechnology is imperative thereof.

Sri Lanka is amidst of one of the worst economic crises since its independence with a huge burden resulted by foreign debts (George, 2013). There is an urgent necessity for the country to take steps for the economic recovery, in order to establish a sustainable economic growth and development of marine biotechnology can play an immense role in that. The use of underutilized marine genetic resources which are still untapped for centuries, will open a new arena to earn foreign income.

The development of marine biotechnology in different disciplines can be discussed in brief, under the following categories:

(i) Food Industry

Use of marine resources as food and in food industry has gained historical credit, as it dates back to at least the Upper Paleolithic period. Even the use of biotechnological applications can be identified in various era of human civilization. Use of MGR to obtain a plethora of compounds such as enzymes, proteins, peptides, polysaccharides, polyunsaturated fatty acids (PUFA), secondary metabolites and pigments which are used in food industry is practiced globally. Marine -based food ingredients are grouped under nutraceuticals, which are nutrients with bioactive substances containing medicinal characteristics or added health benefits. Marine organisms are the main source of ω-3 fatty acids, which exert beneficial effects against various non-communicable human diseases (RubioRodríguez et al., 2012). Most of the nutraceuticals such as eicosapentaenoic and docosahexaenoic ω-3 fatty acids are obtained from oily fishes such as herring, mackerel, sardine and salmon. Other marine organisms such as microbes, microalgae, macro algae and invertebrates are also good sources of nutraceuticals (Gupta and Prakash, 2021).

Marine polysaccharides are proven with numerous applications in food technology. These are abundantly extracted from sea weeds (Venkatesan et al., 2015). Alginate, carrageenans and agar from seaweeds are used as gelling agents, stabilizers and edible films in many food products. Some of these polysaccharides such as carrageenans exhibit various beneficial actions, such as antioxidant, anticancer, antiarteriosclerosis, anti-tumor, and many more (Holdt and Kraan, 2011; Li and Kim, 2011). Chitin, chitosan which are sulphated polysaccharides obtained mainly from crustaceans with broad range of applications are used as gelling and emulsifying agents, natural antimicrobial preservatives, edible antimicrobial films etc. (Hayes, 2011; Kapetanakou et al., 2014). Complex polysaccharides such as dietary fibers are provided by seaweeds and other marine organisms (Holdt and Kraan, 2011; Freitas et al., 2012), which have a wide range of applications as nutraceuticals.

Other than polysaccharides, proteins, peptides and enzymes derived from marine organisms are highly used in food industry as raw materials and enzymes (Debashish et al., 2005). Due to the reason that marine organisms can survive under high pressure and salinity, marine enzymes too are highly stable in high pressure and salty conditions, which are common practice in food processing industry (Shahidi and Kamil, 2001; Trincone, 2011; Zhang et al., 2012). When it comes to amino acid composition, marine peptides are always superior to the terrestrial counterparts, which enables these to serve as potential candidates of amino acids (Holdt and Kraan, 2011).

Pigments and colourants extracted from marine sea weeds and microorganisms are best qualified to be used in food industry. These pigments are less toxic than synthetic colorants and contain health benefits due to the presence of antioxidants (Pangestuti and Kim, 2011). Carotenoids, astaxanthin, fucoxanthin are some of such compounds from seaweeds with proven antioxidant and other activities beneficial in food industry (Ngo et al., 2011; Pangestuti and Kim, 2011). Natural pigments isolated from marine bacteria, microalgae and plants can be used as natural food colorants (Holdt and Kraan, 2011; Baghel et al., 2014).

Production of food ingredients from fish and seafood by-products is a growing area of interest, as it reduces processing waste and is efficient (Ahmed et al., 2020). The fish waste is a major concern as an environmental hazard in many countries. Some non-targeted fish catching results waste, which are underutilized. New product development by extracting commercially important biomolecules from underutilized by catch and processing discards is an important area of research and development. For examples, some countries extract chitin and chitosan, collagen and gelatine from underutilized fish and from fish waste (Ahmed et al., 2020). These compounds serve as promising sustainable resource for many industries, including food industry.

(ii) Health

The marine environment is a rich source of chemical diversity. This diversity has been a source of unique chemical compounds with health benefits (Kijjoa and Sawangwong, 2004). Marine genomes provide new insights in to genetic diversity of bio resources and reveal new sources for drugs (Trincone, 2011). As a result of untiring efforts by scientists to discover drugs from the sea, an array of marine derived compounds or secondary metabolites are lined in the drug development pipeline, to be served as drugs in the future (Plaine, 1977). Close to half of all current anti-cancer discovery efforts focus on marine organisms while more than 1000 new compounds are discovered each year (Hu et al., 2015). Marine invertebrates' microbes, fungi and even marine viruses serve as best candidates for most of the drugs.

Marine bioactive compounds which can be used as drugs are in the pre-clinical and clinical trials (Malve, 2016). Marine pharmacology offers the scope for research on these drugs of marine origin.

(iii) Marine Energy

Organization for Economic Co-operation and Development (OECD) and non-OECD economies are committed to reducing their carbon foot print (Plaine, 1977) by switching to renewable biomass to supplement and replace petroleum-based enrgy. However, this type of turning point is quite challenging for a developing country like Sri Lanka, due to issues related to fuel security or economic vulnerability. To become economically and environmentally viable, biofuel production is proposed as a good alternative. It will address technical and commercial challenges such as disposal of by products, carbon neutrality, production of capital costs, scale up and integration of next generation biofuels (Coyle, 2010). Algal biofuels which are also known as next generation biofuels can be a good source of biofuel. These result from the application of marine biotechnology to algal biomass to generate biodiesel, bioethanol, biogasoline, biomethanol, biobutanol and other biofuels (Plaine, 1977). Several advantages do exist with the production of bioenergy from algae. Microalgae are an alternative natural source of renewable petroleum resources that is capable of meeting the global demand for fuels (Chisti, 2008). Using algae as a source of fuel is practiced for many years (Chisti, 2008; Nagle and Lemke, 1990). The oil content of some microalgae exceeds 80% of the dry weight of algal biomass (Banerjee et al., 2002; Chisti, 2008). The high growth rate of microalgae makes it possible to satisfy the massive demand on biofuels using limited land resources. Production of biodiesel from marine algae is exceedingly popular in countries like China, India and dry coastal regions of the Middle East where the population density is higher than other countries (Li et al., 2008). Extensive studies have been carried out for the cultivation of different marine microalgae using a variety of cultivation systems including both open ponds and various types of closed photo bioreactors. Some examples of marine microalgal species that have been studied for microalgal farming include red marine alga *Porphyridium* sp., N-fixing Cyanobacterium, *Anabaena*, macrophytic marine red alga *Agardhiella subulata*, marine green alga *Dunaliella tertiolecta,* and marine phytoplankton *Tetraselmis suecica* (Li et al., 2008).

(iv) Environment

Use of MGR to environment protection is achieved by using these organisms as bioindicators and mediators of bioremediation (Viricel et al., 2018). Marine microorganisms are widely used as bioindicators to indicate the environmental pollution. Bioremediation after oil spills in oceans by marine microorganisms, monitoring toxic algal blooms, identification of invasive species and probing the marine environment are currently involved in this process. The recent application of molecular tools to biocatalysts may improve bioprospecting research, enzyme yield recovery and enzyme specificity, thus increasing cost-benefit ratios (Viricel et al., 2018). Automated high-resolution bio sensing technologies are used to monitor coastal water quality, including prediction and detection of Harmful Algal Blooms (HAB) and marine toxins (Garmendia et al., 2013). Development of cost-effective and non-toxic antifouling technologies is also possible to be practiced. Precise molecular level identification of microbial strains which have bioremediation ability and their mass production and maintaining of cell cultures are also helpful.

Although most of the research are already initiated on bioprospecting, majority of MGR of Sri Lanka is underutilized for processing, value addition and product development. The screening of MGR for novel bioactive compounds remained as an open area for research, followed by screening and identification. Measures should be taken to isolate these compounds and develop them into value added patented products.

The blooming of marine patents associated with MGRs is largely a result of recent technological advances in exploring the ocean and the genetic diversity. Levels of research activity and product development from MGR can be identified under few main categories; marine pharmacognosy, marine derived enzymes, cosmeceutical industry and bioremediation. Marine pharmacognosy or marine derived pharmaceuticals for drug discover for herpes, cancer and AIDS is valued $100 million, $1 billion and $23 million respectively (Paasivirta, 2015). These drugs are derived mainly from marine sponges, tunicates and microorganisms, which are also available in the Sri Lankan coastal habitats.

The sponges of Sri Lanka are recorded as more than 77 species (Dendy, 1905) but the number may be much higher than this value due to evolution of novel species. This untapped resource of marine sponges is investigated by few Sri Lankan scientists (Ratnaweera et al., 2016; Gunathilake et al., 2020) for various bioactive compounds. However, the transformation of knowledge from research to industry has not adequately addressed. Similarly, soft corals, cnidarians, echinodermates and marine microbes can be investigated for their bioactive properties. Extractions of polyunsaturated fatty acids from marine fish can be facilitated. These are components of dietary supplements that deliver health benefits to humans and can alleviate a broad range of non-communicable diseases. Production of reporter to be used in biomedical research and cell and molecular biology is already established and can be performed from jelly fish available in the Sri Lankan coast. These metabolites will serve as potential source for value addition to Sri Lankan MGR.

Sri Lankan fishery is well established and practiced to provide food and nutrition to the people locally and internationally. There are large scale fish processing industries which aims exporting fish to other countries. Most of the underutilized fish belong to the abundantly available pelagic species, which are landed as bycatch, and some are unconventional species such as krill (Venugopal, 1995). Post-harvest fishery losses are also reported high in Sri Lankan Fishery (Weerasekara et al., 2015). Recovery of flesh by mechanical deboning and development of value-added products are probably the most promising approaches which are already practiced in some private firms in the country. Surimi and surimi-based products, sausages, fermented products, protein concentrates and hydrolysates, extruded products are some of the possibilities of which these underutilized fish can be utilized (Venugopal,1995).

Challenges

Use of MGR for product development is still at its infancy in Sri Lanka, creating a large knowledge gap. Marine bioprospecting can be successfully and sustainably used to provide goods and services to Sri Lankan community.

Screening for the resource with pharmaceutical interest is sometimes challenging, when it comes to harvest the same biomaterial. Repetitive harvesting of the same biomaterial is also indirectly result in the exploitation of the valuable MGR. Use of mariculture to cultivate interested organisms can be a good solution.

Extraction of biomaterials which are applicable in industries such as food, pharmaceuticals, cosmetics and agricultural products, will be highly important. This has been already initiated in Sri Lanka by researchers belong to various disciplines. The potential of using Sri Lankan marine invertebrates such as sponges, ascidians and Echinodermates to isolate bioactive compounds to treat type II diabetes and inflammation (Kim et al., 2013; De Silva et al., 2018) is already proven by research. Cytogenotoxicity of several marine organisms (Kuruppuarachchi et al., 2022, 2023) around the country have been experimentally validated, paving path to isolate and characterize them to be used as anti-cancer drugs. Further, sea weeds and their polysaccharides are targeted for various applications (Fernando et al., 2017).

Mining the deep oceans for MGR is also a challenge to a country like Sri Lanka due to lack of technologies and high cost in procedures. Unexplored deep see remains a hidden treasure for various types of MGR, thus future invention should be focused on these organisms.

Attempts can be initiated to isolate bioactive compounds from MGR of fungi and marine bacteria. These have been recognized as a source of pharmaceutical lead compounds (Dissanayake et al., 2016). Most of these microorganisms are symbiotically associated with sedentary species such as sponges, corals etc.

Conclusion

Discovery of novel marine natural products has a long history. However, development of these products in to valuable commercial products is truly challenging, especially to a developing country like Sri Lanka. By following planning strategies, using proper product up scaling and value addition and collaborations to exchange scientific knowledge and technology will help to overcome these challenges.

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CHAPTER 03

Potential of indigenous plant extracts as a natural insecticide to control vector mosquitoes in Sri Lanka

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Abstract

In terms of public health importance, mosquitoes are the most significant category of insects because they spread numerous diseases. Mosquito control includes spraying chemical insecticides on adult mosquitoes or killing the mosquito larvae. However, the application of synthetic insecticides has not been very successful. Therefore, the use of many former synthetic insecticides in mosquito control programs has been limited in recent years. One approach to these arising problems has been to search for new and effective compounds. Phytochemicals could serve as a new weapon in controlling mosquitoes since they constitute a rich source of bioactive chemicals which has insecticidal activities. Sri Lanka is a biodiversity-rich country with so many endemic and indigenous plants. These are having the potential to be used for phytochemical extractions to synthesize novel larvicides and adulticides. While effective, such phytochemicals reduce the risk of potentially adverse ecological effects. Also, they may prevent the possibility of the resistance that synthetic chemical insecticides typically bring about after prolonged use. There is very less chance of pests developing resistance to such substances. Therefore, the country does not have to spend the cost of buying chemical insecticides to be used excessively. They are potentially economically safe while being practical for control measures. This approach would be a beneficial solution for funding through the rational use of finances and human resources in order to achieve better productivity and fruitfulness of mosquito control programs. Hence, this warrants vector control entities to re-think "biological wealth for economic prosperity" with environmentally-friendly country-wide control approaches for medically important disease vectors.

Keywords: *Biological control, Economy, Environmentally-friendly, Phytochemicals*

Introduction

Mosquito-borne diseases are significant public health concerns in Sri Lanka and many other tropical and subtropical countries. The country has suffered from these diseases since ancient times, with a high prevalence of malaria, filariasis, and Japanese Encephalitis (JE). As a result of successive efforts in control programs, Sri Lanka has attained some remarkable achievements by receiving the malaria-free certification in 2016, and filariasis is no longer a public health concern (WHO, 2016). However, despite all the successful vector control measures implemented during the past few decades, some arbovirus infections, particularly dengue, have increased rapidly over the last few years on par with the global situation.

Not surprisingly, these arboviruses have caused vast epidemics with massive numbers of patients and deaths in Sri Lanka. Due to the growing number of mosquito-borne diseases and the higher ability of disease transmission, controlling mosquitoes is crucial and of great public health concern across the country. Furthermore, because of a lack of specific drugs and vaccines for some of these diseases, the main strategy for the reduction and prevention of the spreading of mosquito-borne diseases is mosquito control. Suspending disease transmission can be done by killing, stopping mosquitoes from biting humans or killing the larvae found at vector breeding sites.

Various strategic approaches have been promoted to control mosquito vectors, including chemical control (indoor residual spraying, mass fogging, use of household insecticides), biological control (use of mosquito predators, release of specific genetically modified mosquitoes), source reduction and public education (Gan et al., 2021). For numerous decades, synthetic chemicals have been used to control mosquitoes.

Insecticide-based intervention is the most common method of controlling mosquito populations in the country in the recent past. In Sri Lanka, larvicides (temephos) and adulticides (principally pyrethroids) have been extensively used to control mosquitoes (Fernando et al., 2020). As a result of the reliance on a few active ingredients registered and used in public health, resistance has now evolved in most regions to all four classes of insecticides; organochlorides, organophosphates, carbamates, and pyrethroids. Apart from that, the high cost that the government should bear, the training of health staff for insecticide application, the cost for protective equipment used during insecticide applications, the impacts on other non-target organisms, the bioaccumulation of chemicals along food chains as well as being non-environmentally friendly have strictly pinpointing challenges of using synthetic chemical insecticides for controlling mosquito populations. The uncontrolled usage of insecticides has led to reemergence and an increase in mosquito populations over the years (Demirak and Canpolat, 2022; Yasuoka et al., 2006). Due to the continuing evolution of resistance development, these substances are becoming ineffective. Therefore, nowadays, scientists are targeting other alternative measures, particularly using environmentally friendly strategies for vector control interventions (Yasuoka et al., 2006).

Natural products derived from plants and animals are preferred over synthetic insecticides as an alternative measure because they are eco-friendly (Surendran et al., 2009). Phytochemicals are botanicals that are naturally occurring insecticides obtained from floral resources (Ghosh et al., 2012). They are biologically active chemical compounds and could act as repellents, toxins, feeding deterrents, and growth regulators against insects. They are relatively safe, inexpensive, and readily available in many parts of the globe. Additionally, they are less toxic, target-specific and highly effective when used in smaller quantities and biodegradable. This makes them excellent alternatives to synthetic compounds to be used as mosquito controlling agents (Surendran et al., 2009).

Historically, botanical preparations have been used as insecticides by the ancient people. This information inspired the idea of using bioactive plants as an environmentally acceptable method for vector control. Since the 1920s, phytochemicals have been used for vector control, however, upon discovery of synthetic insecticides like DDT in 1939, phytochemical application got side-tracked. Exploration of plants as natural insecticides will help overcome the difficulties of integrated vector control strategy, sustaining disease elimination as well as preventing its reintroduction. There is a great deal of research that shows plant extracts as promising alternatives to synthetic insecticides against the growing number of mosquito-borne diseases. Environmentally friendly crude extracts from plants with many phytochemicals have synergistic effects on target species. This lessens the detrimental impact on the environment (Sowmyashree et al., 2023).

Many plants consist of insecticidal phytochemicals to protect themselves against herbivorous insects as secondary compounds (Gunathilaka et al., 2022). Plants are known to contain compounds of insecticidal, insect-repelling and insect anti-juvenile properties. Most of these compounds are biodegradable and less harmful to mammals than synthetic insecticides (Ranaweera, 1996). Using plant-based products could be a good eco- and user-friendly strategy for vector control to reduce and eventually eliminate mosquito-borne diseases soon. The biological activity of the experimental plant extracts varied, which may be due to the presence of various phytochemically active compounds in the plants, including phenolics, terpenoides, flavonoids and alkaloids (Kovendan et al., 2014). To produce larvicidal effects, these active principles may have jointly or independently influenced or contributed (Sowmyashree et al., 2023).

Potential of phytochemicals as mosquito controlling agents in Sri Lanka

Different plant parts from *Annona glabra* have shown effective results on larvicidal properties against *Aedes* mosquitoes. A study by Amarasinghe and Ranasinghe (2017) showed; larvicidal properties on leaf and seed extracts *Annona glabra* plant collected from Hunupitiya, in Gampaha District, Sri Lanka. Study revealed that LC_{50} for *Annona glabra* aqueous leaf extract for third instar larvae of *Ae. albopictus* and *Aeaegypti* were 69.86 g/L and 82.45 g/L, respectively in 24 h exposure. Furthermore, LC_{50} for *Annona glabra* aqueous seed extract for third instar larvae of *Ae. albopictus* and *Ae. aegypti* were higher than the LC_{50} for *Annona glabra* leaf extract and were recorded as 93.27 g/L and 102.251 g/L, respectively in 24 h exposure. The same study reveals that when the extracting solvent is ethanol, Annona glabra is more effective on third-instar larvae of *Ae. albopictus* and *Ae. aegypti* than the aqueous extract, with LC_{50} values of 39.88 mg/L and 54.88 mg/L, respectively in 24 h exposure.

The larvicidal properties of the same plant, *Annona glabra* have been confirmed by another study conducted by Wijebandara et al. (2021). Fresh ripened fruits of *Annona glabra* fruit extract showed a dose-dependent effect against *Ae. aegypti* larvae after the 48 hours exposure period. The percentage mortality rates have shown a significant variance among different concentrations ($P < 0.0001$). The recorded LC_{50} and LC_{90} values for aqueous crude extract were $87.71 \pm 5.41\ gL^{-1}$ and $510.79 \pm 12.56\ gL^{-1}$, respectively, after 48 h of exposure. The previous literature supports the *Annona glabra* plant's high larvicidal activity; phytochemicals such as saponins, flavonoids, steroids and tannins showed combination effects in terms of larvicidal action to mosquito larvae. Hence, *Annona glabra* aqueous fruit extract is a potential as a key source for developing an environment-friendly plant-based larvicide against *Ae. aegypti* in Sri Lanka.

A study by Gunathilaka et al. (2022) on the pericarp extract of *Garcinia mangostana* fresh fruits harvested from Kaluthara area in Sri Lanka against *Aedes aegypti* mosquitoes in Sri Lanka. This was the first study in Sri Lanka to evaluate the larvicidal effect of mangosteen crude extract. The pericarp of the mangosteen fruit is enriched majorly with α-mangosteen. This part is not edible and after consumption, it is disposed of as waste. Larvicidal effects against insects by inhibition of SCP-2 (sterol carrier protein-2) have been shown by the chemical constitution in the mangosteen plant. Further, the study results yielded an LC_{50} of 41 μg/mL, showing that the larvicidal activity of the mangosteen pericarp crude extract increased in a dose-dependent manner. But the study reported a lower LC_{50} value when compared to previous studies; anyhow when the ethanolic crude extract is used as the solvent, it indicated a higher larvicidal effect. The same study indicated larvicidal activity due to the potential to inhibit cholesterol uptake in *Ae. aegypti* larvae. Without these necessary levels of cholesterol, the molting process is affected and therefore, the death of the insect causes during larval stages. Therefore, the study by Gunathilaka et al. (2022) concludes that the *G. mangostana* extracts have an effective sterol carrier protein inhibitor for inhibition of cholesterol uptake and thus can be investigated further to be used as a potential plant-based larvicide in Sri Lanka.

Further, Ranaweera (1996) revealed 18 plant species out of 53 tested, showing mosquito-larvicidal activity against the late 3rd instar *Culex quinquefasciatus* larvae. Out of these 18, extracts of *Acorus calamus*, *Cymbopogon nardus*, *Languas galanga*, *Camellia sinensis*, *Cararium zeylanicum* and *Curcuma domestica* exhibited significant activities (LC50 < 10mg/L). These plant specimens were further extracted with solvents hexane, light petroleum (40-60%) and methanol, and their essential oils were obtained. These essential oils were tested against *Culex quinquefasciatus*, *Aedes aegypti*, *Aedes albopictus*, *Anopheles culicifacies, Anopheles tessellatus and Anopheles subpictus*, revealing the potential larvicidal activity (Ranaweera, 1996).

Further, the above study results revealed that the essential oils of *A. calamus* showed the highest mosquito-larvicidal activity (3.6–12.0 mg/l) against all mosquito species tested except *An. culicifacies* and *Cx quinquefasciatus* was the most sensitive. The essential oil of *C. nardus* demonstrated significant activity (6.3 mg/l) against *Cx* *quinquefasciatus* and less against *Ae. aegypti*. Petroleum extract of *L. galanga* was effective against *Cx quinquefasciatus* (8.3 mg/l) and *Ae. albopictus* (9.3 mg/l). Therefore, these results showed that there is a possibility of isolating effective mosquito-larvicidal compounds might from *A. calamus, C. nardus, L. galanga* and *C. domestica* growing in Sri Lanka.

Another study conducted by Surendran et al. (2009) tested crude extracts of the fruits of the medicinal plant *Sapindus emarginatus* in Sri Lanka against Ae. aegypti for larvicidal properties. The fruits were gathered from Vavuniya in mid-2006 and were sun-dried. The solvent used was ethanol and the LC_{50} and LC_{90} values obtained from the bioassay experiment as 92.9 and 152.6 ppm, respectively. In addition, the extract was positive for the presence of saponin. Hence the results obtained by this study revealed that the medicinal plant *S. emarginatus* could be used as a source for the production of effective mosquito larvicides.

Table 1: List of plants experimented in Sri Lanka for mosquitocidal properties.

