



[Review Article]

Medicinal Plant-Based Immunostimulants for Sustainable Shrimp Farming: From Bioactive Compounds to Reproductive Enhancement



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Abstract

THE expansion of intensive shrimp aquaculture, driven by global food demand, is challenged by disease outbreaks and reliance on unsustainable practices like eyestalk ablation to induce reproduction in broodstock. This review explores the potential of medicinal plant-based immunostimulants as a sustainable alternative, with a specific focus on enhancing the reproductive performance of the Pacific white shrimp, *Penaeus vannamei*. We detail the complex endocrine regulation of crustacean reproduction, highlighting the roles of the X-organ-sinus gland complex, mandibular organ, and key hormones like methyl farnesoate. This foundation underscores the mechanistic drawbacks of eyestalk ablation, creating a compelling need for natural interventions. The paper then synthesizes evidence on how bioactive phytochemicals, including flavonoids, alkaloids, and sterols, can stimulate growth, immunity, and gonadal maturation. A thorough analysis of two promising plants, *Melastoma malabathricum* (Karamunting) and *Cyperus* spp., is presented. *M. malabathricum*, rich in lanosterol, acts as a precursor for steroid hormones, significantly accelerating ovarian development, increasing oocyte size, and elevating progesterone levels in *P. vannamei*. Conversely, *Cyperus* spp. contains methyl farnesoate, a crustacean juvenoid hormone analog, which promotes vitellogenesis, spermatogenesis, and molting, leading to advanced gonadal maturation stages and improved spawning performance. By compiling the molecular and endocrine evidence, this review positions these herbal extracts as effective, natural, and welfare-friendly alternatives to synthetic hormones and invasive techniques, offering a viable strategy to enhance reproductive efficiency and sustainability in shrimp hatcheries.

Keywords: Medicinal Plants, *Melastoma malabathricum*, *Cyperus rotundus*, Shrimp, Reproductive performance.

Introduction

The global effort to address food security challenges has driven numerous countries to increase their reliance on aquaculture to diversify food sources.

Owing to its ability to yield high-protein foods, aquaculture now represents the most dynamic and fastest-expanding branch of the food industry, experiencing an 8% annual growth rate [1]. As global demand for seafood escalates amid population

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expansion, aquaculture has gained international acclaim as a promising and sustainable alternative to traditional capture fisheries [2]. In recent years, the shrimp farming sector has emerged as a key contributor to global aquaculture, with production levels rising significantly, now approaching three million tons annually. This progress is attributed to the adoption of intensive cultivation methods, including increased stocking densities and the deployment of superior quality post-larvae [3]. Ensuring successful shrimp maturation and the production of high-quality seed is fundamental to sustaining the aquaculture sector amid increasing international consumption needs [4].

The practice of increasing fish density in restricted environments to satisfy the expanding demand has caused a rise in organic pollution, leading to a decline in water quality. This imbalance in important water conditions, such as oxygen concentration and pH, compromises fish well-being, inducing stress and increasing their susceptibility to diseases [1] as shown in the graphical abstract.

The aquaculture sector has employed various approaches to decrease or prevent mortality caused by different diseases. One common method involves using herbal extracts to boost the immune system. Among these approaches, enhancing the immune condition of farmed aquatic species through immunostimulants is prominent. In aquaculture practices, herbal extracts, phytochemicals, and plant-derived secondary metabolites serve as crucial feed additives that stimulate both specific and nonspecific immune responses [5]. Over the past few years, there has been a growing trend toward using botanical extracts in aquaculture as an alternative to conventional chemical agents. These plant-derived additives are widely acknowledged for their role in promoting weight gain and improving feed conversion in aquacultured fish [6]. Furthermore, these compounds serve as an economical and naturally safe source of chemicals. The bioactive constituents of plant materials contribute to fish nutrition by improving growth performance, increasing feed consumption, stimulating digestive enzyme secretion, strengthening immune defense, and offering antimicrobial and antioxidant protection [7].

Phytochemical-based herbal extracts contain diverse bioactive components like alkaloids, flavonoids, phenolic compounds, steroids, and essential oils. These substances have been shown to support multiple physiological roles in aquaculture, including promoting growth, stimulating immunity and appetite, reducing stress, improving reproductive health, and offering antimicrobial benefits in fish and shrimp farming [6, 8]. Unlike synthetic steroids, antibiotics, and other chemical substances, herbal-based compounds rarely produce negative side effects. They are economically accessible and do not

build up in living tissues, as they degrade easily and pose minimal environmental risk [9]. Medicinal plants, known for their limited adverse effects on organisms, including those in aquatic environments, are frequently employed in healthcare and nutrition to boost growth, support egg and larval survival, and aid in generating populations with specific or uniform sexual differentiation [10] (See Fig.1).

The Aquaculture Significance of Penaeus vannamei

Thailand, Vietnam, Indonesia, and other Southeast Asian countries initially focused on *P. monodon* aquaculture, but from around 2001–2002, there has been a widespread shift toward the farming of white-leg shrimp (*Penaeus vannamei*), attributed to the improved availability of SPF and SPR broodstock [11]. The white leg shrimp (*P. vannamei*) is considered ideal for semi-intensive farming systems, owing to the accessibility of pathogen-free broodstock. Nonetheless, optimal production hinges on rigorous biosecurity measures, precise water quality regulation, and a high level of technical proficiency. [4]. Native to the Pacific shores of Mexico and stretching through Central to South America as far as Peru, *Penaeus vannamei* is commonly recognized as the Mexican white shrimp or white-leg shrimp. It predominantly occupies muddy seabeds, dwelling at depths up to 75 meters. Characterized by its pale grey-white hue, wild female specimens can grow up to 120 grams, whereas males typically weigh between 60 and 80 grams. This shrimp species is known for its preference for clay-rich loamy sediments [11].

At 30°C, the species displays markedly improved growth relative to 25°C, with the ideal temperature window ranging from 30°C to 34°C; growth is almost entirely suppressed at 20°C. It endures salinity from 0 to 50 ppt, although steady growth is maintained between 10 and 40 ppt. The organism is capable of growing in freshwater conditions, yet its development is hindered below 10 ppt salinity. A pH range of 7 to 9 is tolerable, and peak growth occurs at pH 8.0. Dissolved oxygen levels above 4.5 ppm are essential for optimal biological function. Enhanced growth has also been observed in turbid water containing flocculated particles exceeding 0.5 microns, likely due to increased nutrient availability from algae and bacteria. It is crucial to keep ammonia nitrogen levels under 0.1 ppm and nitrite nitrogen below 1 ppm to avoid harmful effects [11]. The species *P. vannamei*, characterized as an omnivorous scavenger, is less aggressive and less carnivorous relative to *P. monodon*. Their food consumption tends to peak during evening and nighttime periods. Food retention within their gastrointestinal tract lasts between 2.2 and 5 hours and digestion occurs under moderate acidity, with pH values of 5.5 to 7.0. When cultured in confined conditions, the growth rates of *P. vannamei* closely match those of *P. monodon* up to a size of 20 grams,

after which their growth slows significantly. Depending on stocking density, shrimps reach the 20-gram mark within approximately 100 to 120 days. [11].

Aquaculture shrimp production is predominantly comprised of *Penaeus vannamei* (*P. vannamei*), accounting for 80% of total output. Despite the rapid expansion of cultivated shrimp volumes in recent decades, disease outbreaks have led to estimated yearly financial losses of about one billion US dollars since the early 1990s. [12]. The farming of *Penaeus vannamei* has expanded globally due to several favorable traits. It exhibits growth rates comparable to *P. monodon*, reaching weights of 20 grams in under five months. Its less aggressive behavior allows for cultivation at very high stocking densities, up to 150 individuals per square meter. Additionally, this species can tolerate a broad salinity range from 0.5 to 45 ppt and withstands low temperatures down to 15°C. It also benefits from requiring feeds with relatively low protein content (20–35%). Furthermore, *P. vannamei* is straightforward to breed, facilitating successful domestication and production of specific pathogen-free (SPF) stocks. The commercial availability of SPF and disease-resistant stocks enhances its appeal. Selective breeding programs aimed at generating specific pathogen-resistant (SPR) broodstock are facilitated by its short generation time and ease of captive reproduction. Recently, aquaculture has prioritized improving growth and artificial breeding as key objectives [13].