Family	Scientific Name	Mosquito Species Tested	Reference
Annonaceae	*Annona glabra* Linnaeus	*Aedes aegypti* *Aedes albopictus*	Amarasinghe *et al.*, 2017
Annonaceae	*Annona glabra* Linnaeus	*Aedes aegypti*	Wijebandara *et al.*, 2021
Guttiferae	*Garcinia mangostana* Linnaeus	*Aedes aegypti*	Gunathilaka *et al.*, 2022
Acoraceae	*Acorus calamus* Linnaeus	*Culex quinquefasciatus*	Ranaweera, 1996
Burseraceae	*Canarium zeylancium* Retzius Blume		
Zingiberaceae	*Curcuma domestica* Valeton *Zingiber zerumbet* Linnaeus Smith *Languas galanga* Linnaeus Stuntz *Curcuma zedoria* Berg Roscoe *Elettara repens* sonner. Baill		
Fabaceae	*Derris scandens* Roxburgh Bentham		
Rutaceae	*Atalantia rotundifolia* Thwaites Yu Tanaka		
Theaceae	*Camellia sinensis* Linnaeus Kuntze		
Poaceae	*Cymbopogon nardus* Linnaeus Rendle *Cymbopogon citratus* de Candolle Stapf *Cymbopogon confertiflorus* Stapf *Cymbopogon winterianus* Jowitt		
Lauraceae	*Cinnamomum camphora* Linnaeus T. Nees & Eberm *Cinnamomum litseifolium* Thwaites *Cinnamomum multifolium* Wright. *Cinnamomum zeylanicum* Blume		
Rubiaceae	*Ophiorrhiza mungos* Linnaeus		
Sapindaceae	*Sapindus emarginatus* Vahl	*Aedes aegypti*	Surendran *et al.*, 2009

Figure 1: Potential plants with mosquitocidal properties from Sri Lanka (**a** – *Annona glabra*, **b** – *Garcinia mangostana*, **c** – *Acorus Calamus*, **d** – *Canarium zeylancium*, **e** – *Curcuma domestica*, **f** – *Zingiber zerumbet*, **g** – *Languas galanga*, **h** – *Curcuma zedoria*, **I** – *Elettaira repens*, **j** – *Derris scandens*, **k** – *Atalantia rotundifolia*, **l** – *Cymbopogon nardus*, **m** – *Camellia sinensis*, **n** – *Cymbopogon citratus*, **o** – *Cymbopogon confertiflorus*, **p** – *Cymbopogon winterianus*, **q** – *Cinnamomum camphora*, **r** – *Cinnamomum litseifolium*, **s** – *Cinnamomum zeylanicum*, **t** – *Ophiorrhiza mungos,* **u**–*Sapindus emarginatus*)

Global records on phytochemicals with mosquitocidal properties

Many different species of plants around the globe have been shown to possess phytochemicals that can bring about a variety of acute and chronic toxic effects. Roark (1947) described approximately 1,200 plant species having potential insecticidal value, while Sukumar et al. (1991) listed and discussed 346 plant species that only exhibited mosquitocidal activity. These 346 species have belonged to 99 plant families and 276 genera. Members of the plant families Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various types of larval, adulticidal, or repellent activities against different species of mosquitoes (Ghosh et al*.,* 2012). Other studies tested plant species such as the abundantly wild *Ipomoea cairicaia,* and also *Annona reticulata, Annona squamosa, Chrysanthemum indicum* and *Tridax procumbens* for larvicidal activity against different types of mosquitoes and the results show they can be used as eco-friendly methods of mosquito control (Kamaraj et al., 2011; Govindarajulu et al., 2015)

Larvae from the three medically important mosquito genera *Aedes, Anopheles* and *Culex* are all susceptible to a greater or lesser extent to some phytochemicals (Shaalan et al., 2005). The results of many studies have shown the potency of essential oils as natural larvicides and at the same time are advantageous as they reduce the chance of resistance and have minimal side effects on non-target species (Osanloo et al., 2019).

Table 2: Some potential plants experimented for mosquitocidal properties around the globe.

Family	Scientific Name	Mosquito Species Tested	Reference
Annonaceae	*Annona reticulata* Linnaeus	*Aedes aegypti*	Govindarajulu *et al.*, 2015
	Annona squamosa Linnaeus	*Anopheles subpictus, Culex tritaeniorhynchus*	Kamaraj *et al.*, 2011
Salviniaceae	*Azolla pinnata* Robert Brown	*Aedes aegypti*	Ravi *et al.*, 2018
Lamiaceae	*Mentha longifolia* Linnaeus Hudson	*Aedes aegypti*	Abbas *et al.*, 2022
Amaranthaceae	*Salsola imbricata* Forsskal	*Aedes aegypti*	Abbas *et al.*, 2022
Hydrocharitaceae	*Elodea canadensis* Michaux	*Aedes aegypti*	Abbas *et al.*, 2022
Simaroubaceae	*Ailanthus altissima* Miller Swingle	*Aedes aegypti*	Abbas *et al.*, 2022
Rutaceae	*Zanthoxylum armatum* de Candolle	*Aedes aegypti*	Abbas *et al.*, 2022
Rutaceae	*Aegle marmelos* Linnaeus	*Anopheles subpictus*	Elango *et al.*, 2010
Bignoniaceae	*Panjenelia longifolia* Willdenow K. Schum	*Anopheles stephensi*	Sowmyashree *et al.*, 2023
	Millingtonia hortensis Linnaeus filius	*Anopheles stephensi, Culex quinquefasciatus & Aedes aegypti*	Kaushik & Saini, 2008
Asteraceae	*Chrysanthemum indicum* Linnaeus	*Anopheles subpictus, Culex tritaeniorhynchus*	Kamaraj *et al.*, 2011
	Tridax procumbens Linnaeus	*Anopheles subpictus, Culex tritaeniorhynchus*	Kamaraj *et al.*, 2011
	Eclipta prostata Linnaeus	*Anopheles subpictus*	Elango *et al.*, 2010
	Tagetes erecta Linnaeus	*Anopheles subpictus*	Elango *et al.*, 2010
	Artemisia annua Linnaeus	*Anopheles stephensi*	Sharma *et al.*, 2006
Malvaceae	*Abutilon indicum* Linnaeus Sweet		
Lamiaceae	*Hyptis suaveolens* Linnaeus Poit.	*Anopheles culicifiacies*	Kovendan *et al.*, 2014
	Leucas aspera Willdenow Link		
	Ocimum basilicum Linnaeus	*Anopheles stephensi & Culex quinquefasciatus*	Maurya *et al.*, 2009
Acanthaceae	*Andrographis lineata* Nees	*Anopheles subpictus*	Elango *et al.*, 2010
	Andrographis paricutata Burman Nees	*Anopheles subpictus*	Elango *et al.*, 2010
Menispermaceae	*Cocculus hirustus* Linnaeus	*Anopheles subpictus*	Elango *et al.*, 2010
Myrtaceae	*Eucalyptus globulus* Labillardiere	*Culex pipiens*	Sheeren , 2006
Solanaceae	*Solanum xantocarpum* Schrad.	*Culex pipiens pallens*	Mohan *et al.*, 2006

Table 2:

Family	Scientific Name	Mosquito Species Tested	Reference
Papaveraceae	*Argemone mexicana* Linnaeus	*Culex quinquefasciatus*	Karmegam *et al.,* 1997
Piperaceae	*Piper nigrum* Linnaeus	*Culex pipiens*	Shaalan *et al.,* 2005
	Piper retrofractum Vahl	*Culex quinquefasciatus & Aedes aegypti*	Chansang *et al.,* 2005
Euphorbiaceae	*Euphorbia hirta* Linnaeus	*Culex quinquefasciatus*	Rahuman *et al.,* 2000
	Euphorbia tirucalli Linnaeus		Yadav e*t al.,* 2002
Cucurbitaceae	*Momordica charantia* Linnaeus	*Anopheles stephensi, Culex quinquefasciatus & Aedes aegypti*	Singh *et al.,* 2006
Zingiberaceae	*Kaempheria galanga* Linnaeus	*Culex quinquefasciatus*	Choochate *et al.,* 1999
Meliaceae	*Khaya senegalensis* (Desr.) A.Juss.	*Culex annulirostris*	Shaalan *et al.*, 2005
Apiaceae	*Daucus carota* Linnaeus	*Culex annulirostris*	Shaalan *et al.*, 2005
Zingiberaceae	*Curcuma aromatica* Salisbury	*Aedes aegypti*	Choochate *et al.,* 1999
Rutaceae	*Feronia limonia* Linnaeus Swingle	*Anopheles stephensi, Culex quinquefasciatus & Aedes aegypti*	Rahuman *et al.*, 2000
	Citrus sinensis Linnaeus Osbeck	*Anopheles subpictus*	Bagavan *et al.*, 2009
Asphodelaceae	*Aloe ngongensis* Christian	*Anopheles gambiea*	Matasyoh *et al.*, 2008
Sapindaceae	*Paullinia clavigera* Schltdl	*Anopheles benarrochi*	Innacone & Perez, 2004
Commelinaceae	*Tradescantia zebrina* Schinz D. R. Hunt	*Anopheles benarrochi*	Innacone & Perez, 2004

Bioactivity of phytochemicals as mosquito controlling agents

The various phytochemicals reported for their mosquitocidal activity are mainly due to secondary metabolites such as essential oils, alkaloids, phenols, terpenoids, steroids, and phenolics extracted from different plants. Above 2000 species of plants can produce chemical factors and metabolites that are important for pest control programs. There is an indication that active biochemical extraction from plants is determined by the polarity of the solvents used. Polar solvents are for polar molecule extraction and non-polar solvents extract non-polar molecules. Varying types of solvents have a notable effect on the potency of the plant compounds being extracted and in addition, there is also a distinction in the chemo-profile of the plant species.

Kishore et al*.* (2011) reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and have described the mosquito larvicidal potentiality of several plant-derived secondary materials such as alkanes, alkenes, alkynes and simple aromatics, essential oils and fatty acids, lactones, terpenes alkaloids, steroids, isoflavonoids, pterocarpans, and lignans. For example, *Annona glabra* leaf ethanol extract indicated the presence of flavonoids, glycosides, saponins, tannins, steroids, acidic compounds, and anthraquinones while alkaloids, reducing sugars, terpenoids, and phlobatanins were absent (Le Son and Nguyen , 2013).

Saponins are plant bioactive compounds with significant insecticidal action and other biological properties. Furthermore, they are responsible for amplifying pest insects' death intensity, lowering food intake, retardation in development instability in development, and declining reproduction (Gubitz et al., 1999). In addition, saponins are freely soluble and can be extracted in both aqueous and organic solvents and perform their action by attacking with cuticle membrane of the larvae, eventually disturbing the membrane, which is the main cause of larval death (Hostettmann et al., 2005). On the other hand, flavonoids reveal an extensive scope of biocontrol potential of insecticidal activities. Tannins also possess insecticidal properties (Azmathullah et al., 2011). Steroids derived from plants show considerable larvicidal activity against mosquitoes (WHO, 2005). Important phenolic in terms of insecticidal, repellent, and feeding deterrent functions are flavonoids in plants. The *Annona glabra* plant's high larvicidal activity is supported by the presence of phytochemicals such as saponins, flavonoids, steroids, and tannins which showed combination effects in terms of larvicidal action to mosquito larvae. The activity may not be due to a single ingredient but to a mixture of compounds, which may act synergistically.

Generally, the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect from herbivores. Their mode of action inside the target insect body could be several types. The insects that feed on these secondary metabolites potentially encounter toxic substances with relatively non-specific effects on a wide range of molecular targets. The targets range from proteins (receptors, enzymes, signaling molecules, ion channels, and structural proteins), nucleic acids, bio-membranes and other cellular components (Rattan, 2010). This in turn, affects the insect physiology in many different ways and at various receptor sites, the principal of which is an abnormality in the nervous system (such as, in neurotransmitter synthesis, storage release, binding and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway). However, Rattan (2010) reviewed the mechanism of action of plant secondary metabolites on insect bodies and documented several physiological disruptions, such as inhibition of acetylcholine-esterase (by essential oils), GABA–gated chlorine channel, sodium and potassium ion exchange disruption, and inhibition of cellular respiration. Such disruption also includes the calcium channel blockage of nerve cell membrane action, mitotic poisoning, disruption of molecular events of morphogenesis and alteration in the behaviour and memory of the cholinergic system (essential oils), hormonal balance disruption etc. However, the most important activity is the inhibition of acetylcholine-esterase activity (AChE) which is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway. In addition, numerous toxicities to insect larvae result from the physical properties of fatty acids; toxicity by inhalation due to aggregation and formation of thin film at the surface of the water which does not allow respiration, and by penetration due to the amphibolic property of certain molecules, or these toxic compounds may enhance the toxicity of other toxic compounds (Rattan, 2010).

For an effective plant-based insecticide, many parts of higher plants such as leaves, roots, stems, seeds, barks, fruits, fruit peels, resin, and the whole body of little herbs or mixing various plants can be used. The phytochemical activity changes depending on the plant species, plant part, and age, polarity of solvents used for extraction as well as species of mosquito. They show their effect by targeting vital cell components and affecting the physiology of the insect in many different ways (Demirak and Canpolat, 2022).

Conclusion and the way forward

Bioeconomy is an emerging paradigm under which the creation, development, and revitalization of economic systems based on sustainable use of renewable biological resources in a balanced way is rapidly spreading globally (Aguilar et al., 2019). It connects biotechnology, the economy, science, industry, and society. Bioeconomy is a meeting point between biological resources and economic activities as a method of undertaking challenges and accomplishing sustainable development. Further, it involves the production of chemicals, among many other things sustainably and economically for an increasing global population, reducing the dependence on imported and costly products to improve public health.

Plants have proven to be acquiring various defensive chemical compounds that can fight against pathogens and insects. Naturally occurring products like phytochemicals that are less toxic to non-target organisms at a low cost are readily available throughout the globe. Using bioinsecticides that are made up of botanical or plant-based compounds is an excellent alternative because of the minimal hazardous effects to humans and the environment. "Being economically friendly," it becomes more practical and safer for the environment to use such botanical insecticides to control mosquitoes in a country like Sri Lanka.

Although numerous studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae, only a few have been commercially produced and extensively used in vector control programs. The main reason behind the failure in the laboratory to land movements of bioactive toxic phytochemicals is poor characterization and inefficiency in determining the structure of exact active toxic ingredients responsible for the larvicidal activity, both of which are essential for the development of specifications. In addition, the active ingredients can vary both in concentration and composition of the same plant species, in different clones, at different stages of plant growth, and under different climatic and soil conditions. For that reason, the scope of future research should be more focused on the isolation of toxic larvicidal active ingredients. However, the plant extracts may be more effective than the individual active compounds due to a natural synergism that discourages the development of resistance in vectors. The future findings on this aspect would be definitely beneficial to synthesize field-applicable natural mosquito-killing agents for mosquito-controlling programs in Sri Lanka.

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CHAPTER 04

Shaping a sustainable future by igniting tomorrow's game changers: Extremophiles

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Abstract

Extremophiles are magnificent living beings who thrive in extreme environmental conditions. Many scientific studies focusing on identifying extremophiles, their products, and their mechanisms have been recorded in the recent decades. Such studies have mainly focused on extremophiles and the feasibility of using them in various arenas including biofuel synthesis and agriculture. Extremophiles generate the least harmful and biodegradable industrial waste in most cases and produce consistent and higher yields under adverse conditions. Although, several whole-cell extremophiles, extremolytes and extremozymes are currently being used for industrial purposes, many more extremophiles, their products and mechanisms are yet to be discovered. Furthermore, metagenomic studies and genetic engineering of the desired extremozymes into mesophilic hosts and increasing their specificity using nanotechnology would lead to more advanced discoveries. Finding novel extremophiles and their products is expected to revolutionize a vast array of industries. Accordingly, extremophiles are considered as tomorrow's game changers that drive the industrial world towards the development of a bio-based economy.

Keywords: *Biofuel, Extremolytes, Extremophiles, Extremozymes, Sustainable Agriculture*

Introduction

The growth of living organisms depends on specific parameters that are essential for their development. Most of the living organisms thrive on the earth's surface where the temperature is temperate (4 to 40°C), pH is around neutrality, or between 5 and 8.5, and where salinity, hydrostatic pressure and ionizing radiation are low. In the recent decades, many scientific studies have focused on the capabilities of living organisms to survive in extreme environmental conditions. Environments that are beyond the optimal limits for growth and survival of humans due to high or low various parameters including temperature, pH, salinity, pressure, radiation, desiccation, and nutrient availability are considered "extreme" (Rothschild and Mancinelli, 2001). Organisms that can thrive under such extreme conditions are known as extremophiles. Even though most of the extremophiles are microbes, there are multicellular extremophiles as well. For instance, protostome animals found in harsh environments include the Pompeii worm, the psychrophilic Grylloblattodea (insects) and Antarctic krill (a crustacean), (Gupta et al., 2014). Microbial extremophiles can be found in numerous and diverse bacterial, Archaean and fungal communities. Some microorganisms can grow and even thrive in two or more extreme conditions and such microbes are referred to as polyextremophiles. The microbe, *Thermus aquaticus* was the first discovered extremophile and the term extremophiles was introduced by R. D. Mac Elroy in 1971. However, there is another type of organism known as extremotolerant organisms which can withstand extreme conditions only up to a certain degree.

Currently, microbial extremophiles gain much attention due to their potential applications in various arenas including agriculture, dairy industry, biodegradation, chemical processing, bioconversion of hemicellulose, molecular biology, composting, detergent, food, feed additives, feed, leather, paper and cellulose, peptide synthesis, pharmaceutical and therapeutic industries (Yadav, 2021). In this chapter, the feasibility of using the wealth of extremophiles for economic prosperity will be discussed with specific applications. Many more extremophiles, their products and mechanisms are yet to be revealed. There is a variety of potential difficulties of extremophile cultivation which could be the major constraint for identifying the so far unknown extremophiles. However, metagenomics, genetic engineering of the desired extremozymes into mesophilic hosts and increasing their specificity using nanotechnology would lead to advancements in future discoveries.

Classification of extremophiles based on habitat

Polar regions (-2 ^{o}C – 0 ^{o}C), deep marine trenches (0 ^{o}C- 4 ^{o}C and 500 – 1034 atm), terrestrial hot springs (65 ^{o}C – 95 ^{o}C), submarine vents (>100 ^{o}C – 121 ^{o}C), salt brines (> 6% salt), sulfide-rich geothermal zones (pH 3 or lower), soda lakes (pH 10 or above), nuclear power plants (3 – 5 Mrad) can be considered as some of the most important extreme environments in nature. There is a high possibility of finding extremophilic organisms in such environments. Some organisms belonging to different extremophilic groups are presented in Table 1.

In terms of optimal temperature for the growth and survival of living beings, there are three types of microorganisms namely mesophiles (20 – 45 ^{o}C), psychrophiles (below 15 ^{o}C) and thermophiles (above 45 ^{o}C). Out of these three categories, psychrophiles and thermophiles are considered extremophiles. Psychrophiles have been reported from various phyla (Govarthanan et al., 2020) whereas a number of Archaean and bacterial genera have been reported as thermophiles (Elumalai et al., 2019).

Based on the optimal pH range, the extremophiles are categorized into two groups as acidophiles and alkaliphiles. Acidophiles can survive and grow in highly acidic environments of pH below 4. A red algal species; *Cyanidium caldarium* and a green algal species; *Dunaliella acidophila* are reported as extreme acidophiles growing under pH near 0. Several algal and fungal species, and diverse groups of archaea and bacteria found in natural and artificial acidic environments including areas that are associated with human activities such as metal ores and coal mines (Salwan and Sharma, 2020; Tripathi et al., 2021). Interestingly, the archaea, *Picrophilus oshimae* and *Picrophilus torridus* proved that life could even exist at a low pH of -0.2. (Pikuta et al., 2007). Alkaliphiles thrive at higher pH levels (10–13 pH) and *Streptococcus faecalis* was the first isolated alkaliphilic strain. Numerous alkaliphilic bacterial (Preiss et al., 2015) and alkaliphilic cyanobacterial strains belonging to various genera have been isolated in soda lakes, hydrothermal vents, and carbonate-rich soil (Pikuta et al., 2007).

The organisms that can thrive under high salinity conditions (>30% of NaCl concentrations) are known as halophiles. Such organisms can be found in three main domains: bacteria, archaea and eukarya and they can be found in hypersaline environments such as saline lakes (Menasria et al., 2018; Ruginescu et al., 2020; Villanova et al., 2021).

Those organisms that thrive in environments of high-pressure are known as piezophiles or barophiles. Such organisms grow in deep ocean beds (Parihar and Bagaria, 2020). The organisms referred to as radiophiles prefer high levels of ionizing and ultraviolet radiation. The most radiation-resistant microorganism that has been identified so far is *Deinococcus radiodurans* (Gupta et al., 2014). Moreover, *Bdelloid rotifers* has been recorded as the world's most radiation-resistant freshwater invertebrate (Gladyshev and Meselson, 2008). The organisms which grow in the presence of very low water activity are known as xerophilies. Various xerophilic bacteria, fungi, algae and plants have been identified (Rothschild and Mancinelli, 2001). There are other interesting extremophile groups as metallophiles and microaerophiles those that thrive in the presence of high heavy metal concentrations and in the presence of a small amount of free oxygen respectively (Gupta et al., 2014).

Table 1: Different types of extremophilic organisms

Extremophile	Organisms	References
Psychrophiles	*Actinobacteria, Ascomycota, Basidiomycota, Chlamydiae, Chloroflexi, Cyanobacteria, Firmicutes, Euryarchaeto, Gemmatimonadetes, Nitrospirae, Mucoromycota, Planctomycetes, Proteobacteria, Thaumarchaeota, Verrucomicrobia, Flavobacterium, Shewanella Clostridium*	8 and 10
Thermophiles	*Moorella, Gelria, Pseudomonas, Geobacillus, Bacillus, Thermococcus, Thermus, Mycobacterium, Thermotoga, Gallionella, Crenothrix, Sphaerotilus, Leptothrix, Lieskeella, Pyrococcus, Sulfolobus, Metallosphaera, Caldicellulosiruptor, Thermoanaerobacter*	6 and 9
Acidoliphiles	*Cyanidium caldarium Dunaliella acidophila, Aconitium cylatium, Cephalosporium sp., Trichosporon cerebriae, Euryarchaeota, Ferroplasma, Acidobacter, Acidohalobacteria, Leptospirillum, Sulfobacillus, Acidibacillus, Desulfurococcus, Metallosphaera, Pyrococcus, Acidianus, Sulfolobus, Picrophilus, Picrophilus oshimae* and *Picrophilus torridus*	2, 13 and 14
Alkaliphiles	*Streptococcus faecalis, Bacillus pseudofirmus, Bacillus halodurans, Alkaliphilus metalliredigens, Thioalkalivibrio, Pseudomonas alcaliphila, Natranaerobius thermophilus, Spirulina, Chroococcidiopsis, Nostoc, Cyanospira, Gloeocapsa, Nodularia, Synechococcus, Synechocystis, Calothrix, Scytonema, Anabaena, Lamprocystis, Thiocapsa, Thiocystis, Chromatium, Amoebobacter, Thiospirillum, Rhodobacter, Ectothiorhodospira, Chamaesiphon, Oscillatoria*	1, 2 and 5
Halophiles	*Bacillus, Brevibacterium, Dunaliella* (Algae), *Dactylococcopsis* (Cyanobacteria) *Halobacillus, Halococcus, Haloferax, Halogeometricum, Halomonas, Haloterrigena, Marinococcus, Natrialba, Natrinema, Salinibacter, Salinicoccus, Salinivibrio, Virgibacillus*	7, 12 and 15
Piezophiles	*Pyrococcus, Moritella, Methanococcus* and *Shewanella*	11
Radiophiles	*Deinococcus radiodurans, Bdelloid rotifers*	3 and 4
Metallophiles	*Cupriavidus metalliduran* and *Rhodobacter sphaeroides*	4
Microaerophiles	*Campylobacter jejuni* and *Helicobacter pylori*	4

References: [1]Downie and Cruickshank, 1928; [2]Pikuta *et al*., 2007; [3]Gladyshev and Meselson, 2008; [4]Gupta *et al*., 2014; [5]Preiss *et al*., 2015; [6]Zeldes *et al*., 2015; [7]Menasria *et al*., 2018; [8]Chaudhary *et al*., 2019; [9]Elumalai *et al*., 2019; [10]Govarthanan *et al*., 2020; [11]Parihar and Bagaria, 2020; [12]Ruginescu *et al*., 2020; [13]Salwan and Sharma, 2020; [14]Tripathi *et al*., 2021; [15]Villanova *et al*., 2021

Whole-cell extremophiles

Extremophiles display unique enzymatic features and physiological properties which led them to be used in various aspects as whole cells. For instance, they can be used in bio-catalysis. It is very important to study the structural features of extremophiles. The cell membrane of thermophiles consists of saturated fatty acids thus increasing the cell rigidity and lipids linked to their cell walls which increase the heat resistance (Kumar and Nussinov, 2001). In contrast, the psychrophilic membranes are comprisedof a high amount of unsaturated fatty acids and thus help maintain fluidity and nutrient transportation at low temperatures (Georlette et al., 2004). Both acidophiles and alkaliphiles possess certain strategies to thrive under extreme pH environments. Other than peptidoglycan, alkaliphiles possess negatively charged cell-wall polymers and saturated and mono-unsaturated straight-chain fatty acids. Acidophiles also display several strategies to withstand environments with low pH including a positively charged membrane surface, a high internal buffer capacity, over-expression of H^+ ions exporting enzymes and unique transport systems (Gomes and Steiner, 2004).

There are numerous arenas where whole-cell extremophiles can be used including bioremediation, biomining, agriculture, medicine and industrial purposes. Interestingly, *Deinococcus radiodurans* is currently being engineered for the remediation of radioactive wastes. Moreover, metallophiles have gained much demand for utilization in the removal of toxic heavy metals from soils, sediments and wastewater (Gupta et al., 2014).

Extremozymes

The enzymes produced by extremophiles are known as extremozymes. They can be used in various industries as they can withstand harsh industrial process conditions. Most of the enzymes that are currently used in various industries are of mesophilic origin and they have limited applications due to their instability in extreme conditions. Utilization of extremozymes as biocatalysts has gained much attention lately and accordingly, it is very important to study the structural modifications of extremozymes. The structure of thermophilic proteins is characterized by increased surface charge, less exposed thermolabile amino acids and the ability to recover from the denatured state at high temperature with the aid of 'chaperonins'. Chaperonins are special thermostable proteins that are produced by thermophilic extremophiles. Accordingly, thermophilic extremozymes are used in numerous industries including detergent, food, feed, starch, textile, leather, pharmaceutical, pulp and paper industries (Cherry and Fidantsef, 2003). Interestingly, the use of thermostable DNA polymerase which is isolated from *Thermus aquaticus* plays a vital role in molecular biology. On the other hand, psychrophilic proteins display decreased ionic interactions and hydrogen bonds resulting in the ability to produce antifreeze proteins at extremely low temperatures. As reported by He et al. (2004), psychrophilic extremozymes are used in various industries including dairy (to hydrolyze lactose in milk using b-galactosidas), textile (for biopolishing and stone washing using cellulases) and bakery industry (to improve products using glycosidases). Both acidophiles and alkaliphiles use proton pumps to maintain internal pH conditions so that there are no specific modifications of intracellular proteins produced by these extremophiles. Nevertheless, the extracellular protein adaptations of these organisms delivering the ability to survive and operate at extreme pH conditions are yet to be revealed (Gomes and Steiner, 2004). Alkaliphilic enzymes such as cellulases, xylanases, amylases, proteases, lipases, pectinases, chitinase, catalase, peroxidase and oxidoreductase can be used in various industrial applications such as laundry and dishwashing detergents, dehairing of hides and skins, bio-bleaching of pulp and paper, degumming of ramie fibers and textile processing (Gomes and Steiner, 2004).

Extremolytes

Organic compounds such as polyol derivatives (ectoine, hydroxyectoine and betaine), carbohydrates such as trehalose and the mannose derivatives, glucosylglucosylglycerate, glucosylglycerate (GG) and various amino acids can be counted as extremolytes. As a result of exposure to stressful conditions, extremolytes can be accumulated inside microorganisms even up to 25 % of dry cell weight. For instance, numerous UV-radiation-protective extremolytes are produced in radiophiles upon exposure to high doses of UV radiation (Raddadi et al., 2015). Mycosporin-like amino-acids (MAAs) can be considered as one of the most useful UV-radiation-protective extremolytes as it is being used in cosmetic and pharmaceutical industries in manufacturing sun creams and anticancer agents respectively (Raj et al., 2021). Moreover, deinoxanthin carotenoid isolated from *Deinococcus radiodurans* can be used as a chemo preventive agent as it induces apoptosis of cancer cells (Choi et al., 2014).

Furthermore, extremolytes can be used to stabilize proteins in producing therapeutic protein-based medicines, due to their ability to stabilize macromolecules (Raddadi et al., 2015).

Applications of extremophiles and their products in various industries

The metabolic wealth of living beings including microbes has been used in various industries since time immemorial. At the very beginning mesophilic organisms were used to extract biomolecules which were found to be effective generally at moderate conditions only. The use of extremophiles and their biomolecules for industrial purposes has gained much attention in recent times. It has been recognized that extremophiles and their products are sustainable resources that can be utilized in a vast range of industries towards the development of a biobased economy. Out of such industrial purposes, only biofuel synthesis and agricultural purposes are critically examined in this chapter. Further, it is important to note that, most of the extremophiles are believed to produce harmless and biodegradable industrial waste.

Uses of extremophiles in biofuel synthesis

Several types of biofuels are produced and used out of which bioethanol, biodiesel, biobutanol, biogas (Methane) and Hydrogen are the most known. The majority of extremophiles that are currently used in biofuel production belong to thermophile group whereas methanogens and psychrophiles are also being used in biogas and biodiesel production respectively (Barnard et al., 2010).

Even though *Saccharomyces cerevisiae* has been well developed to produce bioethanol, it cannot ferment many pentose sugars which leads to reduce the efficiency of the overall conversion process. Yet, the utilization of organisms that possess the capacity for the fermentation of pentose sugars tends to decrease the final ethanol yield (Demain, 2009). This is where the utilization of extremophiles in advancing bioethanol commercialization from biomass came to the fore and gained wider attention. Accordingly, thermophiles such as *Thermophilic clostridia*, *Thermoanaerobacterium saccharolyticum*, *Thermoanaerobacter* sp. and *Geobacillus* sp. are regarded as highly potential candidates in bioethanol production.

It is important to replace the highly alkaline catalyst used in the transesterification process with a biocatalyst such as lipase for economically sound biodiesel production.

In this regard, it is highly important to continue research on cold active lipases (Joseph et al., 2007). Kim et al. (2020) reported that the psychrophilic *Chlamydomonas* sp. KNM0029C can be efficiently used in the production of biodiesel. Furthermore, extremophilic microalgae such as *Cyanidium caldarium* and *Galdieria sulphuraria*, which tolerate both high temperatures and low pH can be used to minimize contamination within the photobioreactors in the biodiesel production process (Barnard et al., 2010).

As recorded by Barnard et al. (2010), it is important to use mixed bacterial colonies for higher efficiency in biogas production. In this regard, methane producing organisms including thermophilic *Methanobacterium* sp., *Methanosarcina thermophila* and *Methanothermococcus okinawensis*, psychrotolerant and psychrophilic *Methanosarcina lacustri*, *Methanolobus psychrophilus* and members of the genus *Methanosaeta* can be considered as potential extremophilic candidates.

Uses of extremophiles in agriculture

In the current context, the application of agrochemicals such as fertilizers and pesticides has become one of the most crucial issues in agriculture. There is a current trend towards developing more sustainable agricultural practices based on environmentally friendly approaches. In this scenario, the use of plant growth promoting microbes (PGPM) as an alternative to chemical fertilizers can be considered an environmentally friendly approach. Beneficial plant-associated extremophilic microbes (PAEM) are considered to possess a high potential to be used as bio-resources such as bio-fertilizers, bio-inoculum, and bio-controlling agents in agriculture because of their capability to produce agriculturally important bioactive compounds even under harsh environmental conditions. Under stressful environmental conditions, PAEM are known to contribute to crop production, survivability, and fitness by involving in nutrient cycling, nutrient fixation, mineralization, and solubilization confirming the suitability of using them as bio-fertilizers or bio-inoculants. For instance, extremophiles such as *Enterobacter* and *Gluconacetobacter* are involved in nitrogen fixation whereas *Methylobacterium*, *Microbacterium*, and *Ochrobactrum* produce phytohormones. Also, PAEM have been reported to promote resistance-inducing characteristics in plants which make them better candidates as biocontrol agents or bio-pesticides (Kochhar et al., 2022).

Crops face several abiotic environmental stresses including temperature stress (cold or heat), water stress (flooding conditions or drought conditions), salt stress and higher amounts of available heavy metals in the soil. In most cases, stress can be a combined effect of multiple stresses (Yadav, 2021). However, abiotic stresses affect plant growth and lead to decreases in crop yields. In certain parts of the world, especially in temperate regions during cold seasons, low temperature leads to cold stress becoming a major limiting factor for crop cultivation. Interestingly, psychrophilic extremophiles play a major role in nutrient circulation even under cold stressed conditions. Psychrophilic phosphate solubilizing bacteria (PPSB) possess the ability to convert the inaccessible complex phosphorous into plant-available forms by acidification, chelation, exchange reactions, and polymeric substance formation. Also, some of PPSBs (e.g. *Pseudomonas*, *Serratia*, and *Flavobacterium*) are capable of producing phytohormones. They also produce ACC deaminase which can lower plant ethylene synthesized under biotic or abiotic stress to induce senescence, chlorosis, and abscission in plants leading to stress resistance or tolerance (Rizvi et al., 2021). Also, psychrophiles can be applied in crop cultivation as bio-inoculants to promote growth under low temperatures as they solubilize nutrients, fix nitrogen, and produce phytohormones and siderophores which are low-molecular-weight molecules that chelate iron with very high and specific affinity (Yadav, 2017). Rizvi et al. (2021) reported that *Pseudomonas* strains recovered from the Andean glacier enhanced the biological performance of wheat plantlets at low temperatures. As stated by Junge et al. (2019), several psychrophiles including *Arthrobacter nicotianae*, *Brevundimonas terrae*, *Paenibacillus tylopili* and *Pseudomonas cedrina* exhibit multifunctional plant growth promoting (PGP) attributes at low temperatures. Specifically, *Arthrobacter* sp. and *Bacillus* sp. are reported to produce cold-active enzymes and anti-freezing compounds (Verma et al., 2017). Soil becomes acidic due to the application of ammonium-based fertilizers; urea and sulfur in legume cultivation. Acidophilic extremophiles such as *Azotobacter*, *Bacillus*, *Flavobacterium* and *Pseudomonas* can be used as growth promoters of crop plants that are grown in acidic soils (Verma et al., 2017). Another brutal environmental condition that suppresses the productivity of crop plants is salinity. A vast majority of crop plants are sensitive to salinity stress. Some halophilic extremophiles possess the capability to promote seedling germination, increase root and shoot length, biomass, yield, and chlorophyll content under salinity stress. Moreover, *Haloarcula argentinensis* and *Haloferax alexandrines* are astonishing candidates in hypersaline soils to enhance phosphorus solubilization (Yadav and Saxena, 2018). As reported by Verma et al. (2017), drought-tolerant and phosphorus-solubilizing extremophiles are suitable bio-inoculants and thus they can be suitable candidates to be used in desert landscapes to ensure global food security for the overgrowing human population.

Biotic stresses including macro or micro pathogens such as insects, bacteria, fungi, viruses and weeds in or on plants negatively affect the growth and development of plants (Yadav, 2021). Plant growth promoting microbes (PGPM) support the plants to withstand such biotic stresses by promoting nutrient availability and releasing various types of antibiotics and hydrogen cyanide (Yadav, 2021). The most fascinating fact is that plant-associated extremophilic microbes (PAEM) have the potential to support the survival of plants experiencing biotic stresses even under environmentally harsh conditions. In this regard, psychrophiles are potential candidates to be used as bio-controlling agents as they induce resistance in plants against pathogens (Yadav, 2017). Ferreira et al. (2019) reported that there is a high possibility of using psychrophilic Antarctic yeast in the biological control of post-harvest diseases of fruits that are stored at low temperatures. Some psychrophilic *Pseudomonas* spp. strains have been shown to inhibit the phytopathogens including *P. ultimum*, *F. oxysporum*, *P. infestans*, *Fusarium* sp., *R. solani*, *A. solani*, and *P. capsica*. Furthermore, PPSBs are reported to be capable of protecting plants from phytopathogens like *Phytophthora* and *Phytium* species (Rizvi et al., 2021).

Concluding remarks and perspectives

There is a huge potential for the use of extremophiles and their biomolecules in industrial purposes leading to greater economic returns. Moreover, the extremophiles have the ability to generate the least harmful and biodegradable industrial wastes, and yet provide consistent and higher yields even under adverse environmental conditions. However, there are still a vast majority of extremophiles to be explored and only a few extremophiles are currently being used at the industrial level. Notwithstanding their few number and technical difficulties in cultivation, the applications of whole-cell extremophiles, extremozymes and extremolytes hold great potential in biofuel synthesis, agriculture, food, beverage, feed, pharmaceutical, detergent, textile, leather, pulp, and paper and bio-mining industries. A variety of studies conducted by different groups have focused on the identification of novel extremophiles and their products. Furthermore, the application of other advanced biological techniques such as, metagenomics and genetic engineering on extremophiles and related areas would be highly advantageous and more efficient in future discoveries on enhancing the use of extremophiles in industries and agriculture. As discussed in this chapter, it is strongly believed that such novel discoveries will shape a sustainable future in industries, specifically in biofuel synthesis and agriculture. Accordingly, extremophiles can be counted as sustainable resources that could be utilized in several arenas towards the development of a bio-based economy.

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CHAPTER 05

Fungi-based meat analogues: Next generation meat alternatives

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Abstract

As part of their dietary habits, people worldwide including Sri Lankans are looking for nutritionally rich and ecologically sustainable foods due to the concerns about animal suffering and the adverse effects on human health and the environment. Fungi-based meat analogues, especially mycoproteins, have been studied for their ability to resemble animal meat in terms of taste, texture, and sensory and olfactory properties. The nutritional value is similar to that of meat proteins, making it a better choice over plant-based proteins. Based on the high carbohydrate-to-protein conversion rates, the microfungi *Fusarium venenatum* Nirenberg and *Aspergillus oryzae* (Ahlburg) E. Cohn are commonly used to produce mycoproteins using submerged fermentation. The main challenge in popularizing mycoproteins in Sri Lanka is the cost-effective manufacture. Product diversification and alternative technologies to circumvent the requirement of submerged fermentation, which is costly, are gaining momentum. Campaigns along with technological development are needed to improve consumer knowledge and make mycoproteins a viable, cost-effective meat replacement in the near future in Sri Lanka.

Keywords: *Fungi-based protein, Mycoprotein, Sri Lanka, Vegetarian*

The global search for innovative protein sources

The projected global population is estimated to reach 8.5 billion by 2030 and 9.7 billion by 2050, according to the United Nations (2022). With this population growth, there will be an increased demand for meat production, which is currently the most popular source of protein. However, it is important to acknowledge that meat production has significant negative environmental impacts, including deforestation, greenhouse gas emissions, water pollution, and biodiversity loss (Katare et al., 2023). Additionally, excessive meat consumption has been linked to various health risks, such as cardiovascular diseases, obesity, and certain types of cancer. The high levels of saturated fat, cholesterol, and sodium found in meat products contribute to development of these conditions (Geiker et al., 2021). Research has also indicated that processed meats, like bacon, sausage, and hot dogs, are associated with an increased risk of colorectal cancer (Cantwell and Elliot, 2017).

Moreover, the way meat is produced and processed can pose additional health risks. The use of antibiotics and growth hormones in livestock farming has been linked to antibiotic resistance and other health issues (Herago and Agonafir, 2017). Furthermore, the handling and processing of meat can increase the risk of foodborne illnesses (Lee and Yoon, 2021). Conversely, studies have shown that adopting a plant-based diet can offer health benefits, including a reduced risk of heart disease and certain types of cancer (Qin et al., 2022).

It is worth noting that approximately 1.5 billion people worldwide, which accounts for 22% of the global population, identify as vegetarians (Dorgbetor et al., 2022). For 75 million of these individuals, vegetarianism is a personal lifestyle choice driven by concerns about animal welfare, the environment, and personal health. In contrast, for others, poverty leaves them with no alternative (Dorgbetor et al., 2022). The growing prevalence of veganism/vegetarianism is evident in the reduced demand for meat products, the increased availability of vegan/vegetarian options in supermarkets and restaurants, and the rising number of plant-based choices at social gatherings and events. Additionally, celebrities, athletes, and influencers' endorsement and adoption of vegan and vegetarian practices have further contributed to its visibility and acceptance in mainstream society.

Mycoproteins as fungi-based meat analogues

Literally, mycoproteins insinuate proteins from fungi. It is a protein-rich food made of filamentous fungal biomass (Filho et al., 2019). The Food and Drug Administration (FDA) in the United States has designated mycoproteins as Generally Recognized as Safe (GRAS) since 2002, indicating that they pose no health risks and can be consumed as an alternative to meat or as a base ingredient in various food products (Denny et al., 2008). *Fusarium venenatum* Nirenberg and *Aspergillus oryzae* (Ahlburg) E. Cohn are commonly used to produce mycoproteins due to their high carbohydrate-to-protein conversion rates (Denny et al., 2008). The filamentous nature of fungal bodies gives mycoproteins a fibrous texture, making them suitable for mimicking the texture of meat in meat analogues. They can be processed into various forms, such as nuggets, cutlets, and grounds, making them versatile for use in different food products.

Mycoprotein can be grown vertically in airlift pressure cycle fermenters, which require minimal arable land compared to animal or plant-based protein sources (Finnigan, 2011). High-quality mycoprotein biomasses with excellent nutritional value can be produced by using specific microbial strains and substrates under specific conditions. Research indicates that mycoprotein has a lower environmental footprint per unit of mass compared to animal-derived proteins (Tuomisto, 2022). Studies also suggest that by replacing 20% of ruminant meat consumption with microbial protein derived from fermentation, deforestation and related carbon dioxide emissions could be halved by 2050 while reducing methane emissions and offsetting global pasture expansions (Humpenoder, 2022).

In terms of nutrition, mycoprotein contains valuable nutrients suitable for all age groups. It provides all nine essential amino acids and has a high-quality protein digestibility-corrected amino acid score of 0.996 (Derbyshire and Delange, 2021). According to European Commission standards, mycoprotein is classified as a high-fibre food, providing at least 6 g of fibre per 100 g of mycoprotein (Derbyshire and Delange, 2021). It is low in total saturated fat and contains very little cholesterol. Mycoprotein also offers several micronutrients of concern in vegetarian diets, such as vitamin B12, riboflavin, folate, phosphorus, choline, zinc, and manganese (Derbyshire and Delange, 2021). Plant-based proteins often lack certain amino acids like lysine, methionine, isoleucine, threonine, and tryptophan, with lysine being the most commonly absent, particularly in cereal grains (Craig, 2010). Fungi provide all the necessary nutrients for a vegetarian diet and have a low caloric value, making mycoproteins a healthy substitute for meat (Table 1). Combining mycoproteins with other plant-derived foods can address malnutrition concerns.

Furthermore, mycoproteins are less allergenic compared to common food allergens such as soy, gluten, and beef, making them a suitable option for individuals with food allergies. Additionally, the consumption of mycoproteins has been associated with the reduction of non-communicable diseases such as hypercholesterolemia and diabetes (Derbyshire and Delange, 2021).

Table 1: Comparison of the nutrition profile of fungi-based food with that of plant-based food and animal-based food.

Nutrients	Fungi-based food		Plant-based food		Animal-based food	
	Myco-proteins	Mushrooms	Tofu, Soya-beans	Chickpeas	Chicken Breast	Beef mince
Energy (kcals/100 g)	85	55	73	129	160	209
Protein (g/100 g)	11	1.6	8.1	8.4	28.4	21.8
Carbohydrates (g/100 g)	3	12.3	0.7	18.3	0.0	0.0
Fat (g/100 g)	2.9	0.2	4.2	3.0	5.2	13.5
Of which saturates (g/100 g)	0.7	0.1	-	0.29	29.6	47.5
Fibre (AOAC) (g/100 g)	6	N	-	7.1	0.9	0.0
Vitamin B6 (mg)	0.1	N	0.07	0.38	0.36	0.17
Vitamin B9 (µg)	114	N	15	35	6.0	5.0
Vitamin B12 (µg)	0.72	0.0	0.0	0.0	Tr	0.8
Calcium (mg)	48	3	N	48	9	11
Phosphorous (mg)	290	29	95	141	210	93
Iron (mg)	0.39	0.4	1.2	1.9	0.5	0.83
Magnesium (mg)	49	14	23	44	25	11
Zink (mg)	7.6	N	0.7	1.1	1.1	2.1
Potassium (mg)	71	120	63	281	270	163
Choline (µg)	180	NR	NR	NR	NR	NR

Source: Derbyshire and Delange, 2021

Production of mycoproteins

Production of mycoproteins involves the use of solid-state fermentation (SSF) and liquid-state fermentation (LSF) or submerged fermentation (SuF) methods. The choice of method depends on factors such as product requirements, production scale, available resources, and process control considerations (Adamatzky, 2021). The production process can be summarized as follows: the fungus is initially cultivated in an aerobic fermentation system, primarily LSF/SuF, using carbohydrate and nutrient substrates (Finnigan, 2011). Once the fungus mycelium is fully grown, it is heat-treated to kill the fungus and facilitate the release of RNA from the mycelium. After reducing the RNA levels from about 10% to 2%, the mycelium, which becomes slightly firm, is harvested as mycoproteins. In the final stages of manufacturing, the mycoprotein undergoes steaming, chilling, and freezing processes to achieve a meat-like structure resembling chicken. These processes, combined with adding egg albumen or dairy proteins to facilitate hyphae binding, functional additives, herbs, spices, and other flavours, result in a product that mimics the texture and taste of flesh (Finnigan, 2011). The process is illustrated in figure 1.

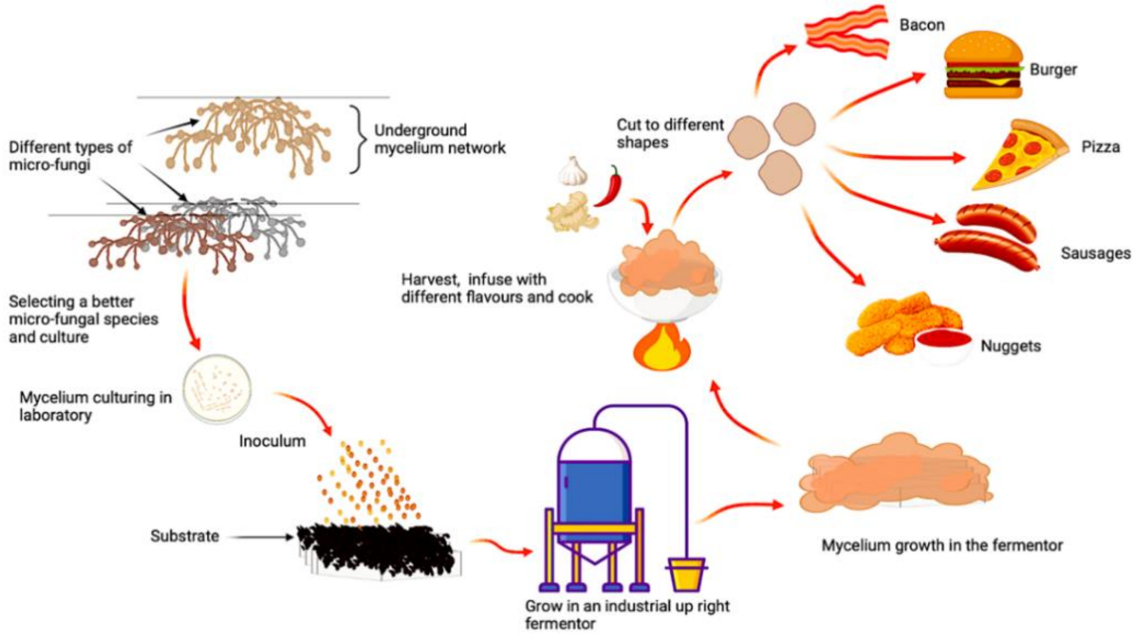

Figure 1: Graphical representation of different meat analogues production using filamentous micro-fungi

Fermentation technology in the production of protein alternatives is experiencing significant growth. According to the State of the Industry Report 2021 by the Good Food Institute, companies in the alternative protein sector raised 1.69 billion USD in 2021, triple the amount raised in 2020. Future Market Insights (FMI) predicts that the global mycoprotein industry will be valued at approximately $976 million by 2032. One well-known brand that utilizes mycoprotein in its products is Quorn™, based in the United Kingdom. Key players in the vegan meat sector in the United States include Myco Technology Inc., Tyson Ventures, General Mills, Beyond Meat, and Impossible Foods. While some companies focus on enhancing the nutritional content of mycoprotein products, others are exploring the addition of mycoprotein to meat alternatives (Future Market Insights, 2022). Mycoproteins have also made their way into the seafood sector, with companies like Aqua Culture Foods in the USA producing mycoprotein-based seafood products such as sushi, minced shrimp, tuna, and calamari for cooked dishes.

Challenges in fungi-based meat analogous production

Challenges in producing fungi-based meat analogues must be addressed to scale up production and make it a viable alternative to traditional meat. The primary challenge is the cost of production, which is influenced by factors such as the specific growth medium, energy requirements, and contamination prevention (He et al., 2020). Fungi require specific nutrients and environmental conditions to grow, and the cost of these materials can vary. Additionally, the equipment and facilities needed for production can be a significant expense. Currently, fungi-based meat production is in the early stages and has yet to achieve the economies of scale seen in traditional meat production, resulting in higher costs. However, as production methods improve and economies of scale are realized, the cost is expected to decrease. Achieving the exact texture and flavour of traditional meat can be challenging, and consumer acceptance is crucial. Texture can be improved by using different types of fungi and adjusting the production process. Additionally, mycoproteins have a slight umami flavour that can be enhanced with flavouring to create various tastes (Cordelle, 2022).