Reproductive Biology of Crustaceans

Reproductive Mechanisms and Hormonal Interaction

Crustaceans represent a vital component of the fisheries and aquaculture sectors. Nevertheless, significant gaps persist in our understanding of the physiological processes that govern their reproduction. For aquaculture practices to become independent of wild-caught broodstock and natural seed, mastering reproductive control in captivity is crucial [14]. This underscores the importance of fully elucidating reproductive mechanisms in economically valuable crustacean species. Our research has predominantly focused on species such as the freshwater prawn *Macrobrachium nipponense*, the giant freshwater prawn *M. rosenbergii*, and the kuruma prawn *Penaeus japonicus* to investigate gametogenesis and reproductive cycles. We have compiled comprehensive data on molting hormone fluctuations and vitellogenin levels in these and related species. This review highlights findings from our laboratory within the broader context of current knowledge on crustacean reproductive biology [15].

Due to their substantial biological significance and strong market demand, crustaceans such as shrimps, prawns, crabs, and lobsters are economically valuable. This has encouraged

scientists to establish breeding strategies for these animals, achieving notable advancements so far [16]. The reproductive control in crustaceans, governed by the neuroendocrine system, shows similarities to that of fish but also exhibits some distinct variations. Being invertebrates, their reproduction depends on both external and internal environmental cues. Reproduction in crustaceans is controlled by the hormones secreted by the glands present in different parts of the body [17].

X-organ: Found within the eyestalks, the neurosecretory organ contains the X-organ, a group of neurons located in the medulla terminalis. These neurons project their axons to the neurohaemal organ, creating the sinus gland, a hub for hormone storage and secretion. As the sinus gland holds and releases neurohormones produced by the X-organ's neurons, the entire system is termed the "X-organ–sinus gland complex". Crustaceans produce several neurohormones, such as Ovary Inhibiting Hormone (OIH/GIH) and Molt Inhibiting Hormone (MIH), which are crucial for reproduction and molting regulation, respectively. MIH works by blocking the function of molten-stimulating hormone (MSH) in a feedback loop known as bihormonal control. Additional hormones include the Light Adapting Hormone (Distal Retinal Pigment Hormone) for visual adjustment, Crustacean Hyperglycemic Hormone (CHH) for glucose regulation, Erythrophore Concentrating Hormone for pigment cell activity, and Neurodepressing Hormone for neural modulation. Both OIH and MIH are directly involved in reproductive functions, with MIH specifically inhibiting MSH to maintain molting cycles [18].

Growth Stimulating Hormone (GSH), Molt or Gonad Stimulating Hormone (MSH).

GSH is produced in the thoracic ganglia after being stimulated by GSHRF, which originates from neurosecretory cells in the supra-oesophageal ganglia. In *Procambarus borverii*, this hormone is stored in the sinus gland [19]. Its key roles are enhancing gonad growth, supporting gamete production, conserving energy for spawning, and preparing the organism for breeding. As a secondary function, GSH combines with other components to regulate ions and physiological processes related to reproduction, employing a modulator-activator-inhibitor system that can trigger the release of GIH or MIH from the X-organ and sinus gland [20].

Y-organ: The Y-organ is situated in the front part of the body within the antennary or maxillary segment. It releases ecdysone, a steroid-based molting hormone (crust ecdysteroid). Its secretory function is regulated by two opposing mechanisms: stimulation from the mandibular organ and inhibition by molt-inhibiting hormone (MIH), which is secreted

by the X-organ to suppress the Y-organ's activity [21].

Mandibular organ: In crustaceans, the mandibular gland functions as an endocrine organ by producing methyl farnesoate (MF), a hormone present in species such as lobsters, crayfish, and shrimp [17]. MF plays a crucial role in reproduction by promoting ovarian development, aiding in vitellogenesis, and inducing the Y-organ to synthesize ecdysone, thereby significantly influencing their reproductive processes. Crustaceans rely on the androgenic gland, rather than the testes, for hormonal activity, as it produces androgenic hormone responsible for male traits and sperm production. Interestingly, excising this gland from a male can cause it to develop female reproductive organs, while introducing it into a female can trigger male features, highlighting its pivotal role in determining sex [17].

The ovarian maturation and feedback mechanism of the X-organ-sinus gland complex regulates reproductive processes in crustaceans [22]. Ovarian development is influenced by the hormones OIH (Ovarian Inhibiting Hormone) or GIH (Gonad Inhibiting Hormone) secreted by this complex. When MIH (Molt Inhibiting Hormone), also termed VIH (Vitellogenesis Inhibiting Hormone) due to its role in suppressing vitellogenesis, is inhibited, the ovary undergoes rapid development, vitellogenesis, and maturation. To promote ovarian maturation in hatcheries, eyestalk ablation is performed, which removes the X-organ-sinus gland complex, eliminating the inhibitory effects of OIH and MIH/VIH on ovarian growth in shrimps, prawns, and crabs [23].

Processes of Oogenesis and Spermatogenesis

Oogenesis

In *Penaeus japonicus*, the ovaries are sac-shaped and lobulated, situated bilaterally along the dorsal side, stretching from the top of the carapace to areas above the hepatopancreas. As maturation progresses, the ovaries expand anteriorly towards the cephalothorax and posteriorly toward the tail [24]. The oviducts emerge from each ovarian lobe and open externally through ovipores located at the base of the third pair of pereopods. During oogenesis, the oogonia multiply via mitosis and increase in number. At a particular stage, these cells enter meiosis and transform into primary oocytes, halting at Prophase I. During this stage, they accumulate yolk substances and proceed to mature. Once meiosis resumes, the process continues until ovulation occurs. In *Macrobrachium nipponense*, this developmental sequence has been histologically examined and categorized into seven distinct stages [15].

The oogonia stage involves oogonia located in the ovarian core, featuring large nuclei and sparse cytoplasm, which proliferate through rapid mitotic

divisions [25]. During the meiotic stage, primary oocytes emerge from oogonia after mitotic proliferation, entering meiosis with a large nucleus and minimal cytoplasm, containing multiple chromosomes. In the previtellogenic stage, oocytes pause at Prophase I and grow in size, displaying one or two prominent nucleoli and basophilic cytoplasm due to RNA accumulation. Endogenous vitellogenesis is marked by PAS-positive cytoplasmic granules, likely glycoproteins, while follicle cells begin surrounding the oocytes. Exogenous vitellogenesis involves lipid and yolk globule accumulation, significantly enlarging oocytes as vitellogenin, synthesized in the hepatopancreas or fat body, is transported from the hemolymph [26]. During maturation, a thin membrane separates oocytes from follicle cells, and meiosis resumes with germinal vesicle breakdown (GVBD), relocating the nucleus to the animal pole. Ovulation releases yolky primary oocytes, followed by spawning, fertilization, and meiotic divisions with polar body extrusion [27]. In species like the kuruma prawn, a cortical reaction forms a fertilization envelope, derived from vitelline envelope granules and cortical vesicles. Penaeids exhibit distinctive rod-shaped cortical structures that discharge upon spawning, creating a protective jelly layer around the eggs [27].

Spermatogenesis

Situated on the left and right flanks, the testes are made up of multiple small lobules. On either side, the spiral-shaped vas deferens connect to the testes and terminate in spermatophores that discharge through the fifth pair of pereopods [20]. The processes of spermatogenesis and spermiation proceed through five sequential stages.

Spermatogenesis begins with the spermatogonia stage, where spermatogonia undergo mitotic division within the seminiferous tubules to increase in number [28]. Next, in the primary spermatocyte stage, some spermatogonia enlarge and differentiate into primary spermatocytes, which have a round, darkly stained nucleus. These primary spermatocytes then undergo the first meiotic division to form two smaller secondary spermatocytes, which retain a similar shape but are half the size [29]. Subsequently, during the second meiotic division, each secondary spermatocyte divides into two spermatids, producing four spermatids per primary spermatocyte, with nuclei that appear irregular and sickle-shaped. Finally, in the spermatozoan stage, spermatids transform into mature spermatozoa without further division, as the nucleus condenses and excess cytoplasm is shed, resulting in the distinctive pinhead-shaped sperm cells [30].