The efficient conversion of carbohydrates into protein by fungi is essential for fungi-based meat production (Lubeck and Lubeck, 2020). Researchers are exploring improved strains of fungi that can convert carbohydrates into protein more efficiently (Strong et al., 2022). They are also investigating enzymes involved in this conversion process to optimize their activity and efficiency. Genetic engineering of fungi is another approach explored to produce specific proteins with high nutritional value. Different growth conditions and substrates are also being studied to improve the efficiency of carbohydrate-to-protein conversion (Barzee, 2021).

Consumer acceptance is influenced by factors such as taste, texture, nutrition, health benefits, price, and availability (He et al., 2020). Taste and texture are crucial, as consumers expect a meat-like experience. Mycoproteins meet these expectations and provide an attractive alternative for consumers looking to reduce their meat consumption (Cordelle, 2022). It is worth noting that advancements in technology have eliminated the need for egg albumin as a binder, making some mycoprotein-based meat analogues suitable for vegetarians and gaining the approval of organizations like the Vegan Society (Finnigan et al., 2019).

Nutrition and health benefits also play a role in consumer acceptance. Mycoprotein is a rich source of protein, fibre, and other nutrients, with low levels of saturated fat and cholesterol. It has been associated with lower risks of heart disease and other chronic illnesses (Derbyshire and Delange, 2021). As more consumers become aware of these health benefits, acceptance of mycoproteins will likely increase.

Regulatory and policy implications need to be considered as fungi-based meat analogues differ from animal and plant-based products. Safety is a primary concern for consumers, as the fermentation process involved in producing mycoproteins may raise doubts about potential allergens and mycotoxins. However, the FDA and OECD have recognized mycoproteins as Generally Recognized as Safe (GRAS), and their extended history of safe use addresses these concerns.

Sri Lankan perspective on fungi-based meat analogues

In Sri Lanka, traditional meat consumption is primarily focused on chicken and beef. Plant-based meat substitutes like soy meat are popular, particularly among low-income communities. However, plant-based proteins may lack certain essential amino acids, necessitating food supplementation. The current economic crisis, exacerbated by the post-COVID situation, has resulted in a decline in purchasing power among Sri Lankans, leading to a significant prevalence of malnutrition in children and pregnant/lactating mothers. To address this issue, developing a low-cost protein source has become crucial.

Although large-scale commercial producers of fungi-based meat analogues or mycoproteins are currently absent in Sri Lanka, the opportunity exists to combat the escalating child and maternal malnutrition caused by the country's economic crisis. According to the Nutrition Month 2022 report published by the Family Health Bureau of the Ministry of Health, the percentages of undernourished children under 5 years of age, including those who are underweight, experiencing growth faltering, wasting, and stunting, have increased compared to 2021. Throughout the country, this situation spans across all age groups, from infants to children aged 1-2 years and 2-5 years.

Given that the capital and maintenance costs associated with submerged fermentation are quite high, alternative production technologies need to be adopted. Sri Lanka's abundance of cereals makes solid-state fermentation (SSF) an easily employable method. Protein bars or cereal bars enriched with mycoproteins could serve as better breakfast options for children under 5 years of age, school children, and lactating mothers. This approach would help popularize mycoprotein-based products and expand the product range without requiring significant capital investment.

While mycoprotein production currently relies mainly on the fungus *F. venenatum*, there is a global search for alternative species. Sri Lanka is home to a wide range of fungal species, including many potential candidates for mycoprotein production. The country's diverse ecosystems, such as rainforests, wetlands, and agricultural lands, host numerous fungi that can be explored for their mycoprotein-producing capabilities. Sri Lankan cuisine also includes a large number of edible wild mushrooms, whose mycelia could be potential candidates for mycoprotein production. The existence of a wide range of edible wild mushrooms provides endless possibilities for flavour and texture.

Furthermore, Sri Lanka's unique biodiversity offers opportunities to discover new fungal strains with untapped potential for mycoprotein production. Exploring unexplored habitats and biodiversity hotspots may lead to discovering novel edible fungi with superior characteristics for mycoprotein production. These newly identified strains can be studied and optimized for commercial cultivation to enhance mycoprotein yields and quality. Additionally, by utilizing locally available substrates and waste materials, such as agricultural residues, crop by-products, or organic waste, Sri Lanka can minimize resource inputs and reduce the environmental impact associated with mycoprotein production. This approach promotes cost-effectiveness and aligns with circular economy principles.

However, one potential challenge in adopting fungi-based meat analogues in Sri Lanka is consumer acceptance. Sri Lankans tend to be conservative in their food choices, and there may be resistance to trying a new protein source that is unfamiliar to them. Nevertheless, the growing interest in plant-based diets and alternative protein sources suggests that there is a definite market for mycoprotein-based meat substitutes in Sri Lanka. Another challenge is the lack of a regulatory framework for alternative protein sources in the country. While Sri Lanka has strict regulations for traditional meat products, no specific regulations currently govern the production and sale of mycoprotein-based meat substitutes. However, considering that mushrooms have been a delicacy in Sri Lankan cuisine for a long time, a similar approach can be used to promote mycoprotein-based foods.

In conclusion, the production and development of mycoprotein products in Sri Lanka have the potential to address protein malnutrition and stimulate economic growth. This endeavour could result in establishing local manufacturing facilities, generating employment opportunities, and exporting the products if they gain popularity both within the country and internationally.

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CHAPTER 06

Fruits and nuts as a plausible option to sustain Sri Lankan economy

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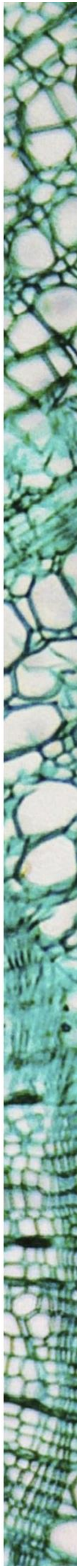

Abstract

Sri Lanka is a tropical country blessed with a wealth of crop genetic resources and diverse agro-climatic conditions. The tropical climate, fertile soil and different agricultural systems in Sri Lanka have paved the way for successful year-round cultivation of many fruits and nuts throughout the country. Among these fruits and nuts; cashews, peanuts, mango, pineapple, papaya, avocado, banana, durian, king coconut etc. are highly popular. However, there is an abundance of less cultivated orphan fruits such as wood apple (*Limonia acidissima*), amla (*Phyllanthus emblica*), June plums (*ambarella*) (*Spondias dulcis*), Ceylon olive (*veralu*) (*Elaeocarpus serratus*), guava (*Psidium guajava*), ripe jackfruit (*Artocarpus heterophyllus*), langsat (*gaduguda*) (*Lansium domesticum*), governor's plum (*ugurassa*) (*Flacourtia indica*), *lovi* (*Flacourtia inermis*), *rambutan* (*Nephelium lappaceum*) tamarind (*Tamarindus indica*) and soursop (*Annona muricata*) etc. which can be commercially cultivated targeting year-round production and export income. There is a growing awareness and a demand world-over for such less popular fruits and nuts possessing various nutritional benefits. Such potential benefits would provide immense opportunities for utilizing them in various value-added food and pharmaceutical production, foreign capital investments, developing fruit orchards as means of tourist attraction while generating numerous novel employment opportunities for Sri Lankans. Sri Lanka is yet to realize the economic potential and the avenues for getting maximum use of these fruits and nuts. Thus, this chapter focuses on the potential of fruits and nuts as a means of enriching the Sri Lankan economy by creating novel job opportunities and income sources.

Keywords: *Economic-prosperity, Health-benefits, Tropical fruits and nuts*

Fruits and nuts; biological wealth to improve quality of human life

Fruits and nuts are gaining much attention all over the world owing to their immense health benefits and the high economic returns provided by them. Many studies have shown the fruits and nuts to be good sources of vitamins, minerals, dietary fiber, and dietary bioactive compounds. Recent studies have also revealed their potential-health promoting effects that stretch beyond the provisioning of basic nutritional needs of humans as they play a major role in preventing non-communicable diseases, infections and promoting immunity, mental health and cognition (Sayago-Ayerdi et al., 2021; Wallace et al., 2019; Carughi et al., 2016; Pem and Jeewon, 2015; Ros, 2010).

Fruits are mature ovaries of flowering plants that usually produce sweet-tasting products with tiny seeds and soft skin. Nuts are hard-shelled one-seeded fruits that do not split open to release their single seed. Globally, there is a wide array of fruits and nuts allowing humans to enjoy a nutritionally and aesthetically diverse diet while bestowing a lot of health benefits. The World Health Organization recommend the consumption of at least 400 g of fruits per day by a healthy adult. However, this requirement is not fulfilled in a majority of the world population (Wallace et al., 2019) despite the availability of a variety of fruits and nuts within easy access. This leads to a considerable gap between the availability and sustainable use of fruits and nuts in many countries. Sri Lanka (SL) as a tropical country is blessed with a broad range of tropical and wild fruits and nuts. These species act as an important part of the structure and maintenance of ecosystems and also possess a tremendous potential to sustain human life by provisioning high economic and health returns. SL, being a tropical paradise of such vegetation, provides ample opportunities in exploitation of cultivation, processing and introducing into domestic markets, exporting them as diversified products, using them in promoting eco-tourism and developing into pharmaceutical ingredients leading to novel entrepreneurial ventures for economic gains. Yet, SL is yet to fully identify and explore the potential of this biological wealth to prosper its people and the economy. Moreover, inadequacy in planning, monitoring, public-private partnerships, government attention, intensives for poor farmers, attempts to breed new fruit cultivars, technologies and heavy postharvest losses act as major barriers to achieve such targets in SL. Accordingly, this chapter discusses the potential of fruits and nuts as an integral component of biological wealth to sustain the Sri Lankan economy and thereby improve the quality of human life.

Increasing demand for tropical fruits and nuts in the global market

Fruits and nuts provide opportunities to consume a nutritionally diverse diet (Harris et al., 2022). Throughout history, fruits and nuts have been commonly used in preparing various dishes and as an important component of main meals under different cuisines. Accordingly, a high global demand exists for fruits and nuts and this will be increased in future with the rising global population.

Apart from general usage, fruits and nuts are gaining increased attention in the global markets due to various health benefits and high economic returns provided by them to sustain human lives (Wallace et al., 2019; Ros, 2010; FAO, 2023). The demand for fruits in developed countries has contributed to increased fruit production in developing countries (FAO, 2021) leading to economic growth *via* enhancing the growth of small farms and creating novel employment opportunities and addition of new products.

Currently, there is a growing interest among the public on the inclusion of fruits and nuts in their diet plans as fruits and nuts provide essential macro and micro nutrients which help maintain a healthy life. In addition, most of the tropical fruits are rich sources of various bioactive compounds such as carotenoids, phenolic compounds such as flavonoids and non-flavonoids phenolics, dietary fibers, sterols and stanols etc. Fruits and nuts offer various beneficial biological effects such as anti-cancer, antioxidant, hypo-cholesterolemic, anti-diabetic, anti-inflammatory, immunomodulatory, anti-hypersensitive properties. For example, antioxidants are important in clearing free radicals that develop in the human body and thereby prevent harmful effects caused by them. Dietary fiber is involved in forming bulky stool leading to reduced rates of intestinal passage, more gradual nutrient absorption and prevention of constipation. Anthocyanins, procyanidins and flavanols in fruits are known to be effective in reducing cardiovascular diseases (Pem and Jeewon, 2015). Nuts are rich sources of protein, mono-unsaturated fatty acids and polyunsaturated fatty acids and are also associated with reduced risks of cardiovascular diseases, cancer mortality, and improvements in the regulation of levels of blood glucose and insulin (Carughi et al., 2016). Yellow xanthophyll carotenoids that are present in colorful fruits are strongly correlated with enhanced cognition (Wallace et al., 2019). Moreover, high levels of fruit intake is reported to be associated with better mental health due to reduced levels of depression and anxiety, maintaining healthy hair and skin and achieving better bone health (Wallace et al., 2019).

Tropical fruits are highly recognized in both domestic and international markets for their nutritional composition, health promoting properties and pleasing organoleptic properties such as unique taste, sensory properties and mouthfeel (Sayago-Ayerdi et al., 2021). Fifty percent of the world share of tropical fruits are used in fresh fruit markets while the rest are used in various processed fruit industries. Majority of the tropical fruits are used to produce tropical fruit beverages while others are being used in the manufacturing of various types of desserts, marmalades, sauces, snacks, jellies, syrups, nectars, flours, wines and etc. (Sayago-Ayerdi, et al., 2021). Accordingly, fruits and nuts are playing a vital role in improving human health and promoting the economic growth of the most developed and developing countries.

Current status of fruits and nuts in Sri Lanka

Diversity of fruits and nuts in Sri Lanka

Fruits are grown throughout SL extending from coastal belts to central hills and covering all 46 agro-ecological regions in the island (Rajapaksha et al., 2021). The tropical climate, fertile soil and different agricultural systems in SL have paved the way for successful year-round cultivation of many fruits and nuts and thereby facilitating the country to cherish a high biodiversity of fruit crops. As stated by Ratnayake et al. (2019), there are over 237 recorded species of fruits in SL that are included in 56 plant families. Further, there are more than 60 species of underutilized fruit crops that are grown in marginal environments in SL.

Sri Lanka provides home for several tropical and sub-tropical fruits and nuts such as mango, pineapple, papaya, avocado, banana, watermelon, wood apple, amla, sweet orange, June plums (*ambarella*), Ceylon olive (*veralu*), durian, guava, ripe jackfruit, king coconut, langsat (*gaduguda*), governor’s plum *(ugurassa*), lovi, mangosteen, rambutan, passionfruit, pomegranate, rose apple, tamarind, soursop, cashews, and peanuts (Export Development Board, 2023). Moreover, cultivation of a few temperate fruit species such as strawberries, apples, pears and peaches has also been carried out (*Flacourtia indica*), Ceylon gooseberry (*Dovyalis hebecarpa*), and Barbados cherry (*Malpighia emarginata*) are some of the examples for the under-utilized fruit crops in SL (Perera et al., 2022). According to Pushpakumara et al. (2016), various endemic, indigenous, and exotic fruit crop species create the species diversity of Sri Lankan fruit crops (Figure 1).

This rich genetic diversity provides unprecedented potential to improve the quality of Sri Lankan fruit crops and thereby diversify the fruit-based products. Moreover, it provides ample opportunities for both local and international communities to enjoy fruit diets enriched with a wide array of nutrients and health benefits.

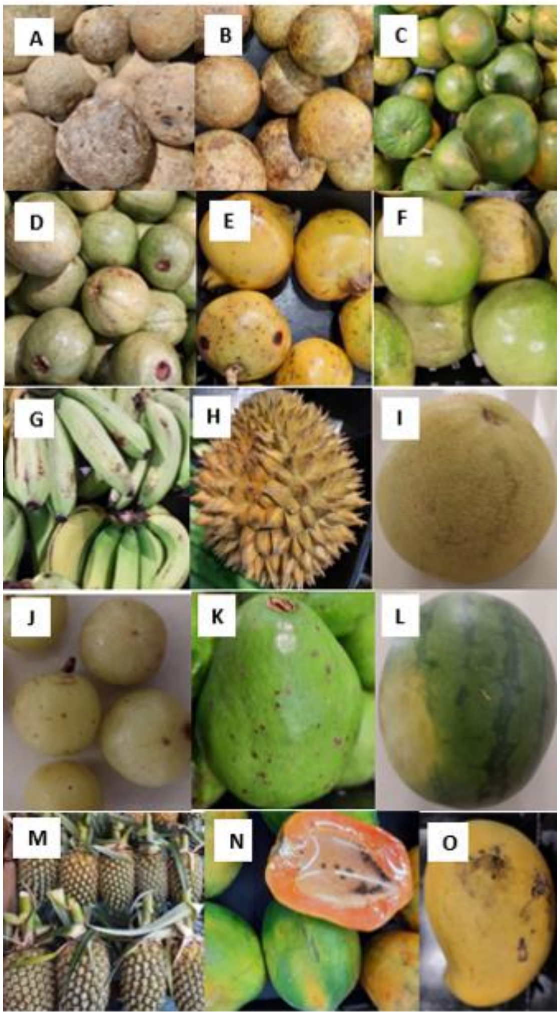

Figure 1: Some of the popular fruits in Sri Lanka (A-Wood apple, B-*Beli* fruit, C-*Naran*, D-Guava, E-Pomegranate, F-Passion fruit, G-Banana, H- Durian, I- Pomelo, J-Amla, K-Avocado, L- Water melon, M- Pineapple, N- Papaya, O- Mango)

on a small scale, especially in the central regions of the country. Madan (*Syzygium cumini*), Himbutu (*Salacia chinensis*), Maha Karamba (*Carissa carandas*), Ugurassa

Fruit production and cultivation in Sri Lanka

The average annual fruit crop production in SL was about 590,000 metric tons by 2018 (Rajapaksha et al., 2021). The tropical climate, with warmer temperatures and high humidity make SL an ideal home for a wide range of tropical fruits with unique color, flavor and aroma. Currently, an extent of about 69,800 ha is utilized for cultivation of fruit crops in SL. Even though numerous fruit crop species can thrive well in different agro-ecological regions, there is a significant variation with respect to fruit crops grown in each region depending on the type of climatic and soil conditions present (Export Development Board, 2023).

According to the Export Development Board of Sri Lanka (2023); mango, papaya, banana, pineapple, avocado, watermelon, mangosteen, rambutan, wood apple, pomegranate, guava, and jackfruit are the major fruit crops grown in SL. Of them, banana, pineapple, and papaya are commercially grown while most of the other fruit crops are produced in small scale home gardens. In addition, a variety of tropical grapes and oranges are also grown in SL (Rajapaksha et al., 2021) and most of them are cultivated under rain-fed conditions. Other than banana, papaya and pineapple, many of the fruit crops grown in SL are highly seasonal and are subjected to large quantities of waste during peak production periods (Rajapaksha et al., 2021).

In terms of nuts, SL is blessed with cashew nuts and peanuts. More than 50 % of the cashews are grown in the dry zone of the country. Nearly 79% of the Sri Lankan cashews are cultivated in home gardens in the major cashew growing areas such as Puttalam, Batticaloa, Kurunegala, Anuradhapura, Hambantota and Mannar (Department of Census and Statistics, 2022). Groundnut or peanut (*Arachis hypogaea* L.) is mainly grown in Hambantota, Moneragala, Puttalam and Kurunegala districts. During *Maha* season, ground nut is mainly cultivated in the highlands under rainfed conditions and in *Yala* season, it is mainly grown in paddy lands of the dry and intermediate zones under irrigation (Ravichandran and Geretharan, 2015).

Current market trends

About 50-55% of the average annual fruit crop production in the year 2018, in SL, has been used for local consumption while 40% has been wasted with about 11% been used for exportation (Rajapaksha et al., 2021). This indicates that, out of the total production, the majority is used for local consumption and only a very small share is exported while there is a huge wastage.

Sri Lanka's export share of fruits includes both fresh and processed fruits. In 2020, SL exported 38,725 metric tons of fresh fruit, earning an export income of US $ 33.1 million (Dissanayake et al., 2022). However, over the last decade, the export-oriented fruit industry in SL has shown more than a three-fold growth with some fluctuations (Gamage et al., 2020). In 2018, total fruit production in SL increased with a substantial contribution by avocado, guava, *rambutan*, orange and lime (Dissanayake et al., 2022). In international trade, the major buyers of Sri Lankan fresh fruit products are the United Arab Emirates (UAE), Maldives, Saudi Arabia, Qatar, United Kingdom, Germany, Switzerland, Bahrain, USA and France. Most of these international markets have shown steady growth in imports over the years (Gamage et al., 2020; Export Development Board, 2023). In terms of processed fruits; Germany, USA, UAE, Italy, the Netherlands, France, Saudi Arabia, Australia, New Zealand, and Canada were recorded to be the major importers from Sri Lanka in 2020 (Dissanayake et al., 2022). Sri Lanka mainly exports processed fruits in the form of fruit pieces, pulp, juice, preserves, and dehydrated form. Pineapple, mango, papaya, melons, and guava are the most popular fruit varieties used in the manufacturing of processed fruit products. Seasonal fruit crops such as *rambutan* and mangosteen also show a growing trend in processed fruit manufacturing (Export Development Board, 2023).

In terms of nuts, SL produces cashew for both local and international markets with a range of value-added products such as raw, roasted, fried and spiced kernels. In 2021, SL exported around 31,000 kg of cashew (Department of Census and Statistics, 2022). Sri Lanka mainly uses ground nuts in producing snacks and confectionaries (Ravichandran and Geretharan, 2015). According to the Department of Census and Statistics, in the year 2021, SL produced 36,947 metric tons of groundnuts.

Beyond the boundaries - Ways and means of using fruits and nuts as a plausible option to sustain Sri Lankan economy

Opportunities available:

1. Fruits and nuts as a commodity in local markets

As reported by Dissanayake et al., (2022), in the year 2020 SL exported 38,725 metric tons of fresh fruits, earning a foreign revenue of 33.1 million USD. In the same year, SL imported 52,778 metric tons of fresh fruits, spending 58.1 million USD. Accordingly, despite being a country blessed with a high diversity of fruits & nut crops SL annually spends more than the export revenue earned on fruit importation. In addition, the average dietary fruit consumption in SL is quite below the required daily intake (Udari et al., 2021). It shows the limited accessibility of locally grown readily available fruits to the general public of the country. This reveals a huge potential for popularizing fruit and nut crop industries as a means of generating national income and employment opportunities while providing opportunities for Sri Lankans to fulfill their essential nutrient requirements (Rambukwella and Samantha, 2013). Accordingly, the local farmers should be encouraged to grow fruit and nut crops targeting year-round supply for domestic markets. In addition to commercial farming, home garden-based fruit farming and cottage industries need be promoted for producing various snacks, jams, jellies, sauces etc. as a source of additional income.

2. Fruits and nuts as a commodity in export markets

Although there is a growing demand for fresh and processed forms of fruits and nuts in the global market, SL currently exports only a minor portion of its production targeting only a few international markets (Dissanayake et al., 2022; Rajapaksha et al, 2021). Despite the promotional initiatives of the export development board of SL, the country has not been able to fulfill the demands in foreign fresh fruit markets (Dissanayake et al., 2022). However, the available fruit and nut crop diversity, the human and other resources available in SL can be effectively used to secure a greater contribution in the international fruit and nut trade simultaneously generating much needed export earnings. Development of novel fruit cultivars, diversification of processed products and identification of new foreign markets are crucial for the success of this endeavour (Dissanayake et al., 2022; Gamage et al., 2020).

3. Fruits and nuts as valuable ingredients in various pharmaceutical, nutraceutical and cosmeceutical applications

As a country, SL mainly focuses on utilizing fruits and nuts either in fresh form or in a few limited types of popular processed forms such as beverages, confectionaries, snacks, jams, etc. However, the applications of the fruit industry go far beyond extending to fruit and nuts being used to extract different chemical compounds in the production of various pharmaceuticals, nutraceuticals, and cosmeceutical formulations (Alalwan et al., 2022; AlAli et al., 2021; Dini and Laneri, 2021). Accordingly, beyond the traditional fruit and nut farming and marketing, SL can aim to widen its market arms to fulfill such demands in the world market ensuring higher economic returns.

4. Fruits and nuts for promoting eco-tourism

Throughout history, the seasons and places where flowers bloom and bear fruits become popular tourist destinations; such as the cherry blossom festival in Japan, the tulip festival in the Netherlands. Similarly, visiting fruit orchards, observing fruit-bearing trees, and involving in fruit picking have currently become highly popular among tourists (Liu et al., 2016). In countries such as Australia and Japan; strawberry, apple and cherry picking have become novel trends in eco-tourism. Similarly, there is a strong potential for SL to promote eco-tourism while utilizing the high diversity of fruit and nut crops. Modernized exterior environments of tourist accommodation facilities with fruit orchards where travelers can enjoy nature is one such example. In addition, fruit orchards and fruit picking areas can be established in different regions of the country representing fruit crop diversity while providing refreshing experiences to travelers. Moreover; fruit spas, juice bars or mocktail bars can be established targeting promotion of eco-tourism by utilizing the existing fruit and nut crop diversity to strengthen the Sri Lankan economy.

5. Fruits and nuts as a novel venture for income generation among local communities

As a developing country, and under the current economic crisis, SL is experiencing low levels of daily income amidst high levels of unemployment. As described above, it is possible to introduce fruit farming and processing industries as a source of extra income or even as a source of main income for the low-income groups of SL communities. In addition, fruits and nuts can be further utilized for strengthening Sri Lankan economy by the establishment of novel entrepreneurships such as; mocktail shops, standard beverage shops, gift shops, fruit spas etc. targeting the higher strata of customers to create a flourishing economy while generating employment opportunities. However, creative thinking, smart policies, strong government and private sector initiatives, appropriate incentives and obtaining local and foreign expertise as required are essential for the initiation and maintenance of such ventures.