Final Maturation, Spawning, and Mating Interrelationships

Female sexual maturation in penaeid shrimp is characterized by two key processes, namely

vitellogenesis and the ultimate maturation of oocytes. The final maturation of ovarian oocytes occurs immediately prior to spawning, which for *P. japonicus* typically occurs following dusk [31]. The sequence consists of two stages: first, the maturation of ova, and second, the breakdown of the germinal vesicle (GVBD) as preparation for fertilization after the eggs are released. Ovulation is marked by the movement of nuclei, which have contracted in the late pre-maturation phase, toward the edge of the oocyte cytoplasm [20]. During the final stage of the maturation cycle, meiotic metaphase becomes halted and can be observed immediately beneath the cytoplasmic membrane of the ovarian oocyte, signifying that GIRD concludes following ovulation [20]. In *P. japonicus*, the nucleus in the ovary undergoes rapid condensation shortly after sunset, with meiotic metaphase occurring between 2100 and 0300 hours. This indicates that germinal vesicle breakdown (GVBD) begins in the evening and concludes overnight, spanning several hours [32].

Once released from the female gonopore, the mature eggs, which remain in metaphase, undergo fertilization by sperm discharged into the seawater from the spermatophore located in the thelycum [33]. It has been well documented that immediately after spawning in penaeid shrimp, small aggregations of immature eggs often become attached to the spawning tank surfaces, along with isolated fertilized eggs [33]. The histological analysis of the maturation process indicated that eggs were enveloped by a multitude of immature oocytes. These immature oocytes, present in early and late perinucleolus stages, are connected, forming a surrounding coat around the egg. In the early pre-maturation stage within the ovary, this immature egg coating remains unformed [31]. This indicates that after the final phase of pre-maturation, there is a rapid proliferation of immature oocytes, which then promptly encase the mature oocytes immediately before spawning. The immature egg coating possibly serves to lubricate the mature oocytes as they exit the ovary [31]. To stimulate spawning, ripe females are transferred during the night period (2100 to 0300) from a dark spawning tank to smaller tanks that are brightly lit [34]. This implies that ripe female individuals, having undergone final maturation in favorable settings, can still proceed to spawn despite experiencing suboptimal environmental conditions [34].

Final maturation can be triggered by various factors, including dietary supplementation with eicosapentaenoic acid (which serves as a precursor to prostaglandin), exposure to temperature shock, periods of darkness, seawater filtration or ultraviolet irradiation, as well as through mating and the transfer of spermatophores, particularly in penaeid shrimp that possess an open thelycum [35]. After spawning begins, it proceeds continuously as female shrimp

release successive batches of eggs from their ripe ovaries alongside sperm from the spermatophore into the surrounding seawater, enabling fertilization. As a result, females need to repeatedly go through the cycles of molting, mating, and sexual maturation to spawn multiple times during their lives [36].

Interrelationships among final maturation, spawning, and mating

In closed thelycum species, mating accompanied by spermatophore transfer occurs before the onset of vitellogenesis. In the spent kuruma prawn (*Penaeus japonicus*), egg-yolk protein (vitellin) begins to accumulate in developing oocytes at the yolk-granule stage approximately one month after spawning, even in the absence of mating. Even after copulation and the transfer of spermatophores in the late autumn to early spring season, female *P. japonicus* possessing a closed thelycum do not reach maturity for several months [37]. These findings suggest that in species with a closed thelycum, mating does not directly speed up the process of vitellogenesis. In *P. vannamei* exhibiting an open thelycum, the mating process involving spermatophore transfer occurs in close association with ovarian development, specifically when oocytes reach the mid-stage of pre-maturation, and happens directly before spawning [37]. Ripe females containing spermatophores exhibited final maturation and spawning within two hours post-mating, implying that such behavioral events are essential for inducing final maturation in species with an open thelycum. Conversely, ripe *P. vannamei* females that remained unmated with mature males did not undergo spawning, despite a month-long observation [38].

Factors regulating final oocyte maturation and spawning

Certain nutrients necessary for final maturation and spawning are available only when various foods are combined, which can stimulate these processes [38]. To illustrate, all five female *P. monodon* provided with a diet consisting of frozen squid, frozen shrimp, and fresh crab underwent spawning, compared to only six females out of twenty that spawned when fed individually on frozen squid, frozen shrimp, or fresh crab [39]. Out of twenty females receiving a uniform diet, four had ovaries that were nearly ripe with oocytes in the early maturation stage, but these did not progress to complete maturation, resulting in unsuccessful spawning [40].

A pelleted diet enriched with vitamin E and fish oil was designed to promote and hasten the final maturation and spawning process in *P. monodon* [41]. In *P. monodon*, feeding a pelleted diet high in vitamin C and containing fish oil promoted and hastened the completion of maturation and spawning [12]. It is considered that a high concentration of vitamin E or C can protect eicosapentaenoic acid

(EPA), an important precursor of prostaglandins necessary for reproduction in crustaceans and various invertebrate species [12]. Oxidative reactions during digestion or lipid peroxidation inside shrimp can affect EPA, an important fatty acid in crustaceans. Spawning shrimp need to acquire EPA because it acts as a precursor for prostaglandins necessary for their final maturation and reproductive process. High concentrations of vitamins E or C may facilitate the conversion of active EPA into prostaglandins [42].

The secretion of prostaglandin in shrimp, induced by UV-treated seawater, seems to stimulate the completion of maturation and subsequent spawning [43]. Temperature shock or seawater filtration are known to induce the completion of maturation and the spawning activity in *P. japonicus* and *P. monodon*. [42]. From these observations, I deduce that final maturation and spawning may be induced by prostaglandin originating from EPA in the food and whose secretion is accelerated by UV-irradiated seawater, temperature shock, or filtration of seawater in penaeid shrimp [44]. In contrast, the act of mating, accompanied by spermatophore transfer, directly initiates the last stages of maturation and spawning in *P. vannamei* with an open thelycum [45]. Prostaglandin secretion, crucial for initiating the last stages of maturation and spawning in crustaceans, could be activated by mating behavior along with the transfer of spermatophores in open-thelycum species [4, 38]. Following unilateral eyestalk removal, ripe female *P. japonicus* exhibited swift spawning within half an hour [46]. The findings imply that spawning in penaeid shrimp might be hindered by compounds, possibly a spawning-inhibiting hormone produced by the eyestalk X organ-sinus gland complex.

Reproductive Rhythms and Environmental Factors

A majority of animal species have a defined breeding season determined by their ecological environment. These periodic reproductive alterations, termed “reproductive rhythms,” occur in response to seasonal changes. Such rhythms are thought to be influenced by external factors, including photoperiod, aquatic temperature, food supply, and other environmental conditions. In crustaceans, many species exhibit these seasonal reproductive trends, with significant attention given to how environmental factors affect them [47]. This paper highlights some research outcomes centered on the freshwater prawn *M. nipponense* found in Lake Kasumigaura [48].

Annual rhythms

The timing of annual biological cycles varies across species, and even within the same species, different strains may display distinct rhythms influenced by their environments. Generally, species inhabiting mid to high latitudes breed from spring to autumn, while those in lower latitudes have longer breeding seasons that lack a clear seasonal

correlation. In deep-sea organisms, breeding is commonly observed during winter months. In the case of the freshwater prawn from Lake Kasumigaura, individuals typically live close to three years and reproduce during the summers of their second and third years. [49, 50] studied the development of female gonads over a year in this lab, revealing that the gonadosomatic index (GSI: gonad weight multiplied by 100 and divided by body weight) increases rapidly from April through June, a period characterized by ovaries full of oocytes undergoing exogenous vitellogenesis. Between June and August, the gonadosomatic index (GSI) remains elevated, with nearly all prawns actively spawning eggs. During this timeframe, spawning occurs continuously among individuals. Most prawns with oocytes that have finished vitellogenesis also possess ovulated follicles [51]. Conversely, those with oocytes still in the vitellogenic phase exhibit a variety of reproductive stages within these oocytes. By September, prawns brooding spawned eggs become uncommon, and GSI values decline. Atretic eggs appear along the ovarian periphery, while oogonia and immature oocytes, ranging from pre-meiotic to pre-vitellogenic stages, occupy the central ovarian regions. From October through March, GSI values remain low. During October and November, oogonia proliferate mitotically within the ovaries, increasing immature oocyte counts; however, these oocytes typically degenerate before reaching early vitellogenesis, marking a phase of ovarian inactivity. Between December and March, immature oocytes resume development, advancing into early vitellogenic stages in preparation for the upcoming breeding season [48].

From April to June, as breeding season nears, vitellogenesis accelerates, causing rapid yolk accumulation in the oocytes. Between June and August, spawning occurs multiple times, with individual fish releasing eggs on several occasions. From September to November, reproductive activity declines, halting spawning and resulting in the degeneration of remaining mature oocytes in the ovaries. Finally, from December to March, during the pre-vitellogenic phase, oocyte proliferation resumes but does not progress into the early stages of vitellogenesis [49, 50].

The gonadosomatic index (GSI) in male freshwater prawns rises from April to June. Histological studies of the testes demonstrate that spermatogonia undergo mitotic division beginning in April, followed by spermiation in May. During June to August, the GSI remains high, with seminiferous tubules densely populated by sperm, reflecting active spermiation [52]. From September until March, GSI values steadily decline. Some spermatogonia are still present in the seminiferous tubules but cease proliferation and gradually deteriorate. In conclusion, male reproductive activity intensifies from April,

reaching its peak spermiation phase between June and August, and sperm production stops from September through March, similarly to the pattern observed in females [53].

Environmental factors

The timing of reproductive activities over the course of a year is predominantly determined by various environmental cues. Species adapt to their habitats to maximize reproductive success, typically aligning breeding periods with optimal conditions such as peak prey availability or favorable temperatures. In regions with moderate latitudes, substantial seasonal changes in water temperature and day length exert a strong influence on reproductive patterns. In contrast, tropical species experience relatively constant temperature and photoperiod, so their reproductive cycles are mainly dictated by factors like seasonal rainfall and food supply [48]. The freshwater prawn experiences oogenesis and spermatogenesis between April and June, approaching the breeding season. During this period, the average water temperature rises from 10°C to 18°C, while day length extends from 13 hours of light (13L) to 14 hours of light (14L). Conversely, at the conclusion of the breeding season in September, water temperature declines from 26°C to 22°C, accompanied by a reduction in day length from 14L to 13L. In controlled laboratory settings, it was hypothesized that the onset and cessation of the breeding season are influenced by water temperature and day length [54]. Consequently, multiple experiments were conducted to examine the effects of these factors. To identify the parameters triggering reproduction, six groups of *Macrobrachium nipponense* were raised under different combinations of daylength (12L and 15L) and water temperatures (10°C, 16°C, 22°C), and ovarian development was monitored from April to June. Initially, the gonadosomatic index (GSI) values were low, and yolk deposition in oocytes had yet to begin [55].

In females maintained at 22°C, an increase in the gonadosomatic index (GSI) was observed, with egg-brooding individuals emerging within one month, independent of photoperiod. By the end of two months, nearly all specimens were engaged in brooding. Conversely, in the 16°C group, GSI showed a slight rise after one month, although no egg-brooding was detected, and this was also unrelated to day length [56]. However, signs of yolk protein incorporation in the ovaries were evident, with oocytes progressing through secondary vitellogenesis. Egg-brooding individuals only appeared after two months of exposure to this temperature. In the group held at 10°C, no increase in GSI or spawning activity was noted even after two months. These findings suggest that the initiation of breeding is influenced more by rising water temperature than by changes in day length [57]. Following this, actual coastal temperature data were

examined in the context of reproductive timing, showing that *M. nipponense* initiates vitellogenesis when water temperatures surpass 12°C. Females begin responding to rising temperatures in April, with vitellogenesis becoming more pronounced as temperatures increase, leading to spawning in June. To identify factors that end the breeding period, female prawns were raised from August to November under four conditions combining two day lengths (12L and 15L) and two water temperatures (28°C and 22°C) [58]. The proportion of individuals stopping spawning was recorded: at 28°C with 15L, spawning continued until late September in all specimens; however, at 28°C with 12L, 90% ceased spawning. Similarly, at 22°C with 15L, 80% maintained spawning activity, whereas at 22°C with 12L, spawning halted entirely.

After September, prawns raised under a 12-hour light regimen stopped spawning regardless of water temperature. However, in the 15-hour light group, all individuals maintained at 28°C continued spawning, while 70% of those kept at 22°C also sustained spawning activity. These findings indicate that the primary environmental factor governing the cessation of spawning in September is the reduction in day length rather than a decline in water temperature [59]. Conversely, in other *Macrobrachium* species such as the tropical *Macrobrachium rosenbergii* (giant freshwater prawn), neither water temperature nor day length exerts a significant influence. When this species is subjected to various combinations of temperature and photoperiod, the boundary between spawning and non-spawning conditions is ambiguous, with only minor variations in spawning frequency observed [60]. The breeding period of *M. rosenbergii* in India's Hoogly River spans from December to July, peaking during the North monsoon season between March and May. In contrast, populations residing in southern India experience their breeding season beginning in August or September, with breeding activity reaching its height during the South-West monsoon months of October and November. The monsoon season brings wet conditions that alter water quality and levels, consequently increasing the availability of food resources. Such conditions are thought to act as a breeding stimulus for this species [48].

Eyestalk Ablation: A Common Endocrinology Manipulation Technique and Its Drawbacks

Environmental signals in natural ecosystems trigger neurosecretory mechanisms in shrimp, leading to ovary maturation and the onset of spawning [61]. As a result, maintaining optimal environmental conditions is fundamental to the protocols of maturation facilities. Adjustments may be made to water temperature, salinity, oxygen concentration, nitrogenous compounds, lighting duration and strength, the physical setup of tanks and rooms, as well as the feeding regimen [61]. Although

broodstock can be induced to meet production requirements through external stimuli, females typically take considerable time to mature and initiate reproduction. Nonetheless, the limited yield is generally acceptable in hatcheries that specialize in genetic selection and supplying broodstock to other facilities [62]. In contrast, hatcheries aiming to produce sufficient postlarvae for grow-out face challenges due to the limited reliability of environmental cues alone. As a result, eyestalk ablation has been implemented to better regulate the internal physiological controls of reproduction [62]. Eyestalk ablation is a commonly employed technique in commercial hatcheries, serving as a basic hormonal intervention to trigger reproductive maturation and spawning in various crustacean species, notably *Penaeus vannamei* [63]. To suppress gonad inhibiting hormone (GIH/MO-IH) secretion, which is generated by the X-organ and sinus gland complex in the optic ganglia of the eyestalk, one or both eyestalks are either removed or constricted through surgical cutting, cauterization, or ligation [64].

Studies have indicated that eye stalk ablation positively influences the reproductive efficiency of shrimp used for breeding [63]. Eyestalk ablation promotes rapid reproductive development in shrimp; however, it also poses several issues, such as elevated stress-induced mortality, impaired physiological regulation, diminished gamete viability, and conflict with principles of animal welfare [61]. Strategies for inducing hormone production represent a substitute approach to guarantee the presence of internal steroid hormones required for the regulation of reproductive processes [65]. Despite their efficacy, the application of synthetic steroid hormones presents financial burdens and environmental risks, notably the possibility of residual contamination in water bodies [65]. Since these hormones originate from environmentally sustainable plants, their use in enhancing the reproduction of tiger shrimp broodstock is deemed safe [61].

In commercial hatcheries, ablation continues to be the predominant approach used to increase egg output by improving the frequency of spawning. Unilateral and bilateral ablation procedures exert additional impacts on females, influencing almost every physiological function governed by the X organ Sinus Gland Complex. Nonetheless, these changes eventually lead to physiological disturbances, resulting in a deterioration of reproductive efficiency in females as time progresses [61]. The process of eyestalk ablation is viewed as a cruel and intrusive technique for shrimp, as it can lead to bodily trauma and elevate stress levels [66]. Hormonal levels, including MIH and CHH, have been shown to diminish after ablation. The consequent decline in MIH induces more frequent

molting in females, requiring considerable energy that detracts from the energy reserved for reproductive processes. However, this increased energetic cost might be partially alleviated through elevated food intake combined with improved physiological efficiency in energy utilization [67].

Female Pacific white shrimp that have undergone ablation exhibit significant weight loss and experience greater reductions in hemocyanin concentration in their hemolymph as well as glucose levels in the hepatopancreas during reproduction compared to females that have not been ablated [64]. In addition, the eggs produced by ablated females contained increased amounts of acylglycerides. However, due to the fast and repeated maturation and spawning events occurring in quick succession, the reproductive efficiency of ablated *L. vannamei* females deteriorates over time, a phenomenon attributed to fatigue and manifested in the reduced quality of their progeny [68].