Measures to develop the fruit and nut industry

Appreciating the rich diversity of fruit crops in Sri Lanka

It is vitally important to identify and appreciate the current fruit and nut crop diversity in the country prior to establishing novel avenues for their utilization. It will help realize the enormous market potential and thereby employ measures for sustainable utilization of fruits and nuts to prosper the economy and the health status of the population. Recording of the diversity should follow with the identification of the most suitable regions within the country to grow a particular fruit crop or to establish a particular fruit-based industry. There is a high agro-ecological specificity in crop cultivation and thus yield and profit generation would highly depend on the designation of suitable areas for each crop species. Consequently, when aligning fruit crops and economic gains, it is of utmost importance to identify the current diversity, potential and limitations on utilizing them in a particular location. Improper and incorrect decision making in this regard would lead to heavy economic losses in the long run.

Identifying the current trends in local and international fruit markets

International fruit markets are highly diverse. Novel trends dominating these markets emerge from time to time. Accordingly, aims to step into these global markets should be facilitated with close scrutiny and timely decision making to align with the novel trends and emerging demands in order to secure and sustain our share in the international market. For example, the present-day consumers are highly concerned about the quality and safety of food that they consume. Accordingly, there is a huge demand for organic and GAP (good agricultural practices)-certified products in the domestic as well as the world market. Moreover, the buyers would appreciate being aware of the health benefits provided by different fruits and expect information such as added preservatives, sugars and salts etc. in the package information. As a country, if SL can cater not only to the demand of fruits but also for such other requirements a stable position would be ensured in the global market (Dissanayake et al., 2022; Gamage et al., 2020).

Ensuring a stable, high quality fruit supply

Ensuring constant supply of high-quality produce, is a must in targeting the global market and obtaining a competitive advantage in the world trade. For that, continuous monitoring and evaluation are needed to meet the requirements of food standards such as HCCP, GAP and ISO. Adhering to these standards and certifications would provide much needed recognition for our products in the world market (Dissanayake et al., 2022; Gamage et al., 2020). In addition, it is required to maintain acceptable and uniform quality parameters such as; flavour, colour, aroma etc. from farm to consumers to develop a product brand for Sri Lankan fruit products for maximum benefits (Figure 2).

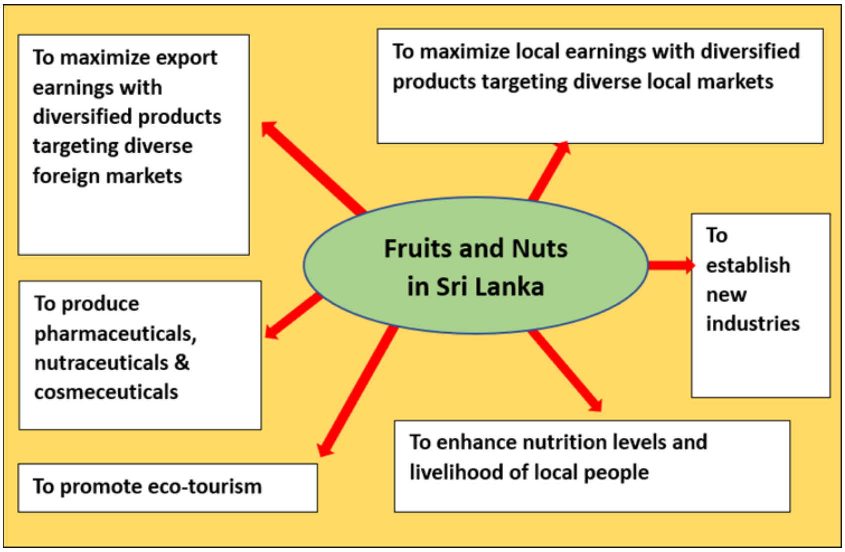

Figure 2: Potential avenues for utilizing fruits and nuts in Sri Lanka to achieve economic prosperity

Product diversification

SL currently exports both fresh and processed forms of fruits and nuts. However, to sustain itself in the domestic and international markets, SL needs to diversify its products and for this it is required to cater to the diverse needs of different consumers and promote value addition. For example, even for domestic markets, it is essential to produce a variety of products which will provide opportunities for consumers to enjoy a wide array of tastes, flavours and aromas. In terms of fresh fruit marketing, different sizes, shapes and colours could be promoted using attractive, innovative marketing strategies.

It is possible to extend the horizons of processed fruit and nut products, while introducing newly developed value-added products rather than limiting to traditional processed products. Sri Lankan researchers have already taken a lot of initiatives and efforts on this. Production of health-friendly, ready-to-serve fruit drinks from under-utilized local fruits is one example for such attempts (Karawita et al., 2018; Sarananda et al., 2017).

According to Perera et al. (2015), diversification of export-oriented fruit and nut products would be achieved either by introducing new fruit items to the export market or by promoting value added products.

Identifying new markets

Most of the Sri Lankan fresh and processed fruit and nut products are exported to a few limited markets. For example, fresh fruits are mostly exported to the middle eastern and Maldivian markets while the majority of the processed fruit products are exported to the European market (Dissanayake et al., 2022). But it is essential to capture potential new markets representing European union (Gamage et al., 2020) and Eastern markets such as China, Jordan, Singapore, Korea, Japan and Russia (Dissanayake et al., 2022).

Strengthening of public-private partnerships and Industry-academic collaborations

Compared to other countries, SL is lagging behind in public-private partnerships and industry-academic/research collaborations. To be a leader of fruit and nut trade, SL needs the active involvement of all these stakeholders contributing to the development of fruit and nut crop production and industries. Currently, knowledge generated through related research conducted in governmental organizations, research institutes and universities are not effectively transmitted to the industrial community due to poor linkage among these organizations. This leads to poor adaptation of the innovations and the technologies developed and prevent their commercialization. Due to this hindrance the fruit and nut industry is stagnated reiterating the importance of collaboration of all the sub-sectors for further improvement.

Introducing new technologies to minimize postharvest losses

About 40% of the annual fresh fruit production in SL is wasted due to various postharvest losses (Rajapaksha et al. 2021). It is vital that such post-harvest losses at all stages of production; such as harvesting, handling, transporting, storage and marketing be minimized to reap maximum benefits of the fruit crops sector. Hence, it is crucial to introduce novel technology at all stages of post-harvest practices to minimize post-harvest wastage (Rajapaksha et al., 2021; Gamage et al., 2020).

Reducing fruit imports and promoting usage of local fruits

To achieve maximum benefits of the domestic fruit marketing, it is necessary to promote local fruit consumption within the country. This should be complemented by limiting the importation of fruits by substituting such imported fruits and nuts, with locally available comparable varieties of fruits and nuts. Moreover, rather than utilizing fruits such as apples, oranges and grapes which are of temperate exotic origin, it is important to promote the usage of locally available tropical fruits and nuts in the hotel industry in the preparation of main dishes.

Finding means of popularizing under-utilized fruits

Other than the commonly available major and minor tropical and sub-tropical fruits and nuts, SL is blessed with over 60 varieties of under-utilized fruit crops (Ratnayake, et al., 2019). Most of such fruit and nut crops are rich in unrevealed health benefits and market potentials that will be instrumental in assuring high economic returns. Thus, to achieve economic sustainability via promoting fruit crops, it is crucial to discover the beneficial properties of under-utilized fruits and using them in developing new products.

Maintaining and promoting current fruit & nut diversity in Sri Lanka through fruit crop breeding

For a sustainable fruit crop industry, it is vitally important to retain and promote existing diversity by crop breeding and conservation. In the meantime, it is essential to promote the development of new fruit cultivars addressing a diverse range of biotic and abiotic stresses and consumer demands followed by providing high quality plant material for farmers to obtain higher yields and adequate, year-round production.

Supporting fruit and nut-based research and innovations

Research and innovations play a key role in the development of any industry. In fruits and nuts-based industries, it is essential to have research breakthroughs to unravel the hidden potentials and thereby to develop new products that cater to the needs of consumers.

Encouraging fruit and nut cultivation and industry with adequate policy and regulatory mechanisms

Even though there are certain policies and regulatory mechanisms targeting the development of fruit and nut crop cultivation and related industries, they are not adequate to cater to the needs of local economies. Thus, it is required to implement necessary changes in existing policies and to develop new policies. These measures should also target the regulation of export barriers, ensuring the quality standards of locally available and export quality fruit and nuts, empowering fruit crop growers, strengthening public-private partnerships and industry involvement, encouraging product development and diversification etc.

Factors that act as barriers to achieve maximum benefits from fruits and nuts in Sri Lanka

Inadequate planning, monitoring, and government attention, on strengthening and promoting the fruit and nut crops and the related industries, lack of intensives for subsistence level farmers to start small-scale cultivations or industries, inadequate public-private partnerships, lack of knowledge on new market trends and new technologies, insufficient attempts to breed new fruit cultivars, lack of new harvesting, processing, handling, storing and packaging technologies and heavy postharvest losses act as major barriers to achieve maximum benefits from fruits and nuts in SL (Dissanayake et al., 2022; Gamage et al., 2020).

Conclusions and future directions

Sri Lanka is a country blessed with a huge diversity of tropical and wild fruits and nuts that act as important components of the structure and maintenance of ecosystems. These vegetations possess a tremendous potential to sustain human life by their health benefits and economic returns. There are tremendous opportunities for Sri Lanka to obtain maximum economic returns from fruits and nuts via exporting them as diversified products, supplying to domestic markets, using them in promoting eco-tourism and in developing of various pharmaceutical, nutraceutical and cosmeceutical products and developing novel entrepreneurships targeting high-income groups. However, Sri Lanka is yet to fully explore the immense benefits that could be achieved by this biological wealth in order to prosper its people and the economy. Moreover, inadequate planning, monitoring, public-private partnerships, government attention, intensives for rural farmers, attempts to breed new fruit cultivars, technologies and heavy postharvest losses act as major barriers to achieve such targets in Sri Lanka. Thus, as a country struggling to achieve its economic stability Sri Lanka should identify the importance of fruits and nuts as an integral component of its biological wealth and act promptly and adequately to reap the maximum benefits from the fruit and nut industry to sustain the Sri Lankan economy and thereby improve the quality of human life.

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CHAPTER 07

Mangrove conservation and restoration in Sri Lanka: Biological wealth for economic prosperity

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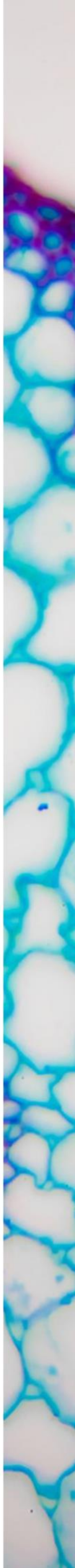

Abstract

Ecosystem restoration has become a significant feature of global policy agendas. United Nations Decade on Ecosystem Restoration (UNDER 2021-2030) prioritizes conservation and restoration of wetland ecosystems, including mangroves. The values of mangroves were best reflected after the Tsunami that hit Southeast Asia in 2004. It is now seen as a great potential in climate change mitigation. In this context, mangrove conservation is given a special priority as its rate of destruction has been higher. Sri Lanka is the first nation to declare the entire Mangrove ecosystem as protected by decree and also planned to restore 10,000 ha of coastal wetlands under the UNDER programme. In addition, Sri Lanka takes a key role in the Commonwealth Blue Charter, as the Champion, aiming conservation and sustainable utilization of mangroves. Valuing environment is a crucial task and it is generally performed using ecological, economic and social values as equivalent to monetary terms for various benefits. This chapter mainly focuses on conservation and restoration approaches of mangroves in Sri Lanka to establish a biological wealth of the country, through which the economic prosperity can be achieved. The economic prosperity of mangroves is discussed in this chapter under two themes viz, ecological wealth and social wealth.

Keywords: *Conservation, Ecology, Economy, Restoration, Valuation*

Introduction

Mangrove forests, are valuable components of coastal regions, provide critical and irreplaceable services around the globe to both human populations and the ecosystems they occupy. However, approximately 47% of global mangrove areas have been lost (Bhowmik, 2022) due to forest clearing and pollution, whereas Sri Lanka has lost more than 50% of its mangrove forest over the last three decades (Mombauer, 2019) due to development, encroachments, conversion of mangroves areas to agricultural lands, shrimp farms, saltern and wastelands (Gunathilaka et al., 2022).

Mangroves cover a very susceptible landscape and are also an asset of biological wealth (Blue Carbon Source) for any nation who plans to meet the SDGs for future generations. The importance of the mangroves has been more recognized in Sri Lanka, especially after the tsunami in 2004. This led to several actions to conserve and protect them with the support of national and international agencies. Sri Lanka has declared all Mangrove Forests as conservation forests and had initiated conserving all remaining mangrove forests while planning to restore 10,000 ha of coastal wetlands under the United Nations Decade of Ecosystem Restoration programme (Cooray et al., 2021), which is considerably higher for a country like Sri Lanka, which has about 2000 km coastline with hundreds of wetlands. Sri Lanka is perhaps the first or only country, which has decided to protect 100% of mangroves by decree.

What are Mangroves?

Mangroves are a specialised group of plants comprising of trees, shrubs and ferns that live in the coastal intertidal zone. They are confined to tropical and sub-tropical regions of the world, and may be at the riverine estuary (Bentota, Nayaru) or lagoons (Batticaloa, Trincomalee, Puttalam, Rekawa) and rarely seen in open coast in Sri Lanka. The ecosystem that they create is referred to as mangrove ecosystem or mangal, which has unique physio-chemical conditions created by tides, freshwater flows and silt.

They have specialised mangrove plants (true mangroves) and consists of other species commonly associated (mangrove associates) with these ecosystems. This ecosystem is recognized as habitats with exceptional biodiversity with endless richness. Furthermore, they coexist with a variety of other ecosystems, including mudflats, salt marshes, inshore, upstream, flooded forests, seagrass beds, and coral reefs (health and extent largely depends on coastal mangrove forest) in the open ocean. This interconnectedness enhances species mobility, nutrient exchange, and helps to support diverse faunal wealth in the mangrove ecosystems (Romanach et al., 2018; Martin et al., 2019).

Significance of mangroves

Mangroves have a range of values as species and ecosystem to the landscape and form an economic base to the residents around it, which is often seen/perceived only when the system is damaged or lost.

Mitigation of climate change

Mangroves contribute significantly in the reduction of atmospheric carbon thus mitigating global warming and associated issues related to climate change. Recent studies of Buditama (2016) revealed that approximately 24 Tg of atmospheric carbon has been captured by mangroves per year globally and accumulated approximately 6.5 Pg C in the process.

Shoreline protection from natural calamities

Mangrove vegetation with its complex root systems reduce the impact of wave actions, storms, tsunami, floods, inundation, and coastal erosion whereby protect the coastal ecosystems thus the associated livelihoods.

Benefits to associated coastal communities

They protect communities from the impacts of climate change, serve as sites of ecotourism and also facilitate provision of various sea foods and other products to people (food, fruits, fodder, charcoal, seedlings, etc).

Specialized habitats and refuge for other organisms

Diversity of mangrove roots act as a refuge for numerous aquatic organisms (fish, molluscs, polychaetas, shrimps, fish, crabs). The evergreen vegetation also provides habitats for many arboreal fauna (snakes, monkeys, mongoose, wildcat, etc.) and migratory species (plovers, sandpipers, spoonbills, ibis, etc). They serve primarily as the hatchery for many aquatic organisms and serve as hide out during their early stages of growth.

Visual amenity

Mangroves along with its fauna provides a clear synopsis of the functions and links between marine ecosystems and therefore attract "green-minded" tourists. They also form an attractive nature walk or canoeing among these dense patches of vegetation in many parts of the word including Sri Lanka (e.g., Bentota)

Mangrove conservation and restoration

Mangroves, under the strict guidelines, consist of about 54 true plant species belonging to 16 different families worldwide (Smithsonian Institution, https://ocean.si.edu), often with locally distinctive features, making them unusual and genetically diverse. Forest Department, in 2015, reported that around 15,669 ha of areas are covered with mangroves in Sri Lanka. Twenty-one true mangrove species and 18 mangrove associates reported in West coast of Sri Lanka (Jayatissa et al., 2002) whereas 10 true mangroves and 12 non-mangrove species reported in the eastern coast of Sri Lanka (Mathiventhan and Jayasingam, 2010). Conservation of such eminent ecosystem is essential to support the life of the coastal region directly and indirectly (Mathiventhan et al., 2018). Sri Lanka takes several initiatives to conserve, restore and to protect the mangroves which are elaborated below.

National policy: Mangrove conservation and sustainable utilization of mangrove ecosystems in Sri Lanka

This policy was approved by the Cabinet in January 2020. It is in line with the article *27 subsection 14* of Sri Lanka's constitution, "the state shall protect, preserve and improve the environment for the benefit of the community" (Ministry of Environment and Wildlife Resources, 2020). This policy was prepared, aiming at conserving the mangrove ecosystem and its sustainable utilization to enrich the biological diversity and its wealth for the economic prosperity of the country.

National guidelines for the restoration of mangroves ecosystems

Even though many initiatives had taken to restore the degraded mangroves and to re-plant them at various places in Sri Lanka, the success rate was low due to the failure of scientific restoration and continued follow up (Mathiventhan and Jayasingam, 2016; Kodikara, 2017; Ministry of Environment, 2021). Therefore, to overcome such issues, the Ministry of Environment has published a national guideline (2021), which serves as a key reference to those who engage in mangrove restoration in future with suitable adaptive management strategies. However, facilitation of natural regeneration has been found to be more successful, which on the other hand makes it sown composition to suit the present-day conditions.

Mangrove Ecosystems and Livelihoods Action Group (MELAG)

MELAG is a subcommittee of the Blue Charter of the Commonwealth organisation, and Sri Lanka is committed to the champion of MELAG, to share best practice and expand cooperation in the conservation and sustainable utilization of mangroves. It further shares three important aspects such as (i) technical know-how and best practices on the restoration of mangroves, (ii) strengthen community partnerships relating to mangrove ecosystems and (iii) develop strategies to strengthen legal frameworks for the conservation of mangroves.

Biological wealth and economic prosperity

Biological wealth is a dynamic feature/entity, which directly related to biological-richness. In broad sense, biological richness of the earth represents the collective value of the biological diversity of the environment. Therefore, the biological wealth shall be defined as the cumulative value of genes, species, ecosystems, and ecological events/functions in a region. An environment with a high biological wealth will better sustain the life of its people and the other surrounding environment.

Economic prosperity does not simple mean as the wealth of the country, but beyond this *viz*. economic growth, economic security and economic competitiveness.

Economic prosperity shall be defined as a balance between the interest of the present society and that of tomorrow (Gunawardena and Rowan, 2005).

Valuing wealth of an ecosystem (mangroves) in terms of economy is a complex process and is reliable if relevant and accurate information/data available based on ecosystem processes, functions and applications of appropriate economic valuation (Morse-Jones et al., 2011). Economic values associated with healthy mangrove ecosystems can however be generated through economic analysis that attempts to measure the use and non-use values of these ecosystems. The values attributed to mangroves are therefore dependent on the ecological properties of them and the productive structural and consumptive use of the communities that depend on them. Considering the importance of mangroves and its associated ecosystems, this section focuses on different types of wealth produced by mangroves and how it shall be related with the economic prosperity of the country.

Ecological wealth of mangroves and economic prosperity

Mangroves provide countless ecological services regionally, nationally as well as globally in terms of direct and indirect use values such as shoreline protection, freshwater regulations, flood barriers, nursery habitats, biodiversity, aesthetic value, maintain bio-geochemical cycles, carbon sequestration and many more. As measured by UNEP, their annual ecosystem services are worth of US$33-57,000 per ha.

(a) Shoreline protection is considered as one of the prime services of the mangroves. This was assessed by different methods such as Replacement Cost Method (RCM), which derives the value of a man-made seawall as having the same protective effect for the shoreline, the value then applied to mangroves (Kairo et al., 2009). The same research group further valued the shoreline protection as a major service of mangroves with close to 55% of the total economic value; and the protection not only limited to coastline but it also extended to inland areas too (Barbier et al., 2008), depending on the use of coastal lands.

Alternatively, another method used is the Damage Coast Avoided Method (DCAM), for example, it calculates the potential damage a tsunami would have on the urban infrastructure or losses in agriculture if mangroves did not exist (Ruitenbeek, 1992). Further, Dahdouh-Guebas and Koedam (2006) reported that only 7% of the villages were severely affected in an area with an intact mangrove belt, during the tsunami, whereas it reached 80-100% in areas with degraded or lack of mangrove forests. This could be converted into monetary terms, based on the severity of the damage.

(b) Carbon sequestration/Decarbonizing

Carbon sequestration/decarbonizing continues of more value considering the impacts of climate change. Mangrove forests play a vital role on carbon sequestration due to its higher biomass density and productivity. Total ecosystem carbon stock of mangroves

was reported ranging from 154 to 1484 Mg C ha-1 with a mean global carbon stock of 885 Mg C ha-1 from an estimated global mangrove coverage of 13.8 – 15.5 million hectares (Giri et al., 2011; Kauffman and Bhomia, 2017; Cooray et al., 2021).
According to Giri et al. (2011) mangroves, including associated soil, could sequester approximately 22.8 million metric tons of carbon each year. He further came up with a straight forward result of 18 tC ha-1Y-1 carbon benefit potential. This study assumes a price of US$ 7 per ton. Therefore, it becomes an additional mangrove value of US$126 ha-1Y-1. But the carbon prices change depending on the location of the market, the type of market, and supply and demand. Conserving and or restoring mangrove ecosystems not only reduce the atmospheric carbon but it also has the potential to contribute positively to the earning of millions of dollars through carbon trading or carbon credit globally. In the case of Sri Lanka, a national offset scheme has been established (Sri Lanka Carbon Crediting Scheme-SLCCS) for the greenhouse gas emission reduction. In addition, a private-public partnership company has been established (Sri Lanka Carbon Fund-Ministry of Environment) to minimize the carbon emission and to build a new low-carbon business economy and low carbon life patterns.

(c) Valuation of biodiversity

Valuation of biodiversity is another newer concept frequently used to assess the importance or potential, economically. Mangroves in their undisturbed state are regarded as a refuge for rich biodiversity. Biodiversity value combines direct, indirect and non-use value and is a valuation of human preference rather than actual value (UNEP/GPA, 2003). Ruitenbeek (1992) defines the capturable biodiversity benefit as the potential benefit, ("the country might be able to obtain from the international community in exchange for maintaining its biodiversity base intact"). In general, Contingent Valuation Method (CVM) is mostly widely used to value the non-use values based on the "Willingness to Pay (WTP)" concept to place a monetary value on the mangroves and say, what the local and regional communities are willing to pay to conserve the biodiversity. Alternatively Benefit Transfer Method (BT) is also used to estimates the value of an ecosystem service by transferring an existing valuation estimate from a similar ecosystem (TEEB, 2010).

Ruitenbeek (1992) and Abeysinghe (2010) came up with a biodiversity value of mangroves of US$ 15 ha-1y-1, measuring mainly the pharmaceutical value of the mangroves. Moreover, UNEP/GPA (2003) estimated a value of US$ 18 ha-1y-1 for biodiversity of mangroves (non-use values) of Sri Lanka.

(d) Mangroves, fishery and related activities

Mangroves provide ideal breeding grounds for much of the world's fish, shrimp, crabs, and other shellfish. They may contribute 5–25% of the offshore fishery (Spurgeon, 2002) and meanwhile 31.7% of the production of fish catch (Aburto-Oropeza's, 2008). A study conducted by Gunawardena and Rowan (2005) in Sri Lanka revealed that the annual net value of the Rekawa mangrove–lagoon fisheries per hectare of mangroves was estimated to US$ 268/ha/year.

Shrimp farming is one of the mangrove-based aquaculture operations in Sri Lanka. It was estimated that around 6400 kg of shrimp produced from mangrove-based shrimp farms, which is practiced in an extent of 1758 ha from Puttalam, Batticaloa and Mannar (Fisheries statistics, 2020). The shrimp exports provide roughly US$ 25million as a revenue, accounting for nearly 50% of Sri Lankan fisheries' export earnings. Mangrove-associated estuaries are popular for crab harvest and cultivation in the north and eastern part of Sri Lanka such as Batticaloa, Vaharai/Panichchankerni, Kokkilai. Further, mangal ecosystem is the ideal habitat for crab fattening. The total revenue earned over six months is around US$ 600 from crab fisheries in Kokilai lagoon (Ranawana, 2017). This is an income that looks attractive for a short period of time.

Social wealth of mangroves and economic prosperity

Coastal communities are dependent on a range of mangrove products that provide cash income and subsistence requirements of the community in the location. The income could support and contribute local economic production and significantly contribute towards achieving better development outcomes. Sustained investment in sustainable use of mangrove resources will depend on the clear evidence that benefits and costs associated with this ecosystem compare favourably with those of other social and economic investments (Acharya, 2002).