Screening and Effects of Phytochemical Compounds in Crustaceans

Numerous bioactive constituents present in herbal-derived phytochemicals, including alkaloids, flavonoids, phenols, steroids, and essential oils, are documented to exert positive influences on aquaculture by promoting growth, reducing stress, stimulating immunity and appetite, exhibiting antimicrobial activity, and enhancing reproductive performance in both fish and shrimp farming [6, 69].

Stimulation of Growth Performance

Plant-based additives in animal feed show considerable potential as replacements for synthetic growth promoters, owing to their beneficial influence on digestive efficiency, gut microbial balance, and animal well-being [70]. The use of plants in human diets is primarily grounded in their role as providers of essential nutrients, including amino acids, carbohydrates, minerals, and vitamins [71]. All dietary components that contribute to enhanced growth are associated with increased metabolic rates and heightened digestive enzyme activity, thereby improving the efficiency of digestion and supporting improved growth performance [72]. The application of plants in aquafeed has taken multiple forms, including the use of their whole parts, extracts, powders, or supplementation alongside fishmeal or polysaccharides, to assess their influence on growth metrics, blood profiles, disease prevention, and general performance parameters [70]. According to existing studies, these substances work synergistically to improve the physiological health and growth efficiency of *P. vannamei*, a key species in aquaculture. Despite these benefits, the species remains vulnerable to disease and growth-related issues. The efficacy of plant-derived growth enhancers is largely attributed to their diverse active ingredients that facilitate better nutrient digestion and

utilization [71]. Several botanical extracts, such as those from *Astragalus membranaceus*, *Bidens alba*, *Codonopsis pilosula*, *Glycyrrhiza uralensis*, *Jatropha curcas*, *Morinda citrifolia*, *Phyllanthus amarus*, *Plectranthus amboinicus*, and *Psidium guajava*, have been commonly investigated for their potential to enhance the growth performance of *P. vannamei* [71]. It has also been documented that supplementing the diet of *P. vannamei* post larvae with extract from *M. citrifolia* fruit (Noni) led to significant improvements in shrimp growth parameters, such as body weight and total length, alongside better growth efficiencies measured by specific growth rate, daily growth average, feed conversion efficiency, and condition factor [73]. Conversely, aquatic plants like *Cynodon dactylon* and *Zingiber officinale* have been utilized as feed for freshwater shrimp *Macrobrachium rosenbergii*, resulting in enhanced growth metrics, including mean body weight, average length, specific growth rate, condition factor, weight gain rate, length gain rate, and feed conversion efficiency [74]. Likewise, plants such as *Alternanthera sessilis*, *Andrographis paniculata*, *Cissus quadrangularis*, *Coriandrum sativum*, *Eclipta alba*, *Glycyrrhiza glabra*, *Melaleuca alternifolia*, *Mentha arvensis*, *Murraya koenigii*, *Myristica fragrans*, *Ocimum sanctum*, *Piper longum*, *Piper nigrum*, *Quercus infectoria*, *Withania somnifera*, and *Zingiber officinale* have been documented to enhance growth performance, survival weight, protein content, and specific growth rate in *Macrobrachium rosenbergii* [74]. Correspondingly, the impact of the aqueous leaf extract of *Melastoma malabathricum* on crustacean development was examined, indicating that a concentration of 15 µg per gram of body weight leads to an increased growth rate [75].

Stimulating Sexual Maturation and Inducing Reproduction

In various crustaceans such as river crabs, shrimps, crabs, and lobsters, the hormonal regulation of reproductive processes has been widely investigated. It has been found that hormones secreted by neuroendocrine tissues play a vital role in the development and maturation of reproductive organs [76]. Various alternative ablation approaches to stimulate maturation have recently been introduced. One common practice among shrimp farmers is the use of natural food sources, including mollusks, squid, and polychaetes, which effectively support reproductive performance [77]. Despite their common use, such foods often result in pathogenic contamination in shrimp reproduction. This issue has prompted the adoption of plant-based methods to enhance the reproductive process of these organisms [71]. Medicinal plants contain a variety of compounds, among them phytoestrogens such as isoflavonoids (including flavonoids and isoflavones), along with cholesterol, lignans, anthraquinones,

chalcones, and saponins [78]. Phytoestrogens possess a chemical framework comparable to the steroid hormone estradiol (E2) present in animals [79]. They have been identified as having estrogen-like effects on reproduction, capable of both stimulating and inhibiting reproductive activities [71]. Their low molecular weight allows them to easily pass through cell membranes and interact with estrogen receptors (ERs), resulting in estrogenic or antiestrogenic actions [80]. The estrogenic or antiestrogenic response observed in animals is influenced by multiple factors, including the levels of endogenous estrogen, the activity of aromatase, the species in question, their reproductive condition, the length of exposure, and whether the substance is administered orally or parenterally [81]. In commercially important species like *Penaeus merguensis*, studies have been conducted to enhance reproductive maturation as a response to declining production caused by the intensified harvesting of wild shrimp for consumption. This decline prompted investigations into the effects of dichloromethane and acetone extracts from *Emilia sonchifolia* on promoting ovarian development [71]. Owing to the presence of alkaloids, tannins, flavonoids, sterols, palmitic acid, and honey acid in its makeup [71]. Several studies have focused on germplasm preservation by using extracts from *Moringa oleifera* and ginger (*Zingiber officinale*) in refrigerated spermatophores. The supplementation of these extracts significantly enhanced sperm viability compared to controls, without exhibiting any spermicidal effects [82] (See Fig.2).

Case Study 1: Karamunting (*Melastoma malabathricum*)

Karamunting (Melastoma malabathricum) as an herbal compound and Geographical Regions

The Kalimantan Forest herb, Karamunting (*Melastoma malabathricum* L), possesses multiple chemical constituents such as flavonoids, alkaloids, steroids, anthocyanins, saponins, and tannins, which are present in its roots, stems, leaves, flowers, and fruits. According to Danladi's study, the flower of this plant contains high concentrations of phenols and flavonoids [83]. *Melastoma malabathricum*, commonly known as Karamunting, contains lanosterol in its extract, a phytosterol and precursor to cholesterol used by crustaceans to produce steroid hormones that play a vital role in their reproduction [84]. The presence of lanosterol in Karamunting extract elevated cholesterol hormone levels, which in turn hastened the gonadal maturation of parent white shrimp. Comparable outcomes were also reported in tiger shrimps subjected to eye ablation and serotonin hormone administration [85].

Melastoma malabathricum is a shrub or small tree native to regions such as Australia, Bangladesh, China (including Taiwan), the Himalayas, Indochina,

and Malesia. It has also been introduced to Madagascar. This species predominantly inhabits wet tropical environments. It is recognized by the Weed Science Society of America as a weed present in North America. In multiple U.S. states, *Melastoma malabathricum* is identified as a potential invasive threat and is officially classified as a noxious weed, with regulatory measures including quarantine or prohibition in California, Massachusetts, Minnesota, and Oregon. Additionally, all *Melastoma* species are designated as noxious in Hawaii [86].

Plant Description and Extract Preparation

Melastoma malabathricum is a perennial, spreading shrub typically ranging from 1.8 to 2.0 meters in height, though it has been documented to grow as tall as 10 meters, with a width of approximately 2 meters. Its stems are upright, branching, slender, quadrangular, rough to the touch, and densely covered with tiny, stiff, closely appressed scales that have fine hair-like edges. The leaves measure between 5 and 12 cm in length, are arranged oppositely, simple in form, and possess petioles measuring 0.5 to 1.9 cm. The leaf blades are narrowly elliptical to oblong-elliptical, tapering sharply at both ends, prominently featuring 3 to 5 veins. Both leaf surfaces bear small, rigid, flat-lying hairs, with the upper surface exhibiting rows of white cells at the hair bases. The flowers, which bloom mainly in summer, are pink, violet, or mauve, about 7.5 cm in diameter, grouped in sessile terminal corymbs of 3 to 6 flowers, each divided into 5 to 7 parts and accompanied by two large, deciduous bracts. The anthers show dimorphism. The fruit is a berry characterized by red, sweet yet astringent pulp, enclosed by a scaly calyx tipped with bristles, containing numerous tiny seeds. These seeds, approximately 1 mm wide, are densely marked with pits, coiled in shape, and embedded within the pulp [86].