Example: Community-mangrove interaction and the financial benefits obtained through a mangrove restoration project in Kenya (Environment for Development, UNEP).

Carbon Finance Project, Gazi Bay, Kenya

The Gazi Bay community in Kenya led carbon finance project for the conservation, management and restoration of 117 hectares of mangroves has so far sold certificates of 3000 tonnes of carbon dioxide (CO_2), with funds being allocated to community projects and additional mangrove activities overseen by village leaders. One of the many successes of the project has been a dramatic reduction in illegal harvesting of mangroves, and it is hoped that the success of the community-based initiatives in these countries will pave the way for other developing countries to start establishing new carbon projects to ensure sustainable ecosystem services to local communities.

The economic value of mangrove was estimated, in the South-Eastern coast of Sri Lanka and it shows that at Rs. 119,438 (US$ 1,171) per household per year and Rs. 938,502 (US$ 9,201) per hectare per year. The direct use benefit of mangrove products (Gross Value) is Rs. 9,953 per household per month (IUCN, 2007). This study further revealed that mangroves provide higher proportion (42%) of total benefits to the poor income households while comparing medium (37%) and high income (21%) households.

Future potential

The economic and social benefits of mangroves, which are estimated to run into hundreds of billions of dollars worldwide remain largely untapped due to a lack of carbon finance mechanisms, appropriate policy interventions and rapid mangrove deforestation. UNEP estimates that the economic cost of the destruction of carbon-rich mangroves is about US$42 billion annually. This economic loss may be overcome by conserving and restoring the valuable mangrove ecosystems. Policymakers and financial markets are beginning to take interest, though more needs to be done to develop new methodologies for carbon trading for mangroves. Conserving such valuable mangrove ecosystems are vital for coastal population those who traditionally depends on them and various life support and protection services. It does not undervalue its importance to the country at large over the various sectors that had been mentioned earlier, and for Sri Lanka, we are sitting on a gold mine to be tapped and utilised effectively.

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CHAPTER 08

Production of a cost-effective and eco-friendly bio-piscicide using an endemic plant of Sri Lanka: *Catunaregam spinosa* (Thunb.) Tirveng

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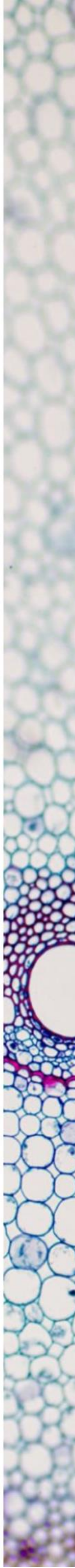

Abstract

Currently, most of the global activities are combined with green concept to ensure the sustainable future of the planet. Fishes are important creatures in different forms as they play an integral role in aquatic systems becoming a vital source of protein in humans' diet. However, overgrown and invasive fish populations distract the ecological balance while threatening the existing native species. *Catunaregam spinosa* is a potent piscicidal plant used by tribal people for harvesting fish which aroused the curiosity in developing a bio-piscicides as an alternative to chemical products which cause numerous hazardous impacts. Thereby, present study developed a *C. spinosa* based bio-piscicide using its aqueous seed extract tested against *Danio rerio* following the guidelines by The Organization for Economic Co-operation and Development. The result indicated the potential fish toxicity of seed extract in dose and time dependent manner on mortality of *D. rerio*. Active ingredient conjugated with suitable adjuvants recorded Lethal Concentration (LC) 50 of 4.074, 3.759, 2.943 and 1.952 mg L^{-1} and LC90 of 12.770, 11.772, 9.217 and 7.807 mg L^{-1} after 24, 48, 72 and 96 h respectively. Behavioural changes and different humane end points determined the mortality of fish. The product showcased satisfying activity by recording 85.71 % of mean mortality after one month of time. Cost-benefit analysis ensured the economic feasibility of the product compared to those chemical piscicides in relative to the low cost and environment friendliness while safeguarding missing of *C. spinosa* from natural population.

Keywords: *Bio-piscicide, Catunaregam spinosa, Cost-effectiveness, Danio rerio, Environmental friendliness*

Introduction

Green approach is extensively employed global concept applying in different platforms like medicine, waste management, electricity, agriculture, industries and transportation to mitigate the inferior human impacts on the environment. Fish play an integral role both in human lives and aquatic systems. They serve as a protein rich source and assist the economic survival of people while maintaining the diversity of aquatic system. Meantime, playing an important role, fish still experience several threats like over-exploitation, deforestation, climate changes, wide spread of exotics species, poor legislation by the government and water quality degradation leading for their mass destruction. Increasing food demand escorts people to over-exploit and poor legislation calls for illegal fishing which ultimately affects the survival of both human and fish lives.

Fishery industry

Among the different means of fish harvesting such as hand-gathering, trapping, netting, spearfishing and trawling, use of piscicidal plants is one of methods especially used by tribal people. They threw cooked, soaked water mixtures or preheated stones rubbed against these plant materials to haunt fish. Also, they buried parts of bark in the underwater sand and sprinkled the infusions of materials to catch fish. The fish toxicants occupy in these materials either mix with blood stream directly or diffuse through tissues in to fish body to make them paralyze and later to die. Leaves of *Diospyros embroypteris* (Timbiri), roots of *Derris scandeus* (Kalaveal) and fruits of *Catunaregam spinosa* (kukuruman) are well known in fish stupefying in Sri Lanka (Ulluwsupwe, 1995). These plants are more popular among farmers who work in small scale fishery industry as fish can easily get captured when they get floated on the water surface after administrated by toxicants. Fabeaaceae, Euphorbiaceae, Apocynaceae and Asteraceae are families rich with piscicidal plants. Researchers from countries like India, Nepal, tropical Africa, west Indies, Polynesian countries and central America studied piscicidal plants found in own countries wherein *Zanthoxylum armatum*, *D. elliptica, Tephrosia purpurea*, *Barringtonia asiatica* (futu or vutu) were renowned for high piscicidal activity (Kulakkattolickal, 1989; Jawale, 2018).

However, these primitive methods ensured the sustainable fishing amidst of satisfying food demand assuring environment friendly practices. The evolving time conquered for drastic changes in human lives while increasing the food demand to use user-friendly commercial piscicides which later invaded the fishery industry causing significant hazardous impacts on fish population and their inhabitants. Conversely, over-populated and invasive fishes also usually lead for different concerns.

Threats from over-populated and invasive fishes

Overpopulated and invasive fish disturb the integrity of the eco-system by creating competition for limited resources including oxygen, food and space. Rodrigo (2020a) reported 74 % of Sri Lanka's freshwater fishes; a total of 139 species including 61 which only can be found in Sri Lanka, are threatened to extinct due to overpopulated and invaded invasive species.

Overpopulation

Fish overpopulation is a scenario where fish population exceeds the carrying capacity of ecological system. It later causes slow and continuous death of fish especially live in stagnant waters due to depletion of dissolved oxygen level, inadequacy of food, space and multiplication of different parasites. Recently, tilapia is becoming a notable invasive species in Sri Lanka as having high reproduction and survival rate with distinctive adaptations like living in a dwarfism by being stunt and elevating the oxygen capacity to compensate the oxygen demand to cope up the increasing metabolic functions during the harsh conditions (Athauda, 2010). A relative scenario happened in Kandy Lake in 2019 which was reported by Dr. A. Arulkanthan where the overpopulation of Tilapia was the main reason of dying fishes in Kandy Lake. Also, he found, caused internal and brain damages due to depletion of oxygen, consequent respiratory harassing of gills and multiplied parasite called 'Isopoda' as main reasons of fish deaths (Seneviratne and Fazlulhaq, 2009).

Invasive fishes

A fish species is referred as an invasive if they impose direct impacts on native species either by competing for resources or directly feeding on larvae of other species. Sri Lankan fresh water bodies are tortured by at least 30 exotic fishes which are either intentionally released to boost aquaculture, mosquito controlling or accidently for aquarium trends (Rodrigo, 2020b). According to the National Aquatic Resources Research and Development Agency (NARA) (2020) *Atractosteus spatula* (alligator gar), *Chitala ornate* (clown knifefish), *Oncorhynchus mykiss* (rainbow trout), *Hypostomus plecostomus* (suckermouth catfish/tank cleaners), *Clarias batrachus* (walking catfish) and *Mayaheros urophthalmus* are common invasive fishes reported in Sri Lanka. Currently these species are wide spread and co-currently impose harmful impacts on native species. For instances, suckermouth catfish lays more eggs than other species and their egg survival rate is around 80 % which only reported 30 % in native species. Also, suckermouth catfish directly feed on larvae of other aquatic animals and have ability to survive few hours outside the water. *Mayaheros urophthalmus* is another introduced species from Central America which aggressively attacks inhabitant aquatic organisms hence need serious concern in controlling (Rodrigo, 2020b). Moreover, the overgrown invasive fishes disturb fishery industry by reducing praying frequency of commercial fishes that used as food source and reduce the richness of fish harvest due to lack of resources for a satisfying fish growth.

Piscicides, active compounds and toxic mechanisms

Anhydrous ammonium substances, quick lime, hydrated lime $Ca(OH)_2$, sodium hypochlorite, calcium hypochlorite and sodium cyanides are inorganic chemicals used by people in regional preference (Das et al., 2017). Antimycin A, rotenone, niclosamide and TFM (3-trifluoromethyl-4-nitrophenol) are some registered synthetic piscicides in USA (U.S. Army Corps of Engineers, 2013). Rotenone, saponins, tannins, nicotine, cardiac glycosides, alkaloids and diosgenin are naturally occurring fish toxicants found in different plant species. These compounds act as neurotoxicants by disturbing the normal activities of nervous system and respiratory organs. Interrupted fish respiration causing them to rise onto the water surface for excess gasping of oxygen and later get paralyzed and died (Dickers et al., 2003).

Over past decades, world mostly tend to use user-friendly chemical piscicides over environmental friendly bio-piscicides and currently the trend has diverged towards green approach to use ethnobotanical products due to negative externalities caused by chemical products. Indiscriminate use of these chemical piscicides leads subsequent disease like asthma, allergy and cancer in human while leaving life of non-target organisms in risk by attacking their endocrine system and reproductive development even at low concentrations of these toxicants (Das et al., 2017). Also, the long-persistence of chemical debris and their toxicity in the environment, high loading of nutrients and resistance built up from targeted species draw the demand of chemical piscicides backward. Unfortunately, chemical piscicides are favored by farmers due to user-friendly approach, effectiveness and long-lasting activity affirmed by laboratory testing and product modifications. Unlike chemical piscicides, bio-piscicides are easily biodegradable, less harmful to farmers and non-target species and low probability in developing resistance. In the practical perspective, bio-piscicides are lack of rigidity and durability of its activity, find difficulties in long term storage and occur inevitable toxicity over human. For an instance, high concentration of rotenone causes skin ulcers and neurological malformations and its non-selectivity imposes toxicity on invasive and native aquatic species (U.S. Army Corps of Engineers, 2013). Thus, consuming of rotenone is controversial and some countries have banned its uses in aquaculture practices.

Rotenone is naturally derived compound and main active ingredient in most of the piscicidal plants which originally found in *Derris* plants and currently used in entomological studies for management of insects (Ling, 2002) (Figure 1). It is a highly toxic compound to cold blooded aquatic animals and harmless to warm blooded vertebrates and human (Rodriguez, 1990). Breaking the controversies, neurotoxicity of rotenone on human and mammals was declared by the World Health Organization (WHO) in a report of "The WHO recommended classifications of pesticides by Hazard and guidelines to classification, 2009" by mentioning insolubility of rotenone in water limiting possible gastrointestinal absorption. Moreover, in natural environment rotenone gets rapidly decomposed after exposing to air and sunlight in 2-3 days (Ling, 2002). Rotenone as a neurotoxicant shows cellular mitochondrial level poisoning and suppress the respiratory chain of cells by inhibiting oxidation of NADH to NAD, block the glutamate and succino-dehydrogenase and then H^+ transportation. Lack of H^+ decelerates oxygen gaining from the water and energy, causing asphyxia of tissues and then paralysis of organs. Isman (2006) identified 44 % of rotenone in *Derris* plant grown in Venezuela and Peru. Saponins interrupt the fish respiration, as the soap-like property lyses the blood cells and increase the rate of toxins by direct uptaking into the blood stream via gills (Figure 2). Later, the asphyxiation occurs to block the respiration causing escape of essential electrolytes (Rodriguez, 1990). However, saponin toxification is reversible if fish moved into untainted water as they can revive back to their pre-toxic state.

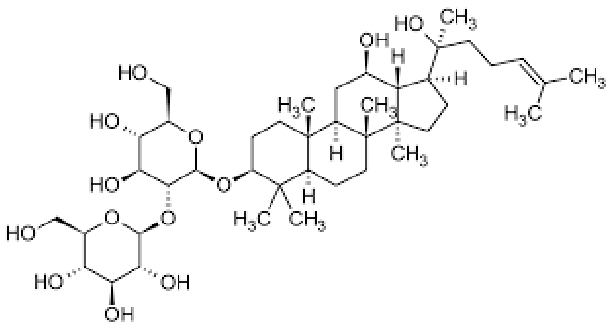

Figure 1: Chemical structure of Saponin
Source: Savage, GP (2003)

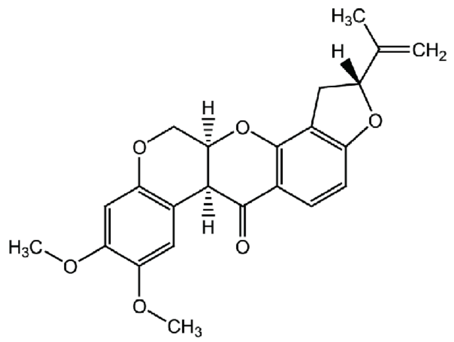

Figure 2: Chemical structure of Rotenone
Source: Sae-Yun et al. (2006)

Cardiac glycosides and alkaloids attack nerve mechanism of heart muscles and central nervous system (Rodriguez, 1990). Alkaloids cause respiratory failures, complete paralysis of muscles and later death of fish. They perform minor effects alone and synergetic effect with major constituents despite having less toxicity compared to rotenone and saponins. Tannins are also slow in activity which depends on relative abundance of the toxicants found in the sources. They bridge cross links between proteins and create resistance against proteolytic enzymes. The producing molecules precipitate on gills of fish interrupting gill respiration and finally leading fish to die by asphyxiation. Unfortunately, correlation between the structures, relative toxicity, biochemical mechanism and environmental impacts of most of these toxic compounds are still ambiguous.

C. spinosa as a piscicidal plant

C. spinosa is a well-known terrestrial fish poisoning plant (family Rubiaceae) where the activity mostly found in fruits, seeds and root bark. Numerous researchers mentioned the moderate activity of *C. spinosa* and few examined the relative active ingredients and their derivatives (Jawale, 2018). However, *C. spinosa* is less toxic compared to those reputed plants of *D. elliptica* and *T. candida* which rich in rotenone and tephrosin respectively (Mohotti and Epa, 2016). Kulakkattolickal (1989) studied toxicity and durability of toxicity of ripen fruits of *C. spinosa* against three predatory fishes;

Ophiocephalus punctatus, Clarias batrachus and *Heteropneustes fossilis* and found LC50 of 0.02 – 0.04 % against all three fishes and 204 h as the durability of toxicity in the environment. Moreover, he noticed a positive correlation between temperature of water and fish mortality. Shirgur (1975) studied comparative toxicity, effective stupefying inducement and biodegradability of aqueous seed, whole fruit and pericarp extracts of *C. spinosa* against *Cyprirtfts carpiocommunis* (Common carp) fingerlings and determined 6 h, LD100 as 3.0, 100.0 and 150.0 ppm respectively. The time taken to kill and stupefy fingerlings were 12, 40, 180 min and 10, 35, 122 minutes exposed to seed, whole fruit and pericarp extracts respectively at the dosage of 1 g L^{-1}. Seed extract recorded highest number of days (18 days) to loss the activity followed by 15 and 13 days for whole fruit and pericarp extracts respectively. Fruits and seeds are high in piscicidal activity unlike stem, stem bark, roots and leaves which cause considerable damage to the whole plant when collecting the raw materials.

Development of biopiscicide

Effective killing of target organism at fairly low concentration, no harm to human after consumption of poisoned fish, toxicants get quickly degraded remaining no precipitation in the water body, easy availability and beneficial economics are characteristics of an acceptable piscicide. Biopiscicides derived from natural resources are safe in fish controlling though the conventional use of whole plant threatens the diversity and conservative aspects due to the mass use of plant materials. Developing a biopiscicide by extracting particular bioactive compounds only uses the small quantities of the raw materials. Optimized concentrations, selective target organism, seasonal and developmental stages-based application protocols, determine the successful management of species while mitigating the side effects on non-target species. Determined lethal dose and suitable adjuvants will enhance the durability and effectiveness of the product. Hashimoto et al. (1991) developed a biopiscicide using secondary metabolites of *Edgeworthia chrysantha* and recorded 100 ppm LC50 against *Oryzias latipes.* Tea seed cake is a potent bio-source in piscicidal activity as it recorded 25 ppm of dose level against *Oreochromis niloticus* (Chiayvareesajja et al., 1997).

Since there is lack of evidences in assessing piscicidal activity of *C. spinosa* grown in Sri Lanka, present study aimed to study neurotoxicity of aqueous seed extract of *C. spinosa* using adult *D. rerio* as the test organism and developing a feasible bio-piscicide.

Determination of piscicidal activity of *C. spinosa*

Any study involves in manipulating animal models is required to grant the permission from respective ethical committees and reported study was ethically granted by the Ethics Review Committee – Animal Studies Faculty of Graduate Studies, University of Sri Jayewardenepura under the reference no. FGS/ERCAS/2022/05/02.

Seeds collected from Ayurveda Herbal Garden, Haldumulla, Sri Lanka (6º45'42" N 80º53'59" E) were air dried and size reduced to prepare the stock solution (20.0 mg L^{-1}) and dilution series (1.25, 2.5, 5.0, 10.0 and 20.0 mg L^{-1}). Seven to eight months old healthy adults of *D. rerio* obtained from Aquarium at Karadiyana, Piliyandala, Sri Lanka were acclimated in a suitable glass tank under the laboratory conditions (Mohotti and Epa, 2016). The assay was conducted using seven fish per concentration at 26 ˚C according to the guidelines by OECD 203 (OECD, 2019). Distilled water was used as the negative control. Mortalities and behavioural changes were recorded after 24, 48, 72 and 96 h. The LC50 and LC90 were computed using regression analysis in SPSS 24.0 software.

Formulation of the bio-piscicide using *C. spinosa*

Bio-piscicide formulation was developed using lethal dose of aqueous seed extract of *C. spinosa* at which 90 % population was killed after 24 hours (12.77 ~ 13.0 mg L^{-1}). Adjuvants of sodium acetate [1.42 % (w/v)] and propionic acid [0.06 % (v/v)] acted as the buffering agent and antimicrobial compound respectively. Molasses [0.25 % (w/v)] played as the adhesion agent, Ultra Violet (UV) protectant and phagostimulant. Carboxy methyl cellulose [0.25 % (w/v)] was added as the emulsifier and stabilizer. The durability of the activity of developed piscicide was evaluated after one month of formulation. After fish were introduced to the test tanks, distended abdomen (dropsy, swelling), preferred tank location, gulping air at the water surface, swollen and bleeding gills and loss of body balance were observed as humane end points. The severity of behavioural changes was concentration dependent which ranged from mild to severe. Rapid gill movements followed by loss of body balance, spinning body, faded body colour, expanded pectoral and pelvic fins, thickened, twisted and deformed caudal fins were observed in dead fish (Plate 1.1).

(a) (b) (c)

Plate 1.1: (a) Discoloured carcasses (b) expanded and damaged pectoral and pelvic fins (c) thickened, twisted and deformed caudal fin of poisoned *D. rerio*

Normal behaviour was observed soon after the fish were introduced to the low concentrations, yet after about 5 min fish started to swim near the water surface gulping air. Fish were collapsed to the bottom of tank in different time intervals respect to the concentration of test solution. Few seconds after the fish were exposed to high concentrations, they tend to aggregate in the corner of the tank and few minutes later, erotic swimming was observed which apparently led all them to die within 5 min followed by several times to escape from the tank (Plate 1.2). Literatures discussed a possible reason of losing body balance as deficiency of coordination between nervous and muscular impulses caused by excess accumulation of acetylcholine in synaptic and neuromuscular junctions (Rao et al., 2005). Increased gill movements and efforts in escaping from aquarium might be due to interferences occurred by toxicants on gill tissues and its functions (Bernardi et al., 2013). Abnormal or deformed caudal fins may be either due to diffused or focal haemorrhages throughout the fin tissues or proximity to the fins. Fins are the locomotors of fish which use to forward and lifting motions also to stabilize the body balance. As fins were physiologically damaged by neuro-toxicants it might triggered the loss of balance, aggressive swimming and after settling the bodies at the bottom.

Plate 1.2: Dead adults of *D. rerio* in treated tank of *C. spinosa*

Based on the data analysis concentration-dependent mortality of adult *D. rerio* was reported against aqueous seed extract of *C. spinosa*. The LC50 was 4.074, 3.759, 2.943, 1.952 mg L^{-1} and LC90 was 12.770, 11.772, 9.217, 7.807 mg L^{-1} after 24, 48, 72 and 96 h. Piscicidal activity of developed bio-piscicide was high due to LC90 was < 100.0 mg L^{-1}. At a dose of 10.0 mg L^{-1} > 80 % mortality was recorded and maximum lethal concentration of 100 % population was killed was 20.0 mg L^{-1} after 24 h. However, prolonged exposure to low concentrations also induced the fish mortality. For an instance, no mortality was recorded at the lowest concentration of 1.25 mg L^{-1} up to 72 h and sudden increase of mortality (14.28 %) was observed after 96 h. These results interpret the possible chronic effects of toxicants after long exposure even to low concentrations of toxicants.

Ramanayaka and Atapattu (2006) studied fish anesthetic property of ten local plants collected from Sri Lanka where *C. spinosa* was one of them and screened potential anesthetic activity of fruits of *C. spinosa* against *Cyprinus carpio* (common carp). They recorded time required to attain total loss of balance and lay down of fish at concentrations of 1.25, 2.5, 3.75 and 5.0 mg L^{-1} and observed fish collapses after 50, 33, 20 and 20 min respectively. Comparatively, reported present study also recorded 60, 30, 25 and 17 min for collapsing respectively at 1.25, 2.5, 3.75 and 5.0 mg L^{-1}. However, none of the fish exposed were recovered. After one month of time the activity of bio-piscicide has been reduced by 4.76 % recording 85.71 % of mean percentage mortality.

The protocol followed in this study is cost effective and highly environmentally friendly compared to those of chemical piscicides. Since responsible active agents and underlying physiological mechanism of fish poisoning of *C. spinosa* is still ambiguous further experiments would help for better clarifications.

Cost-benefit analysis

Cost-benefit analysis ensured the cost effectiveness of the developed bio-piscicide compared to existing chemical piscicides which use high cost and technologies. Cost was calculated from obtaining seeds to formulating the bio-piscicide. The low weight (26.0 mg) of size reduced seeds to prepare 2.0 L of test solution is the first evidence of cost effectiveness of this product. Importantly, use of such a small quantity of raw materials address the issues arising in mass use of plant materials infringing the conservation rules of flora. The required quantity of 26.0 mg can easily be obtained by splitting two fruits of *C. spinosa* since a fruit contains numerous seeds (50-70). Secondly, the use of conditioned water as the extraction solvent; the cheapest solvent in the world, cut off the cost usually incurred in expensive and toxic solvents like ethanol, methanol and ethyl acetate. The total electricity cost for electric balance, electric grinder, pH meter, dissolved oxygen meter, conductivity meter and the stirrer was Rs. 100.00. Adjuvants were high in cost in its first purchase like Rs. 2,000.00 for sodium acetate (500 g), Rs. 2,500.00 for molasses (500 g), Rs. 3,200.00 for carboxy methyl cellulose (500 g) and Rs. 3,500.00 for propionic acid (500 mL). The total cost in developing 1.0 L of bio-piscicide was Rs. 181.34 which included Rs. 56.64, 12.50, 8.00 and 4.20 proportionally for sodium acetate (1.42 % (w/v)), molasses (0.25 % (w/v)), carboxy methyl cellulose (0.25 % (w/v)) and propionic acid (0.06 % (v/v)) respectively. The calculated cost was significantly low compared to production of chemical piscicides amidst of their tremendous hazards, high cost incurred in machinery, equipment and purifying processes.