The *M. malabathricum* samples underwent drying in an oven set at 50 degrees Celsius for a duration of 48 hours, followed by grinding to obtain a fine powder. Extraction of the leaf simplicia was performed via maceration with 96% methanol. Afterward, the methanol extract was fractionated by liquid-liquid extraction using solvents such as hexane, ethyl acetate, and butanol. [87]. Phytochemical screening was performed to identify the levels of alkaloids, terpenoids, saponins, tannins, and flavonoids. The qualitative evaluation of the phytochemical components in the *M. malabathricum* extract adhered to recognized standard procedures [88].

Bioactive Compounds and Effects on Shrimp Reproduction

The plant *Melastoma malabathricum* comprises multiple chemical constituents such as flavonoids, alkaloids, steroids, anthocyanins, saponins, and

tannins, distributed throughout its roots, stems, leaves, flowers, and fruits. [87]. The phytochemical screening results revealed that the methanol extract of *M. malabathricum* leaves contains secondary metabolites such as flavonoids, tannins, and saponins. Similarly, the ethyl acetate fraction comprises the same bioactive compounds as the methanol extract but in greater concentrations, as indicated by the more intense coloration observed upon reaction with specific reagents. In contrast, the butanol fraction was found to contain only flavonoids and saponins, whereas the aqueous fraction exclusively contained tannins [88]. The extract of Karamunting (*Melastoma malabathricum*) contains lanosterol, a phytosterol that serves as a cholesterol precursor. Crustaceans utilize this compound to synthesize animal steroid hormones essential for their reproductive processes [87]. Karamunting extract showed elevated levels of lanosterol α and β amirin. Additionally, research has indicated that karamunting plants contain both sitosterol α and β amylin [87, 89].

One approach to promote faster gonadal maturity in the shrimp species *P. vannamei* involves feeding them diets rich in cholesterol. For example, the plant *Melastoma malabathricum* is frequently utilized for this purpose, as it contains bioactive compounds such as saponins, tannins, triterpenoids/steroids, flavonoids, lanosterol, and plant-based cholesterol, which act as a hormonal precursor that triggers gonadal development in white shrimp [85]. In a similar manner, the plant serves as an agent to promote and speed up the development of ovaries in *Scylla olivacea* crabs [90]. The injection of *Melastoma malabathricum* ethanol extract derived from Karamunting into mature female *Litopenaeus vannamei* shrimp led to an increase in progesterone hormone levels and a faster development of gonads, indicating the potential of this extract to accelerate reproduction and thereby boost production efficiency over a shorter duration [85]. Karamunting extract significantly enhanced the size of oocytes ($P < 0.05$). At the beginning of the study, the mean oocyte diameter was $15.57 \pm 3.15 \mu\text{m}$. After the experimental period, oocyte sizes in the Control group averaged $25.29 \pm 2.69 \mu\text{m}$, while ovarian treatment groups showed the following sizes: T1 at $65.65 \pm 2.64 \mu\text{m}$, T2 at $63.98 \pm 3.06 \mu\text{m}$, T3 at $39.12 \pm 6.01 \mu\text{m}$, T4 at $28.08 \pm 0.84 \mu\text{m}$, and T5 at $27.65 \pm 0.71 \mu\text{m}$. The extract clearly induced greater oocyte growth compared to both initial values and the Control [85]. The lanosterol contained in the karamunting extract seemingly boosted progesterone production, thereby promoting accelerated gonadal maturation and an increase in oocyte size in the white shrimp broodstock. Significant differences were observed in the GSI values between the treatment groups and the control ($P < 0.05$) [85]. Additionally, the injection of serotonin in mature female specimens of the freshwater shrimp *Macrobrachium rosenbergii*

promoted a significant rise in their gonadosomatic index (GSI) [91].

Case Study 2: *Cyperus* spp. (e.g., *C. rotundus*)

Cyperus spp. as an herbal compound and Geographical Regions

Cyperus rotundus is a perennial herbaceous plant that grows up to 40 cm tall. It features slender, dark green stems and elongated, pointed leaves measuring between one-sixth and one-third of an inch in width. The flowering stem exhibits a triangular cross-sectional shape, while the flower itself ranges from 2 to 8 inches in length and contains three stamens and a pistil composed of three stigmas. The flower is bisexual in nature [92]. *Cyperus* species, commonly found growing wild in fields, paddy lands, and coastal areas, hold considerable promise for use in aquaculture. [92, 93].

Cyperus rotundus L., commonly known as purple nut sedge and a member of the Cyperaceae family, is a perennial herb native to India, with a widespread presence in tropical and subtropical regions globally. This species is infamously invasive, often labeled as the “world’s worst weed” due to its detrimental impact on crop productivity. It thrives in sunny and moist conditions and is capable of growing in diverse soil types, from sandy to loamy and even in rainforest habitats. In India’s southern regions, its essential oils are used in fragrance production. Recognized as a major weed in 92 countries, *C. rotundus* negatively affects over 50 types of crops, including rice, maize, sugarcane, cotton, and various vegetables. The plant grows best in light to medium soils and tolerates a wide pH range but does not survive in shaded conditions. Moisture-rich soil is essential for its growth [93].

Plant Description and Extract Preparation

The plant exhibits an inflorescence that may take the form of a spike, panicle, or compact head, with the spikelet serving as the fundamental unit. Each spikelet can contain one or more diminutive flowers, each situated in the axil of a glume. Flowering occurs around July to August, with flowers displaying hues from reddish-brown to nearly black, and they may be either unisexual or bisexual. The leaves are simple, alternate, and arranged in three vertical rows (tristichous), dark green on the upper surface, and emerge from reddish-brown sheaths near the stem’s base. Ligules are absent, and sheaths are closed. Rhizomes are numerous and slender, producing white, fleshy tubers when immature that turn hard and black with maturity. The seeds are albuminous. Stems originate from the tuber base, leafy at the lower part. The culms are glabrous, dark green, solid, and typically triangular in shape [93].

Specimens of *Cyperus* spp. were sourced from agricultural surroundings. The plant’s rhizomes were removed and washed under flowing water to clear off

any adhering soil. Clean rhizomes were diced into small portions and dried in a cabinet set at 40°C. These dried segments were then milled into a fine powder with a blender. The final concentrated extract was purified by recrystallization in chloroform to obtain a methyl farnesoate-rich compound [93, 94].

Bioactive Compounds and Effects on Shrimp Reproduction

Phytochemical investigations have identified essential oils, flavonoids, terpenoids, sitosterol, stearic acid, sugeonol, sugetriol, α -cyperolone, α -rotunol, β -cyperone, β -pinene, β -rotunol, β -selinene, and both mono- and sesquiterpenes such as methyl farnesoate (MF) as the principal constituents of this plant [93].

To promote faster sexual maturation in *P. vannamei* shrimp, another technique includes the use of feedstuffs that are naturally rich in methyl farnesoate (MF), such as those derived from *Cyperus* spp. [94]. *Cyperus* species, commonly found in natural habitats such as agricultural fields, paddy lands, and coastal zones, show promising applications in aquaculture. These wild plants are rich in diverse bioactive constituents, including alkaloids, terpenes, phenolic acids, and sesquiterpenes such as methyl farnesoate (MF). Notably, MF shares structural and functional similarities with juvenile hormones in crustaceans, indicating its potential role in triggering endocrine responses to support reproductive enhancement in tiger shrimp within hatchery environments [94]. Methyl farnesoate (MF) functions similarly to gonadotropins and growth hormones, playing a key role in the presence of vitellogenin in females and promoting reproductive activity in males [94]. Administering CE to domesticated tiger shrimp broodstock led to marked improvements in reproductive development and spawning performance, especially when the dosage reached 100 µg per gram of body weight across four weekly injection sessions [93]. Due to the practical limitations and mortality risks linked to injection-based feeding methods, there has been a transition toward administering treatments through feed. When CE was delivered via feed at a dosage of 300 µg/g body weight, an enhancement in reproductive outcomes was observed [93]. The administration of CE in the feed showed a positive effect on the mating success of female broodstock and promoted gonadal maturation (GML) in correlation with increased CE concentrations. The most significant advancement to GML stage IV in female tiger shrimp occurred at a CE dosage of 500 µg/g BW, where 62.5% of the shrimp reached this stage. While both CE treatment and eyestalk ablation facilitated progression to GML IV, the control group, which did not receive CE, only advanced to GML II [93]. *Cyperus* rhizome extract was found to contain 0.001735% juvenile hormone relative to its dry weight. Administration of juvenile

hormone at 100 µg per gram of body weight, with an application increased to four times this amount, has been shown to significantly enhance the production of vitellogenin and spermatogenin in prospective female shrimp, facilitating optimal reproductive function [94]. Additional findings indicated that the highest molting frequency was observed in the treatment group receiving 150 µg/g BW, with a total of 15 molts during the maintenance period. Meanwhile, the broodstock shrimp exhibiting the greatest gonad maturity (75%) were found in the group treated with 100 µg/g BW. Molecular analysis of vitellogenesis progression, conducted using PCR with vitellogenin primers F and R alongside β-Actin primers F and R, demonstrated positive developmental responses in the broodstock shrimp treated with Cyprus sp. [93,94] (as shown in Table 1).

Alternatives to Eyestalk Ablation: The Role of Phytogetic Compounds

Given the various adverse consequences linked to ablation as previously outlined, and considering the welfare of shrimp broodstock, achieving predictable maturation and spawning of captive shrimp without resorting to eyestalk ablation has become a key long-term objective for the industry [61]. Researchers have pursued different strategies to develop alternatives to eyestalk ablation, primarily concentrating on comprehending and modulating endocrine functions [64]. The central approach for this objective has been biotechnology, particularly focusing on hormones responsible for maturation control. Among the molecules with the potential to stimulate maturation and spawning in *L. vannamei* are peptide and steroid hormones, juvenoid hormones, neurotransmitters, and neurotransmitter antagonists [68]. As demonstrated by the case studies on *Melastoma malabathricum* and *Cyperus* spp., phytogetic compounds offer a promising, natural path to achieve this goal. These plants provide bioactive molecules like lanosterol and methyl farnesoate that directly interact with the crustacean endocrine system, stimulating gonadal development and maturation without the need for invasive procedures.

Conclusion

Medicinal plant phytochemicals present a promising, natural pathway toward achieving sustainable crustacean aquaculture. This review has

consolidated evidence demonstrating that herbs like *Melastoma malabathricum* and *Cyperus* spp. are far more than simple immunostimulants; they are powerful endocrine modulators capable of directly enhancing shrimp reproduction. The efficacy of *M. malabathricum* is linked to its lanosterol content, which serves as a critical precursor for crustacean steroid hormones, thereby accelerating gonadal development and boosting progesterone production. Similarly, *Cyperus* spp., through its active compound methyl farnesoate, effectively mimics native juvenile hormones, upregulating vitellogenin expression and synchronizing gonadal maturation to stages conducive for spawning. The comparative analysis reveals that administration of these extracts, particularly via feed, can deliver outcomes comparable to eyestalk ablation, such as significant increases in oocyte diameter, gonadosomatic index, and the percentage of broodstock reaching advanced maturity, but without the associated physiological stress, mortality, and ethical concerns. For the commercial adoption of these alternatives, future research must focus on standardizing extraction protocols, determining optimal dosages for oral delivery, and conducting large-scale trials to validate hatchery-level benefits.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' contributions

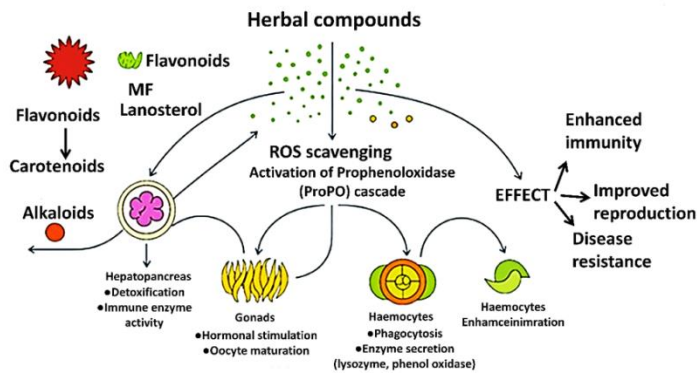
N.N.H., A.E.E., and S.E. collected data from the available published papers; L.Y. and X.D. supervised the work. All team members wrote, revised the original draft, and approved the final manuscript.

Declarations

All data included in this paper are original and obtained from the available freely published papers.

Ethics approval and consent to participate

Not applicable



Graphical abstract: The molecular mechanism of Herbal compounds in the crustacean body.

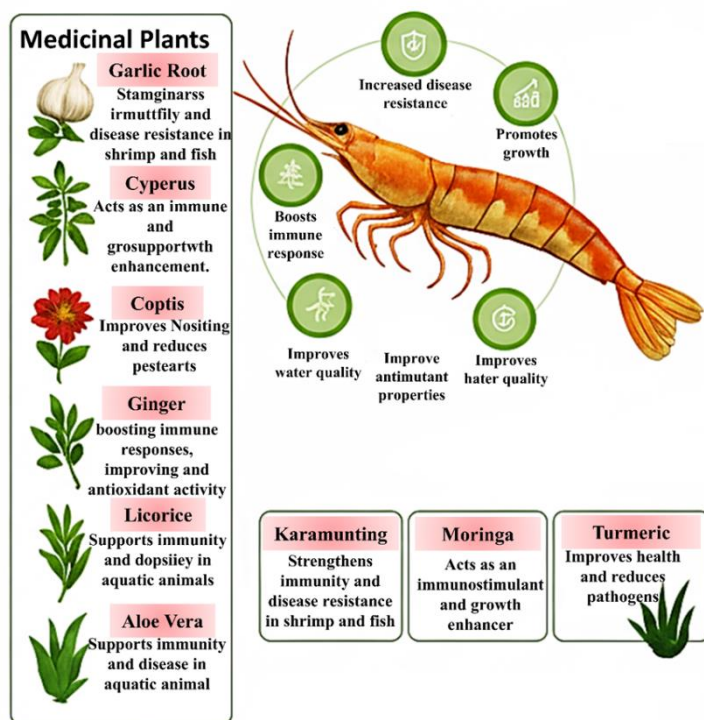


Fig.1. The Role of Medicinal Plants in Sustainable Aquaculture.

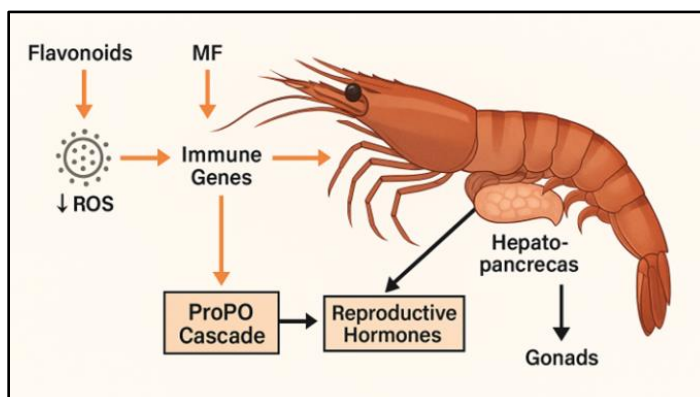


Fig. 2. Mechanistic insights into the role of herbal compounds in regulating shrimp immunity.

TABLE 1. Comparative Benefits of Karamunting (*Melastoma malabathricum*) and *Cyperus* spp. Extracts on Reproductive Performance and Growth of *Penaeus vannamei*.