Further development of product will help for a better performance and extended shelf due to the current frame is insufficient in commercial and large-scale applications. Also, corporation of advanced and suitable adjuvants will enhance the activity and durability of the product. Investigating the fish poisoning mechanism of *C. spinosa* will add value to the product which is already in progress under this study by evaluating acetylcholine esterase activity of poisoned brain and muscle tissues of *D. rerio*.

Environment and toxicity assessments by field trials will ensure the safety use of the product to human and non-target organisms and also the sustainable fish harvesting in fishery industry.

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CHAPTER 09

Exploitation of lactic acid bacteria for growth of the food industry

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Abstract

The microbial diversity on earth presents a massive, largely under-exploited genetic and biological resource pool that could be exploited for the recovery of new genes, metabolic pathways, and valuable products. Further, numerous metabolic traits allow microorganisms to perform a variety of essential ecosystem functions, on which several agriculture, food, pharmaceutical, and chemical productions depend. Lactic acid bacteria (LAB) are a heterogeneous group of Gram-positive, non-sporulating, anaerobic, or facultative aerobic cocci or rods, which produce lactic acid as the main end-product of the fermentation of carbohydrates. The LAB used in the food fermentation process had a long history of safe use and are, therefore, referred to as "food grade" or GRAS (Generally Recognized as Safe) microorganisms. The LAB are well known for centuries as safe starter cultures with many numbers of applications including the production of fermented foods and beverages. Moreover, they produce aroma compounds, vitamins, ethanol, bacteriocins, exopolysaccharides, and enzymes of high value. As a result, LAB improve shelf-life, microbial safety, stability, and texture, and contribute to the pleasant sensory profile of the end product in the food industry. Scientific reports support the probiotic effect of LAB extensively including treatment of various gastrointestinal diseases, immunomodulation, anti-diabetic activities, etc. In this chapter, the exploitation of LAB for the development of the food industry is widely discussed in areas such as sustainable agriculture for food and feed production, improving quality and safety of food, biocontrol agents and bio preservatives, for health and wellbeing**,** biotechnological production of vitamins, organic acids, neutraceuticals, low-calorie sugars, bioactive compounds, etc., biorefining of 'green' crops and food waste management.

Keywords: *Bacteriocins, Biorefining, Bioremediation, Exopolysaccharides, Lactic acid bacteria*

Introduction

Lactic acid bacteria (LAB) are a heterogeneous group of Gram-positive, catalase-negative, anaerobic, or facultatively aerobic, non-sporulating, cocci or rods, which produce lactic acid as the main end-product of the fermentation of carbohydrates. The homofermentative species produce lactic acid (<85%) as the sole end product while the heterofermentative species produce lactic acid, CO_2, and ethanol/acetate. According to the current taxonomic positioning given in the National Centre for Biotechnology Information (NCBI) Taxonomic Browser, LAB has been categorized in the phylum Firmicutes, class Bacilli, and order Lactobacillales. Although LAB include more than 60 genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Enterococcus*, and *Weissella* are the main genera involved in food fermentations (Wang et al., 2021). In general, LAB occurs in nutrient-rich habitats and were first isolated from milk. Occurrence of LAB is common and abundant in fermented food products such as meat, milk products, vegetables, beverages, and bakery items. Moreover, they are common inhabitants of soil, water, manure, sewage, silage, and plants, further, are a component of the microbiota on human and animal mucous membranes such as intestines, mouth, skin, and urinary and genital organs (Quinto et al., 2014). Members of this group are of major importance to human health, well-being, and economic activities such as food, chemical, and pharmaceutical industries. Their application in the food fermentation process has a long history of safe use and, therefore, are referred to as “food grade”. Consequently, LAB are awarded ‘Qualified Presumption of Safety (QPS)’ by the European Food Safety Authority and ‘Generally Regarded as Safe (GRAS)’ status by the US Food and Drug Administration (EFSA, 2010; Qiao et al., 2022). Recently, LAB received great consideration due to their probiotic potential for humans and animals, and important metabolic characteristics including, but not limited to, their ability to degrade indigestible carbohydrates, proteins, and fats, catabolism of amino acids, conversion of non-nutritive and/or unsafe substances present in food and also for producing diverse enzymes, vitamins, antibodies, exopolysaccharides, and various feedstock (Lee et al., 2021; Wang et al., 2021) some of which are detailed below.

Application of LAB in sustainable agriculture for food security

Sustainable agriculture aims at protecting the soil health and the consumer while securing environmental quality. As plant-microbial interactions are integral parts of agriculture, microbial-based agricultural practices are well-defined as briefly discussed below for the roles that they play in maintaining agricultural sustainability leading to food security, which has been defined as all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their food preferences and dietary needs for an active and healthy life (United Nations' Committee on World Food Security).

LAB as biocontrol agents

Nearly 60 billion USD worth of agricultural production (food crops) is lost due to fungal contaminations annually. According to the statistics of the Food and Agriculture Organization (FAO), global mycotoxin (naturally occurring toxins produced by certain fungi) contamination of food produces is around 25%. The ability of LAB to control fungal growth is well documented. Studies have shown that LABs produce substances like reuterin, bacteriocins, diacetyl, reutericyclin, organic acids, acetoin, hydrogen peroxide, etc., with antifungal and antibacterial activity against plant pathogenic microbes, (e.g. members of *Pseudomonas*, *Aspergillus*, *Debaryomyces* *Penicillium* genera), protect some plants from abiotic stress (the negative impact of non-living factors on living organisms in a specific environment), promote growth, perform as plant stimulants and also reduce the *E. coli* counts and their distribution in fermented food and vegetables (Raman et al., 2022).

Some strains of LAB like *Lactococcus lactis* and *Limosilactobacillus reuteri* are vigorous bacteriocin (small peptide units produced by ribosomes with the ability to inhibit the reproduction and growth of numerous bacteria) producers. They are detrimental to microorganisms and, hence, are promising candidates for developing antimicrobials. Further, biosurfactants (surface-active biomolecules) produced by LAB with antifungal, antibacterial and antiviral properties are used in sustainable agriculture (Rooney et al., 2020; Raman et al., 2022). Spoilage of food by molds mainly of *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* genera causes vast economic losses and their toxins such as aflatoxins, ochratoxin, patulin etc., endanger people's health and safety. Filamentous fungi, mostly aspergilli and fusaria, are the key causes of food, crop and animal feed spoilage. Acidic substances produced by LAB fermentation such as lactic acid, acetic acid, propionic acid, phenyllactic acid, and fatty acids reduce the pH level and cause intracellular acidic stress, creating an unfavorable environment for the growth of fungi in food. Control of fungal diseases of cereal crops is important to ensure food and feed safety. Few examples of employing LAB as biocontrol agents in various crops as reported by Li et al. (2020), Raman et al. (2022), and Zhao et al. (2022) are below listed.

01. Controlling Fusarium head blight and pathogenic *Zymoseptoria tritici* and ascomycete fungi causing septoria leaf blotch in wheat
02. Reducing toxic agents produced by filamentous fungi in wheat and maize grains
03. Controlling fruit rot of jackfruit by *Rhizopus stolonifer*
04. Controlling blue mold infection in fresh foods
05. Bio-controlling activity agent toxigenic fungi in table grapes
06. Reducing mycotoxin content in viticulture
07. Antagonism of *Lb. plantarum* species against necrotrophic fungi (*Botrytis cinerea*)
08. Antifungal activity of *Lb. plantarum* IMAU10014 against citrus green rot
09. Antifungal activity of *Lb. pentosus* against filamentous fungi and yeast pathogens
10. Edible films containing 2% LAB prevented banana blackening and prolonged shelf-life
11. Metabolites of LAB neutralized mycotoxin levels and inhibited pathogenic fungal populations in fruit and vegetable crops

Moreover, bio-pesticides like subspecies and strains of *Bacillus thuringiensis*, which are safe, eco-friendly and target specific are receiving huge interest as an alternative to conventional chemical pesticides. For example, metabolites produced by *Lb. sakei* and *Lb. curvatus* tend to kill nematodes (very small, slender worms) and LAB fermented dairy products along with commercial insecticides have elevated the insecticidal activity of the agents (Al-Mahin et al., 2012).

Biostimulants and biofertilizers of LAB origin

Plant growth stimulation and resistance development to abiotic stress by *Lc. lactis* and *Lb. plantarum* is well supported by several authors including Strafella et al. (2021) and Raman et al. (2022). The LAB species are also known to increase crop yield by solubilizing phosphates, accelerating mineral uptake, enhancing organic matter catabolism, decomposing, and bio-stabilizing waste of animal and plant origin. These processes improve the agronomic value of soil by assimilating organic matter, increasing soil structure, aeration, and fertility, neutralizing alkalinity, and endorsing soil moisture retention (Strafella et al., 2021; Raman et al., 2022).

Applications of LAB in food processing, improving food quality and safety

The long history of safe use, GRAS status and, favourable metabolic activities while growing in foods make LAB the most frequently used genera for the production and preservation of foods. As a result, a range of starter, functional and bio-protective, cultures are commercially produced as below discussed.

Production of fermented foods

Fermentation is a first-born biotechnological technique employed in the food production process. Currently, it has become one of the main primary processes of the food industry. Earlier, fermentation was conducted employing undefined cultures with low efficiency and inconsistent quality of the final product. At present, selected, defined starter cultures are used in the industrial fermentations. The frequently used LAB genera in food fermentations include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, *Enterococcus,* and *Weissella*. The main application of LAB as starter cultures is for producing many varieties of fermented foods including dairy (e.g. cheese, yoghurt, fermented milk), meat, fish, fruit, vegetable and cereal products. The most frequently used starter cultures containing LAB are shown in Table 1.

Fermentation ensures a longer shelf-life by reducing microbial spoilage and the development of food toxins such as cyanogenic glycoside, glucosinolates, etc., enhancing safety by restraining the transfer of pathogenic microorganisms like *E. coli*, *Staphylococcus aureus* etc. while improving organoleptic properties (the features of food, that create specific experiences via the senses, e.g. taste, sight, smell, and touch) and digestibility. Fermentation also increases the activity of microbial enzymes such as amylases, lipases, proteases, and phytases. These enzymes modify the raw material through the hydrolysis of polysaccharides, proteins, and fat hence, while improving the nutritional value of the raw material by degrading anti-nutritive compounds, like phytic acid and tannins (Zapasnik, 2022).

Table 1. LAB used as starter cultures in fermented food products.

Product category	LAB species
Dairy products	
Cheese (Mesophilic starter)	*Lc. lactis* ssp. *Lactis, Lc. lactis* ssp. *Cremoris, Lc. lactis* ssp. *lactis* var. *diacetylactis, Leuc. mesenteroides* ssp. *Cremoris*
Cheese (Thermophilic starter)	*S. thermoplillus, Lb. delbrueckii* ssp. *Bulgaricus, Lb. helveticus, Lb. delbrueckii* ssp. *lactis*
Cheese (Mixed starter)	*Lc. lactis* ssp. *Lactis, Lc. lactis* ssp. *Cremoris, S. thermoplillus*
Yoghurt	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus*
Fermented milks	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus Lb. casei, Lb. acidophilus, Lb. rhamnosus, Lb. johnsonii*
Yakult	*Lb. casei* ssp. *Casei*
Acidophilus milk	*Lb. acidophilus*
Kefir	*Leuc. Mesenteroides Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *lactis var. diacetylactis, Leuc. menesteroides*
Fermented meat products	
Dry sausages	*Lb. sakei, Lb. curvatus, Lb. plantarum, Lb. pentosus, Lb. casei,P. pentosaceous, P. acidilactici*
Greek dry fermented sausages	*Lb. sakei, Lb. plantarum, Lb. curvatus, Lb. pentosus, Lc. lactis* ssp. *lactis, W. hellenica*
Fermented fish products	
Thai fish	*Lb. plantarum, Lb. reuteri*
Pickled fruits and vegetables	
Cabbage (Sauerkraut)	*Leuc. mesenteroides, Lb. plantarum, Lb. brevis, Lb. fermentum*
Cucumber	*Lb. brevis, Lb. plantarum, Lb. pentosus, Lb. acidophilus, Lb. fermentum, Leuc. Mesenteroides*
Olives	*Lb. brevis, Lb. plantarum, Lb. pentosus*
Fermented cereal products	
Sourdough	*Lb. brevis, Lb. hilgardii, Lb. sanfransiscensis, Lb. farciminis, Lb. fermentum, Lb. brevis, Lb. plantarum, Lb. amylovorus, Lb. reuteri, Lb. pontis, Lb. panis*
Kimchi	*Leuc. mesenteroides, Lb. plantarum, Lb. kimchii*

Note: *Lc. Lactococcus, Lb. Lactobacillus, Leuc. Leuconostoc, P. Pediococcus, S. Streptococcus, W. Weissella*

Source: Bintsis, 2018a

Food preservation and safety

Preservatives of biological origin (derived from plants, animals, and bacteria) are now becoming popular in the global food additive market, due to their natural origin, non-toxicity or low toxicity, high efficiency, and also their ability to preserve the original flavour of the food (Zhao et al., 2022). The LAB produce a range of biologically active metabolites with numerous antimicrobial activities (lactic acid, acetic acid, hydrogen peroxide, low molecular weight substances such as fatty acids, diacetyl, reutericyclin, reuterin, antifungal compounds like propionate, phenyl lactate, hydroxyphenyl lactate and bacteriocins), hence, are well known for their effective bio-preservative properties. The LAB also neutralize mycotoxin produced by filamentous fungi by producing anti-mycotoxinogenic metabolites with GRAS status received from US Food and Drug Administration (Zapasnik, 2022).

Functional food industry

The LABs own proven therapeutic properties that are essential for protecting and improving human health. The LAB increase the bioavailability of nutrients, produce compounds having antioxidant activity, and also synthesize vitamins (biotin, cobalamin, folates). Antioxidant activity is related to their ability to transform phenolic acids to biologically active forms, positive effect on the content of ascorbic acid, and conversion of individual diet components into group B and K vitamins.

A correlation between high antioxidant activity and anticarcinogenic properties of bacterial lysate is also reported (Zapasnik, 2022). Several LAB genera have also been recognized as good probiotics (the FAO/WHO defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host) and the global probiotics market is characterized by three main applications as listed below.

1. Probiotics in foods and beverages (e.g. dairy, non-dairy, cereal, baked food, fermented vegetable, fruit, and meat products)
2. Probiotic dietary supplements (e.g. single cell protein, nutraceuticals, specialty nutrients, and infant formula)
3. Animal feed probiotics (e.g. substitutes for antibiotics and growth stimulants).

According to Zoumpopoulou et al. (2018), the US and EU market value in these sectors was estimated to be over USD 1.8 billion and 630 million, respectively, in 2017. An increasing number of food and feed companies worldwide are, therefore, trying to utilize LAB in their products to meet customer demand, improve product quality and diversify their product lines. The extensively discussed probiotics of LAB origin include *Lb acidophilus*, *Lb. casei, Lb. fermentum*, *Lb. gasseri, Lb. johnsonii*, *Lb. paracasei*, *Lb. plantarum*, *Lb. rhamnosus,* and *Lb. salivarius*. The other probiotic LAB species include *S. thermophilus*, *Bifidobacterium lactis*, *B. longum* and *B. breve*. The beneficial effects of these probiotics are mostly strain-specific and include a contribution to the healthy gut microbiota, treatment of various gastrointestinal diseases, maintaining a healthy immune system, controlling pathogenic bacteria, supporting the health of the reproductive tract, oral cavity, lungs, skin, circulatory system and gut–brain axis (Hill, 2014; Bintsis, 2018a). Therefore, a large number of LAB are employed as commercial probiotic products with proven health-beneficial properties (Table 2).

Table 2. Commercial probiotic products available in the market.

Brand/trade name	Food type	Sources/ strains	Manufacturer company	Country
Aciforce	Freeze-dried product	*Lc. lactis, Lb. acidophilus, En. faecium, B.bifidum*	Biohorma	Netherlands
Actimel	Yoghurt drink	*Lb. casei immunitas*	Danone	France
Bacilac	Freeze-dried product	*Lb.acidophilus, Lb.rhamnosus*	THT	Belgium
Bififlor	Freeze-dried product	*Lb.acidophilus, Lb. rhamnosus, B.bifidum*	Eko-Bio	Netherlands
Hellus	Dairy product	*Lb. fermentum* ME-3	Tallinna Piimatööstuse AS	Estonia
Proflora	Freeze-dried product	*Lb acidophilus, Lbdelbrueckii* subsp. *bulgaricus, S.thermophilus, Bifidobacterium* spp.	Chefaro	Belgium
Provie	Fruit drink	*Lb plantarum*	Skanemejerier	Sweden
ProViva	Fruit drink and yoghurt	*Lb.plantarum*	Skanemejerier	Sweden
Yakult	Milk drink	*Lb. casei Shirota*	Yakult	Japan

Lc. Lactococcus, Lb. Lactobacillus, Leuc. Leuconostoc, P. Pediococcus, S. Streptococcus, En. Enterococcus, B. Bifidobacterium

Source: Mishra et al. (2015)

Industrially valuable products synthesized by LAB

The LABs produce a variety of valuable products including, but not limited to, organic acids, amino acids, short-chain fatty acids (SCFA), amines, bacteriocins, vitamins (folic acid, riboflavin, vitamin C, pyridoxal), nutraceuticals, low-calorie sugars (mannitol), bioactive compounds (exopolysaccharides, antioxidants), flavour substances (2,3-butanedione, 2,3-pentane-dione). As a result, in recent years, LAB have attracted more attention from science and industry and shown a diversity of extended applications in the food-related industries as discussed below.

Organic acids

Organic acids produced by LAB include lactic acid (main product), acetic, formic, succinic and citric which are of industrial value. Organic acids are extensively applied in numerous industries due to their versatile use as monomers and starting materials for biodegradable polymers, food supplements, and bio-based materials for bio-refinery (Wang, 2021; Ibrahim et al., 2021). Recently, lactic acid has gained more interest due to its utilization in the manufacture of poly lactic acid, which is a green polymer, a substitute for petroleum-derived plastics. It is also applied in medicine for the regeneration of damaged tissues and in sutures, repairs and implants. Moreover, lactic acid is used as flavourings, acidulants, buffering agents, and inhibitors of bacterial spoilage in a wide variety of processed foods and also in chemical, textile, and pharmaceutical industries. Different esters of lactic acid are frequently used as emulsifying agents in baking foods (Lee et al., 2021).

Bacteriocins

Antibiotic resistance is escalating and threatening humans and animals worldwide. Bacteriocins synthesized by LAB could be used as an alternative to antibiotics (Wang, 2021; Ibrahim et al., 2021). Bacteriocins of LAB origin are considered ideal for food industry applications based on characteristics including GRAS status, odourless and colourless nature, preserving the organoleptic qualities of food, easy clearance by the proteolytic enzymes, etc. One of the best-known bacteriocin is nisin, produced by LAB species including *Lactococcus lactis* ssp. *lactis* which are used as permitted food additives in nearly 50 countries around the globe, mainly in canned foods, dairy industry and also for controlling mastitis (Lee et al., 2021).

Exopolysaccharides

Extracellular polymers produced by microorganisms when composed of sugars are referred to as exopolysaccharides (EPS). The LAB have the ability to secrete biopolymers such as xanthan, gellan, dextran, pullulan, scleroglucan, etc. Due to their composition, structure, and physical properties, EPS are used in different food and pharmaceutical industries. For example, EPS improves the viscosity and texture during milk fermentation and these biopolymers act as thickeners, stabilizers, gelling, and viscosifying agents, emulsifiers, and fat replacers in low-caloric foods. The EPS are also recognized as prebiotics (natural substances in some foods that boost the growth of beneficial bacteria in the gut). Further, EPS prompt positive physiological reactions such as lowering cholesterol levels, reducing the formation of pathogenic biofilms and adhesion to epithelial cells and anti-carcinogenic, immune-modulating and prebiotic effects (Nath and Malik, 2016).

Application of LAB in food waste management

An amount of 1,300 billion tons of food waste (FW) per year is produced globally which is one-third of the total global food production (FAO, 2013). Thus, the global community is driving towards valorising FW while taking steps for its reduction. The FW possesses a huge potential for fermentation due to its vast yield and organic content. Among a number of FW fermentation methods such as fermentation towards Mixed Volatile Fatty Acids (VFA), butyric acid, acetic acid, and ethanol production, fermentation them into lactic acid using LAB is the method that requires the least interventions. Compared to chemical synthesis, lactic acid fermentation is a better technique to produce the acid due to the biological process' low energy requirements, low cost of substrates, and low reaction temperature (Wang et al., 2020; Raman et al., 2022). There was an excessive need for lactic acid of 1.2 million tons in 2016 with an anticipated annual growth rate of 16.2% till 2025 (Esteban et al., 2018).

In addition, it has been found that the use of undefined mix cultures of LAB can be more beneficial than using pure cultures due to reasons such as no sterilization requirements, improved adaptive capacity host environment, and the capability of utilizing mixed substrates (Nunes et al., 2017).

Application of LAB in the animal feed and livestock health sectors

Based on the functions of the additives used in the production of animal feed, they can be categorized into several groups such as nutritional, zootechnical, and sensorial (additives affecting the organoleptic properties of animal products). Among them, zootechnical animal feed addresses animal health through feedstuff. The use of LAB as a sensorial or nutritional feed is found to be occasional. Therefore, parallel to the use of LAB as probiotics for human consumption, their usage in animal products has drawn attention. It is true that most of the time LAB are employed as probiotics (*Lb. acidophilus, Lb. bulgaricus Lb. reuteri,* etc.) to enhance animal health and guard against illnesses. The majority of them fall under the category of "zootechnical feed" and focus on benefits for the gastrointestinal tract. The few most significant benefits of using probiotic LAB are enhancing growth rate, disease control, body weight management, and milk and egg production as summarized in Table 3.

Table 3: Benefits of LAB towards animal health.

Category	LAB species	Benefit	Reference
Ruminant	*Lactobacillus gasseri*	*In vitro* inhibitory activity against *Staphylococcus aureus*	Otero et al., 2006
Ruminant	*Pediococcus* spp., *Leuconostoc* spp	Act against urogenital infections	Wang et al., 2013a
Swine	*Lactobacillus sobrius*	Weight gain improvement & reduction in the amount of enterotoxigenic *E. coli* in the ileum, intestinal defenses & modulation of gut microbiota.	Jakava-Viljanen et al., 2008
Poultry	*Lactobacillus* spp.	Increase recovery of broiler chicks after infected with *Salmonella enteritidis*	Higgins et al., 2008

Future prospects of LAB

Due to the safety for human and animal consumption and metabolic versatility (including industrial-scale fermentations), LAB are gaining attention towards novel uses such as (industrial production of green chemicals, fuels, enzymes etc.). Analysing complete genomic information of LAB is at the top among the future prospects to enhance and improve the existing applications (Peng et al., 2022). Thus, one of the major focuses is using whole-genome sequencing (WGS) of individual LAB strains to evaluate the product's safety by identifying potential virulence genes and other factors that could have a detrimental influence on health through food. Two major examples of using genetic modifications in the food industry are the expanding substrate utilization performance of *Lc. lactis* by expressing α-amylase-encoding gene AmyA from *Streptococcus bovis* and improving the metabolite production of *Lb. plantarum* by co-expressing pyruvate carboxylase and phosphoenolpyruvate carboxykinase (Okano et al., 2007; Tsuji et al., 2013).

The use of bioinformatics in WGS is one of the most technically suitable & cost-effective recommended approaches. It can be used to increase understanding on LAB strains in the food safety (Mendoza et al., 2023). However, producing genetically modified LAB using WGS is severely constrained in terms of food applications due to laws and regulations as well as the unfavourable consumer perception of GMOs and their metabolites (Bintsis, 2018b). At the same time, being an alternative for chemical preservatives and many other food-related uses, the applications of LAB will be further enhanced and diversified in the coming decades with the use of WGS.

Necessity of employing LABs for the growth of Sri Lankan food industry

In this chapter, we briefly discussed numerous applications of beneficial LAB for the growth of agriculture and food industry, which plays a major role in sustainable economic development. However, Sri Lanka is still far from fully exploiting and utilizing these valuable microbial resources as extremely limited work has been done on the indigenous microbial strains with industrial prospects in Sri Lanka. As a result, Sri Lanka does not have a starter culture production facility or a functioning industrial culture collection for the country, apart from a depository of an industrially beneficial LAB and yeast isolates obtained from Sri Lankan dairies within the Industrial Technology Institute (ITI) under the Ministry of Industries. Consequently, most basic food industries (bakery foods, production of alcoholic beverages, production of fermented dairy/meat/vegetable foods such as yoghurt, curd, cheese, pickles and salami etc.) completely depend on imported, genetically modified, freeze-dried cultures and/or undefined starters with unpredictable performances. Our industries (dairy industry is on the top of this list) using imported cultures or undefined starters face huge economic losses, due to changes in import/export policies which restricts the importation of certain cultures after designing and establishing production processes, loss of viability and functionality during shipments, price, inability to utilize for multiple applications, not fitting for household and small scale food processing applications, unpredictable bottlenecks in global supplies like covid pandemic, etc.

Furthermore, none of our industries are utilizing microorganisms for the production of lactic acid, antibiotics, enzymes, vitamins, single cell proteins, amino acids, food grade/pharmaceutical solvents, chemicals, etc. which have huge economic prospects. Further, a very limited range of fermented foods are available for the Sri Lankan consumers and the functional prospects of those products are not scientifically proven. Hence, numerous opportunities exist for market oriented new product developments to enhance the colonic and general health status of the consumers and to satisfy the market demand through the introduction of locally developed indigenous functional starter cultures and their technological application process design (Rajawardana et al., 2020). Moreover, bovine mastitis accounting for considerable economic losses in dairy industry is widespread in dairy herds all over the world, including Sri Lanka. The overall prevalence of sub clinical mastitis in major milk-producing areas of Sri Lanka was 27.3%, and *Staphylococcus aureus* was the most common pathogen identified. Unhygienic milking practices, poor health management, and other environmental and farming conditions mainly lead to the high prevalence of mastitis (Ranasinghe et al., 2021). As discussed earlier, nisin produced by LAB is highly effective for controlling mastitis-causing pathogens like *Staphylococcus aureus*. In addition, being a country that produces tons of agricultural waste, and a country that anything can grow, bioremediation is mostly limited only to research so far. Therefore, there is an urgent need to fully and immediately exploit existing microbial biotechnologies to strengthen food value chain to maximize food security, safety and of waste management. This may result in better process control, enhanced food security, safety and quality, and reduction of economic losses.

Conclusion and perspectives

The LAB are the most commonly used microorganisms in the food industry. Their safe and favorable metabolic activities and GRAS approved beneficial metabolites are gaining huge attention hence, a range of starter, functional, bio-protective and probiotic cultures with desirable properties are commercially produced worldwide. The current advances in the biotechnology, nanotechnology and genetics, have provided new insights and applications for the LAB in numerous sectors including, but not limited to, agriculture, food, pharmaceutical and chemical industries. Although analyzing complete genomic information of LAB is at the top among the future prospects to enhance and improve the existing application. However, producing genetically modified LAB is severely constrained in terms of food applications due to laws and regulations of responsible authorities including FDA, European Union (EU) and World Trade Organization (WTO). The LAB being an alternative for chemical preservatives and many other food-related uses, the applications of LAB will be further enhanced and diversified in the coming decades with the use of WGS.

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CHAPTER 10

Pineapple value chain in Sri Lanka: Opportunities and challenges

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Abstract

Pineapple has been highlighted as an economic product with significant potential, increasing demand and grown in Sri Lanka for both the domestic and export markets. Though, the interest in agricultural sector has decreased over the last years in Sri Lanka, there are still opportunities for diversified and commercially oriented farming. As such, pineapple is a crop that offers high potential for further expansion. This chapter explains the significance of contribution and sensitivity analysis as tools to analyse and understand the value chain of a business in Sri Lanka as it helps to assess the impact of various factors on the overall performance and profitability of the value chain. Further, the chapter discusses how value margins change in major trading channels with the impact of COVID-19, considering the pineapple value chain in Sri Lanka. This chapter highlights the price variations among different trading channels (retail, supermarket, and export) and the imbalance in profit margins earned by each stakeholder in the Sri Lankan scenario.

Keywords: *Pineapple, Sensitivity analysis, Value chain*

Introduction

Sri Lanka is classified as a lower-middle income developing country, with agriculture accounting for 6.9% - 8.21% of GDP. The wide variations in precipitation, geography, and soil in Sri Lanka facilitate cultivation of a diverse range of crops. Out of the major plantation crops in Sri Lanka (i.e. tea, rubber, and coconut), the total land area under coconut is around 400,000 hectares, with 70% of the coconut acreage falling within the districts of Kurunegala, Puttalam, and Gampaha, which is typically known as the "Coconut Triangle". Pineapple is a crop that is commonly inter-cropped with coconut, and around 90% of the pineapple farms in the country is present within the "Coconut Triangle". The total pineapple area in Sri Lanka is ~5,543 hectares, with a total production of 40,000 MT/year, 70% of which is produced in the Kurunegala and Gampaha districts (Figure 1). The most popular variety grown here is 'Mauritius', a 'Queen' kind with spiny leaves, golden-yellow flesh colour, and an excellent flavour (Vidanapathirana et al., 2012).

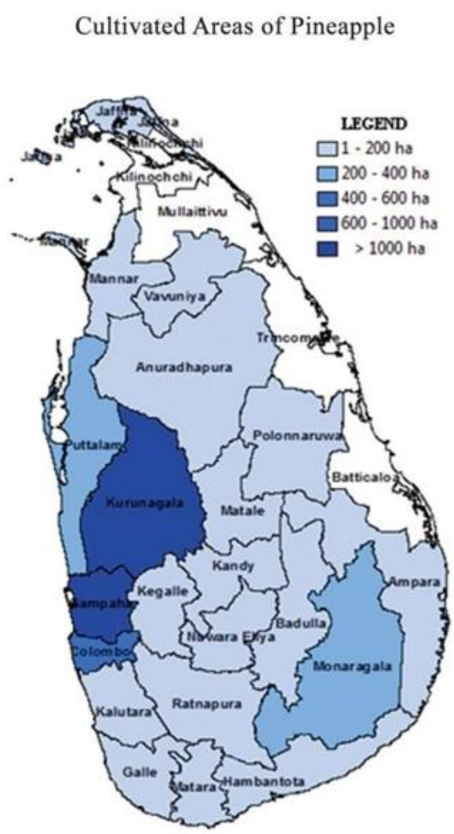

Figure 1: Pineapple expansion over the country
(Source: Department of Agriculture Sri Lanka)

At present, Sri Lankan production has reached the level where domestic demand for pineapple has been met. Pineapple marketplaces have emerged through a variety of trading channels, with final output provided *via* retail, supermarket, hotel, and restaurant outlets, while small-scale farmers cultivate pineapple for weekend fares and self-sufficiency. Moreover, Sri Lanka produces ~540,000 MT of fruits annually and exports both fresh and processed varieties to many destinations in the world. The pineapple trade channel contributes ~1000 MT to this. Around 65% of the fresh product is destined for the Middle East and the Maldives, while over 98% of the processed items are destined for the European market. United Arab Emirates, Saudi Arabia, Maldives, India, UK, Kuwait, India, Germany, Qatar, and Pakistan have been enlisted as top fruit and vegetable importing countries from Sri Lanka. It is noted that the Middle-East countries play a vital role in the Sri Lankan export trading channel mainly due to their geographical location and strict import requirements.
According to the records, selling prices of pineapple are low during peak season (May -June) and are high during off-peak season (December-January). Fruits can be obtained throughout the year by hormone applications, but the price will increase with the hormone application.

The value chain of pineapples

The value chain idea refers to the process of adding value to a product as it moves from input suppliers to manufacturers to consumers (Rathnayake & De Silva, 2022). Input supply, manufacture, assembly, transport, storage, processing, wholesaling, retailing, and utilization are typical value chain linkages, with exportation serving as a crucial stage for products bound for foreign markets (Vidanapathirana et al., 2012; 2020). The increase in value of a commodity as it travels through the supply chain to the final consumer is represented by a value chain. An examination of the supply and value chains in the agricultural sector might present major obstacles and inefficiencies. The major marketing channels in a typical pineapple value chain in Sri Lanka include input suppliers, growers, retailers, collectors, wholesalers, fresh and processed pineapple exporters, and consumers (Vidanapathirana et al., 2020). The vast majority of farmers sell their produce to collectors at the field level. Hence, many farmers can avoid having to pack or transport fruits when they sell the produce. Collectors supply pineapple to commission agents, as well as hotels, restaurants, and institutional buyers.

Wholesalers travel from all across the country to key pineapple-producing areas, where they buy from collectors or directly from growers. The last sellers in the chain are the retailers and they have to cover the transport expenses if they buy pineapple through commission agents, but not if they buy directly from wholesalers (Vidanapathirana et al., 2012).

Contribution Analysis

Contribution Analysis, also known as Margin Analysis, gets started by identifying potential trade channels throughout a specific value chain. For example, trading channels can be as follows.

Farmer → Collector → Wholesaler → Retailer → Consumer

Farmer → Collector → Wholesale → Supermarket → Consumer

Farmer → Collector → (Processor) → Exporter → Consumer

Each trade channel creates value for its stakeholders. This is expressed in terms of the price margin received by each stakeholder. This enables us to determine which trading channel is optimal for the farmer. Contribution Analysis is an examination of the associated costs and potential benefits of specific business activities or financial decisions. The goal is to determine if the costs associated with the change in activity will result in a benefit large enough to offset them. Instead of focusing on overall business output, the influence on the cost of producing a single unit is frequently used as a point of reference.

Sensitivity Analysis

Sensitivity Analysis determines how changes in specific variables or factors impact the outcomes or results of a value chain. It involves varying one or more input variables while keeping others constant to observe the corresponding changes in the output. Different values of an independent variable might affect the particular dependent variable under a given set of assumptions. This helps to understand the vulnerabilities, risks, and uncertainties associated with the value chain. In contrast to the supply chain of a commodity, a Sensitivity Analysis can determine the sensitivity of the profit in the value chain to changes in input costs or to the significance of a key raw material in case of a supply shortage. Lastly, it helps to understand variations in revenue and profit margin due to the changes in the demand for the final product.

Value chain constraints

Contribution Analysis and Sensitivity Analysis on crops such as pineapple are lacking in Sri Lanka, where it would have laid the groundwork for linking financial institutions and the actors involved in the trading channels, benefiting the entire agricultural sector. Further, prior studies of value chain analysis for pineapple have been done before the COVID-19 pandemic, and therefore, an analysis during and post-COVID scenario is vital to determine the post impacts of the pandemic. The marketing process from farm-gate level to retail must convey information not only about prices but also must ensure the reliability of the distribution network. As the distance between the farmer and the consumer widens, the level of credibility becomes more difficult to establish. Therefore, several interesting value chain issues have arisen in connection with the distribution of agricultural foods. Some of the major problems and constraints in the pineapple value chain in Sri Lanka include climate change (i.e. drought and high rainfall), less land availability, high investment, lack of good varieties, lack of planting material, high cost of inputs, lack of laborers, pests, weeds, and diseases, price fluctuations and lack of technical knowledge.

There are various impacts and shocking points in the supply chain of pineapple in Sri Lanka. Climate change and related rainfall and temperature variations are some of the main impacts on farming communities. This impacts the fruit quality/grades hence the farm gate price. Also, yield drops with droughts or heavy rains, and thus the scarcity increases the demand and farm gate prices. The pandemic affected the supply chain mainly in the transportation sector and the labour cost increased with travel restrictions and health issues. Further, due to market discoveries, customers had found new markets than the usual suppliers. Storage costs also had been increased in both fresh as well as value-added products since there were international travel bans. Even though the records are scarce for the statistics on the supply glut in pineapple, due to the high seasonality of production during glut periods, large quantities are wasted (Rajapaksha et al., 2021) and it directly affects the farmers as the price drops. In the year 2021, there was a significant decline in the Sri Lankan pineapple market after two years of growth, and the export performance of fresh pineapple had an upward trend from 1990 to 2004 and a downward trend from 2004 to 2012 (Rupasinghe et al., 2016). The current financial crisis is also impacting the pineapple supply chain. However, as per the farmers, there is a slight increase in the local pineapple market as imported fruits such as apples and grapes are expensive.

Before determining the revenues and margins along the value chain, it is critical to understand the cost of production (COP) borne by the primary actor, the farmer, in each value chain. In this chapter, the value chain primarily identified three categories of pineapple based on their weights (large size → above 1.2 kg, medium size → 0.8 kg – 1.2 kg, and small size → below 0.8 kg). The cost of production is calculated for 1 kg of pineapple (Cost of production = LKR 53.67 per 1 kg of pineapple in 2021).

Trade margins along the pineapple value chain

Besides knowing the cost of production which is a fundamental figure in each trading channel, the margins and costs borne by the other actors along the trading channels are also important for identifying areas for improvement.

Farmer → Collector → Wholesaler → Retailer

From a final retail price per 1 kg of pineapple at LKR 160.00, the farmer/producer gets LKR 74.67. This is composed of production costs of LKR 53.67 and a margin (i.e. profit) of LKR 21.00. From the final retail price, the farmer gets a profit of around 13.1%. On the next level, the collector purchases the pineapple (1 kg) at LKR 74.67, adding transport and handling costs of LKR 2.50 and adds his collector's margin of LKR 7.50, where the pineapple cost goes up to LKR 84.67. From the final retail price, the collector's contribution is around 6.25%, and from that, he makes a profit of around 4.7%. The wholesaler purchases the pineapple adding storing and handling costs of LKR 5.00 and adds a margin of LKR 10.00. From the final retail price, the wholesaler contributes 9.4% and takes his profit as 6.25%. The retailer purchases the pineapple from the wholesaler at LKR 99.67 and sells it at LKR 160.00. The contribution of the retailer to the final retail price is around 37.7% and his profit would be lower than this when considering the handling costs.

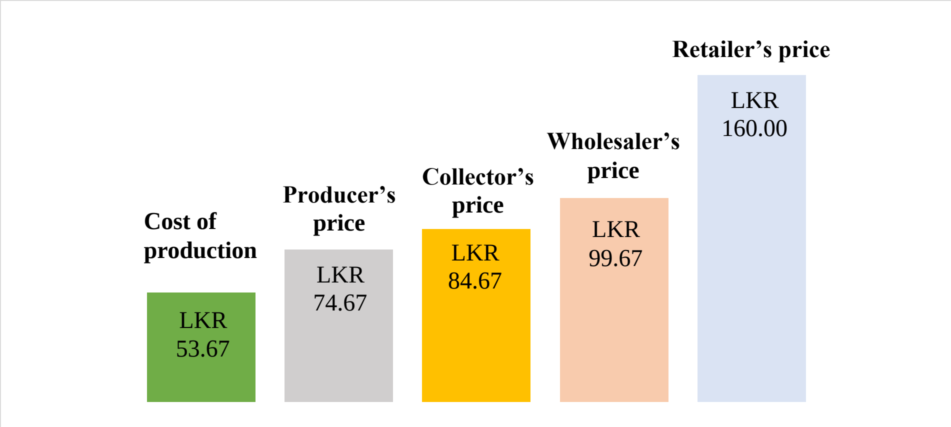

Figure 2: Price variation along the retailer trading channel per 1 kg of pineapple (based on the data collected during survey, 2021)

In conclusion, the contribution of the farmer/producer to the final retail price is around 46.67%, the collector contributes 6.25%, the wholesaler contributes 9.4% and the retailer contributes 37.7%. The total profit is shared between the farmer/producer (13.1%), collector (4.7%), wholesaler (6.25%), and the retailer (<37.7%).

Farmer → Collector → Wholesale → Supermarket

This analysis was done based on one particular supermarket chain (franchise). From the final supermarket price per 1 kg of pineapple which is LKR 175.00, the farmer/producer gets LKR 80.67. This is composed of production costs of LKR 53.67 and a profit margin of LKR 27.00. From the final supermarket price, the farmer gets a profit of around 15.4%.

On the next level, the collector purchases the pineapple (1kg) at LKR 80.67, adding transport and handling costs of LKR 4.50 and his profit margin of LKR 8.00. Thus, the pineapple goes for LKR 93.17. From the final supermarket price, the collector contributes 7.14% and takes his profit as around 4.57%. The wholesaler purchases the pineapple adding storing and handling costs of LKR 6.83 and adds a margin of LKR 10.00, where the pineapple goes to LKR 110.00. From the final price, the wholesaler contributes 9.61% and takes his profit as around 5.71%.

The supermarket purchases the pineapple from the wholesaler at LKR 110.00 and adds a margin of LKR 65.00 and sells it to the final customer at LKR 175.00. From the final supermarket price, the supermarket contributes to 37.1% and its profit would be lower than this when considering the handling costs.

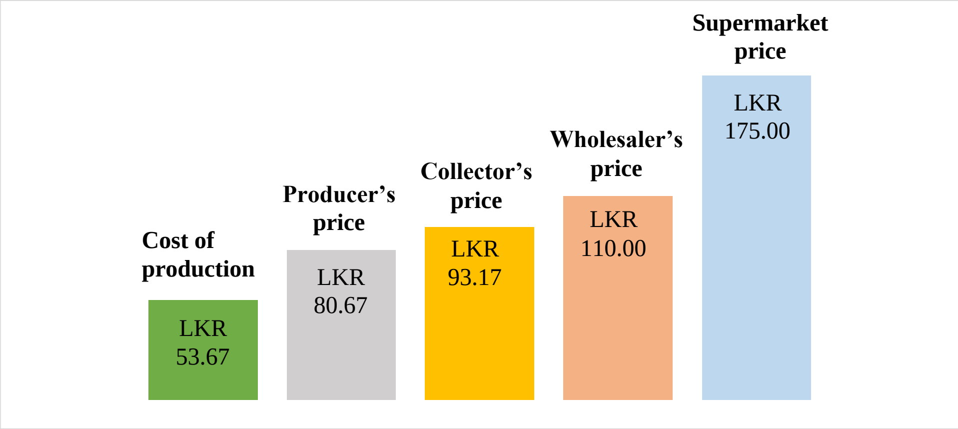

Figure 3: Price variation along the supermarket trading channel per 1 kg of pineapple (based on the data collected during survey, 2021)

In conclusion, the contribution of the farmer/producer to the final retail price is around 46.1%, the collector contributes 7.14%, the wholesaler contributes 9.61% and the supermarket contributes 37.1%. The total profit is shared between the farmer/producer (15.4%), collector (4.57%), wholesaler (5.71%), and the retailer (<37.1%).

Farmer → Collector → Exporter (Fresh)

From the exporter's final price per 1 kg of fresh pineapple of LKR 280.87, the farmer gets LKR 98.67. This is composed of a production cost of LKR 53.67 and a profit margin of LKR 45.00.

The collector purchases the pineapple at LKR 98.67 and adds transport and handling costs of LKR 6.50 and his profit margin of LKR 9.00. Thus, the pineapple goes at LKR 114.17 to the exporter. The contribution of the collector to the final retail price is around 5.51% and his profit is around 3.2%.

The exporter purchases the fresh pineapple adding transport and handling costs of LKR 86.70 and a profit margin of LKR 80.00 and sells it abroad at LKR 280.87. From the final retail price, the exporter contributes 59.35% and takes his profit as around 28.48%.

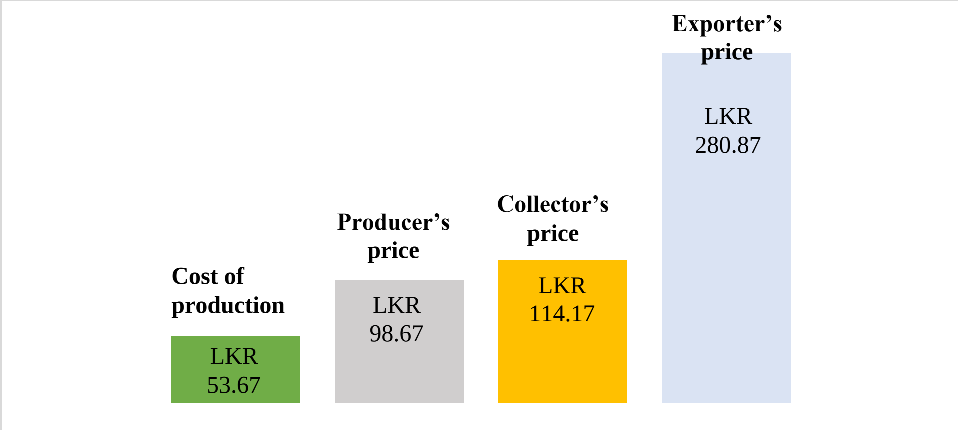

Figure 4: Price variation along the export trading channel per 1 kg of pineapple (based on the data collected during survey, 2021)

In conclusion, the contribution of the farmer/producer to the final retail price is around 35.13%, the collector contributes 5.51%, and the exporter contributes 59.35%. The total profit is shared between the farmer/producer (16%), collector (3.2%), and the exporter (28.48%).

Impact of climate change

Whilst farmers have been in a persistent struggle to adapt to the effects of climate change, the evolving climate variability in Sri Lanka is not conducive to positive agribusiness development within the nation. Average temperature in Sri Lanka is increasing, whilst precipitation patterns are also changing significantly leading to food losses due to maladaptation, which further accentuates food insecurity (Ministry of Environment Sri Lanka, 2022).

Sri Lanka has also been experiencing the effects of changing climate, ranking 30th in 2019 (and 23rd in 2000–2019) among 180 countries affected by climate change based on the Climate Risk Index for 2021 (Eckstein et al., 2021). Erratic rainfall patterns, droughts and water scarcity, floods, rising temperature had been adversely affected the pineapple production. Climate change has led to more unpredictable and erratic rainfall patterns, making it difficult for farmers to plan their planting and harvesting schedules. Further, prolonged droughts have detrimental effects on crop yields, particularly for rain-fed crops. Farmers, especially semi-subsistence farmers have been doing inter-cropping for decades which may be an appropriate method for small farmers who cannot risk losing their entire yield. This degree of moderate climate change hazard shockingly hampers the advancement of more commercial horticulture. This has, however, given an incentive for the farmers to shift towards climate-smart agriculture.

Impact of the COVID-19 pandemic

Sri Lanka's agriculture sector has so far been less affected by the COVID-19 outbreak, as the sector is only loosely integrated with global supply chains. However, many farmers still suffered severe losses due to control measures, and temporary import restrictions hampered trade. Less production of high-value commodities is likely, however not yet noticeable because of the lockdowns and disruption in the value chains.

Amid rising food security concerns, the government started a campaign to motivate people to start their home gardens. As a part of the campaign, packets of seeds were distributed free of charge in some areas. An estimated 2.1 million agricultural households are at risk of losing their livelihoods despite various measures taken by the government to safeguard agricultural supply chains. Though, the lockdown exempted farming operations and food supply chains from the beginning, implementation problems caused severe labour shortages and price collapse in wholesale markets. Increased levels of food loss and wastage due to market closure, blockages to transport routes, and declined demand were visible during the pandemic.

Impact of supply glut (Overproduction) and post-harvest losses

One would think that high production of an export crop would be great, but recently, overproduction of pineapple has caused prices to drop dramatically. However, marketing is governed by the law of supply and demand, and when supply exceeds demand, the price decreases. This is beneficial for local pineapple consumers because the excess is being dumped on the local market at a reduced price.

Another major concern in the pineapple value chain is the losses during the post-harvest handling process as it creates different degrees of quantitative and qualitative losses. Improper and non-scientific post-harvest practices and handling, as well as the lack of knowledge and awareness on many related aspects at the farmer level, appear to contribute to losses that eventually prevent full economic advantages from reaching the small-scale producer (Rajapaksha et al., 2021).

Financial risk

Sri Lanka has experienced strong and sustained growth in recent years for the past three decades. After growing 3.3% in 2018, the country's economic growth slowed down to an estimated 2.3% in 2019. This is primarily due to the aftermath of the terrorist attacks of April. According to the updated IMF forecasts from 14 April 2020, due to the outbreak of COVID-19, GDP growth is expected to fall to -0.5% in 2020 and pick up to 4.2% in 2021, subject to the post-pandemic global economic recovery. It is expected to pick up to 4.7% in 2020 and 2021, according to the latest World Economic Outlook of the IMF (April 2020). The political situation is still very unstable in the country. Moreover, the country's unemployment rate reached 4.9% in 2019 (4.4% in 2018), while the record of poverty has also increased. Even though the data are scare, shortage of chemical fertilizers in the previous year may also add adverse effects on pineapple production.

Conclusions and recommendations

In conclusion, the smallholders will continue to work traditionally when viewing agribusiness as a business. However, it requires changes at all levels such as; land planning and higher density planting, innovations such as drip irrigation, access to provincial funds, and advanced agrarian and administration services. Large-scale producers will be the driving force behind preparing to discover satisfying revenues for their high-quality products. Smallholders may not benefit in the same way from contemporary processing firms. High-end markets will provide greater opportunities in Sri Lanka, where sales will be through supermarkets, hotels, and restaurants. Pineapple may do extraordinarily well if costs are reduced and quantities are increased, as well as by supplying to reliable export markets, particularly in the Middle East. Furthermore, it may develop further into expanded product lines and goals to cover the comprehensive variations within the value chain.

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