Aspect	Karamunting (<i>Melastoma malabathricum</i>)	<i>Cyperus</i> spp.
Plant Common Names	Karamunting, Indian Rhododendron.	Purple Nut Sedge, Java Grass.
Scientific Name	<i>Melastoma malabathricum</i> .	<i>Cyperus rotundus</i> .
Geographical Distribution	Australia, South Asia, China, Indochina; invasive in North America.	Native to India; widespread in tropical/subtropical zones worldwide.
Main Phytochemicals	Lanosterol, saponins, tannins, flavonoids, triterpenoids, anthocyanins.	Methyl farnesoate, essential oils, terpenoids, flavonoids, α -cyperolone, β -cyperone.
Unique Compounds	Lanosterol (precursor to crustacean steroid hormones).	Methyl farnesoate (MF) mimics crustacean juvenile hormones.
Hormonal Impact	Elevates cholesterol-derived hormones $\rightarrow$ triggers gonadal development.	MF increases vitellogenin and spermatogenin $\rightarrow$ boosts reproductive activity.
Effects on Reproductive Organs	Enlarges oocytes significantly (e.g., Control: $\sim 25\ \mu\text{m}$ $\rightarrow$ T1/T2: 63–65 μm). Accelerates ovarian development. Increases GSI values.	Advances in gonadal maturation to GML stage IV. Stimulates vitellogenesis in females. Enhances sperm production in males.
Growth & Molting Effects	Limited data on growth; main focus on reproduction.	Increases molting frequency (up to 15 molts). Promotes overall growth in freshwater shrimp (evidence from <i>Macrobrachium</i>).
Administration Methods	Mainly injection; oral delivery is recommended for practical application.	Injection (less practical) and feed-based administration (more effective for farms).
Optimal Doses	Not standardized; study examples: injections leading to GSI increases and faster maturation.	Feed: 300–500 $\mu\text{g/g}$ BW effective; Injection: 100 $\mu\text{g/g}$ BW.
Benefits for Hatcheries	Reduces time to reach reproductive maturity; shortens breeding cycles; increases production efficiency	Replaces eyestalk ablation; synchronizes gonadal maturation; enhances reproductive output.
Molecular Mechanism Evidence	Elevated progesterone levels; increased cholesterol steroid precursors.	Upregulated vitellogenin gene expression (PCR confirmation); elevated molting hormone-related factors.
Effects on Egg Quality	Enlarged oocytes, improved gonadal health, and potentially better hatch rates.	Higher percentages of advanced gonadal stages; improved egg-laying rates.
Preparation Techniques	Dried, macerated in methanol; fractionated into hexane, ethyl acetate, butanol fractions; phytochemical screening.	Cleaned rhizomes dried at 40°C, milled; extract purified via chloroform recrystallization; isolated MF-rich compound.
Practical Considerations	Injection is feasible for broodstock in research; oral inclusion in feed is preferred for commercial scale.	Feed delivery is practical, scalable, and avoids stress and mortality associated with injections.
Environmental & Sustainability Benefits	Plant-based, natural alternative to synthetic hormones and antibiotics; eco-friendly.	Reduces need for invasive techniques (eyestalk ablation); sustainable reproductive enhancement.
Additional Reported Benefits	Potentially improves the survival of larvae. Improves uniformity of sexual maturation.	Additional Reported Benefits. Promotes synchronous spawning. May increase larval survival rates.
Potential Side Effects	No known negative effects reported in trials.	Overdosing could increase mortality or stress; recommended dosages should be strictly followed.
Relevance for <i>P. vannamei</i>	Demonstrated clear benefits for broodstock maturity; recommended for hatchery phases.	Effective for both male and female maturation in <i>P. vannamei</i> and tiger shrimp.

Aspect	Karamunting (<i>Melastoma malabathricum</i>)	Cyperus spp.
Bioactive Compounds	Lanosterol, saponins, tannins, triterpenoids/steroids, flavonoids, plant-based cholesterol.	Methyl farnesoate (MF), essential oils, flavonoids, terpenoids, sitosterol, sugeonol, cyperolones.
Main Active Mechanism	Lanosterol acts as a cholesterol precursor for crustacean steroid hormones; it triggers ovarian and gonadal maturation.	MF mimics crustacean juvenile hormones; triggers endocrine pathways for gonadal maturation and vitellogenesis.
Effects on Gonadal Maturation	Accelerates ovarian development and increases oocyte size (e.g., up to 65.65 µm). Elevates progesterone levels. Increases the gonadosomatic index (GSI) significantly.	Effects on Gonadal Maturation level (GML) in females, up to stage IV at 62.5% with 500 µg/g BW. Enhances vitellogenin and spermatogenin production.
Optimal Dosage (as per studies)	Injection of ethanolic extract; doses led to rapid gonadal maturity	Feed-based delivery: effective dose at 300–500 µg/g BW; injection doses at 100 µg/g BW are also effective.
Molecular & Endocrine Effects	Boosts cholesterol hormones, leading to increased steroidogenesis and faster reproductive cycles.	MF functions like gonadotropins; it promotes vitellogenin synthesis, molting frequency, and reproductive hormones.
Growth Promotion	Not explicitly highlighted for growth promotion; focus mainly on reproductive enhancement.	Noted in freshwater shrimp (<i>Macrobrachium rosenbergii</i>) for enhancing growth rates, feed conversion, and survival; potential but not explicitly confirmed in <i>P. vannamei</i> .
Other Reported Benefits	Oocyte diameters in treated shrimp significantly exceed control groups. Clear evidence of faster egg development.	Improves molting frequency (e.g., 15 molts in the highest dose group). Enhances mating success and spawning performance.
Preparation & Extraction	Leaves dried, ground, macerated in methanol, fractionated with solvents; extracts screened for secondary metabolites.	Rhizomes dried, milled, extracted, and purified by recrystallization in chloroform to isolate MF-rich compound.
Safety & Practicality	Injection is practical in small trials but less feasible on farms; oral administration could reduce stress.	Feed-based administration is more practical and less stressful; injections carry a higher mortality risk.
Relevance to Sustainable Aquaculture	Potential to reduce use of synthetic hormones and antibiotics for maturation; plant-based, eco-friendly solution.	Potential to replace eyestalk ablation or synthetic hormone injections; promotes sustainability and animal welfare.
References	[95-97]	[98-102]

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مُنشَطات المناعة المُستخلصة من النباتات الطبية لتربية الروبيان المستدامة: من المُركّبات النشطة بيولوجيًا إلى تعزيز القدرة التكاثرية

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الملخص

يواجه التوسع في تربية الروبيان المكثفة، المدفوع بالطلب العالمي على الغذاء، تحديات تتمثل في تفشي الأمراض والاعتماد على ممارسات غير مستدامة مثل استئصال العين لتحفيز التكاثر في الأسماك والجمبري. تستكشف هذه المراجعة إمكانات المنشطات المناعية المستخلصة من النباتات الطبية كبديل مستدام، مع التركيز بشكل خاص على تحسين الأداء التناسلي للروبيان الأبيض في المحيط الهادئ، *Penaeus vannamei*. تقدم تفاصيل عن التنظيم الهرموني المعقد لتكاثر القشريات، مع تسليط الضوء على دور مجمع الغدة الأنفية العضوية X، والعضو الفكي، والهرمونات الرئيسية مثل ميثيل فارنيسوات. يؤكد هذا الأساس على العيوب الميكانيكية لاستئصال العين، مما يخلق حاجة ملحة للتدخلات الطبيعية. ثم تجمع الورقة الأدلة حول كيفية قيام المواد الكيميائية النباتية النشطة بيولوجيًا، بما في ذلك الفلافونويدات والقلويدات والستيرويدات، بتحفيز النمو والمناعة ونضج الغدد التناسلية. يتم تقديم تحليل شامل لنباتين واعدين، هما *Melastoma malabathricum* (Karamunting) و *Cyperus spp.* يعمل *M. malabathricum*، الغني بالانوستيروول، كسلانف للهرمونات الستيرويدية، مما يسرع بشكل كبير من نمو المبيض، ويزيد من حجم البويضات، ويرفع مستويات البروجسترون في P. *vannamei*. على العكس من ذلك، يحتوي *Cyperus spp.* على ميثيل فارنيسوات، وهو هرمون شبيه بالهرمونات اليفوخية للقشريات، والذي يعزز تكوين الصفار، وتكوين الحيوانات المنوية، والتحول، مما يؤدي إلى مراحل نضج متقدمة للغدد التناسلية وتحسين أداء التبويض. من خلال تجميع الأدلة الجزيئية والغدد الصماء، تضع هذه المراجعة هذه المستخلصات العشبية كبديل فعالة وطبيعية وصديقة للرفاهية للهرمونات الاصطناعية والتقنيات الغازية، مما يوفر استراتيجية قابلة للتطبيق لتعزيز كفاءة التكاثر والاستدامة في مفرخات الروبيان.

الكلمات الدالة: النباتات الطبية، الميلاستوما مالاباثريكوم، السعدة المستديرة، الروبيان، التكاثر.