International Journal of Biological Innovations

Available online: http://ijbi.org.in | http://www.gesa.org.in/journals.php

DOI: https://doi.org/10.46505/IJBI.2019.1101

Research Article

E-ISSN: 2582-1032

Medicinal Fruit Lapsi *Choerospondias axillaris* Roxburgh, 1832 (B. L. Burtt & A. W. Hill) Enhances Fish Growth and Immunity in Aquaculture

Shyam Narayan Labh[1*], Shubha Ratna Shakya[1] and Babita Labh Kayastha[2]

[1]Aquaculture Research Unit, Department of Zoology,
Amrit Campus, Tribhuvan University, Nepal
[2]Nobel Academy, Pokhara University, Kathmandu, Nepal
**Corresponding author:* snlabh@gmail.com

Received: 12.01.2019 Reviewed: 25.01.2019 Accepted: 31.01.2019

Abstract: Today we live in a world where poverty, hunger and malnutrition are prevalent. It has been estimated that 14 per cent of the global population or 852 million people are going hungry and of these 690 million are in the Asia-Pacific region. Aquaculture is the farming of aquatic organisms in both coastal and inland areas involving interventions in the rearing process to enhance production. It is probably the fastest growing food-producing sector and now accounts for 50 percent of the world's fish that is used for food. Fish contributes over 20 per cent of the animal protein intake for more than 2.6 billion people around the world. Hence, fish and fisheries make a major contribution to nutritional security and the fight against hunger and poverty in Asia. Immunostimulants known as immunostimulators are attractive substances that activate the immune system of humans and animals for prevention of diseases and improvement of the body's natural resistance to various viral and bacterial infections. These biologically active substances are the products derived from natural sources or synthetically made with different chemical properties and mechanisms of action. Lapsi, *Choerospondias axillaris* is indigenous fruit tree of Nepal found growing within 900- 2000 m above sea level in many parts of the country. Native to Nepal hills (850-1900 m asl), the tree has also been reported from India, China, Thailand, Japan and Vietnam. Nepal is unique for processing and use of Lapsi fruits. Fruits are rich in vitamin C content, and are consumed fresh, pickled and processed for preparing a variety of sweet and sour, tasty food products locally called as Mada and candy. It is grown in 301 village Development committees of 29 hill districts of Nepal for some socio-economic purpose. Like other medicinal fruits, lapsi also act as immunostimulants and enhance the immunity of fish during aquaculture.

Keywords: Aquaculture, *Choerospondias*, Immunostimulants, Lapsi, Medicinal fruit, Nepal.

Introduction

Aquaculture is the farming of all forms of aquatic animals and plants in fresh, brackish and marine environments under controlled conditions (FAO, 2018). It is the fastest-growing food producing sector of agriculture worldwide that provides half of all fish for human consumption (Troell *et al*, 2013). Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for millions of people around the world (Allison 2011).

a) Status of Global Aquaculture

Global aquaculture production grew at an annual average rate of 8.9 per cent since 1970, as compared to 1.2 per cent for production from capture fisheries and 2.8 per cent for terrestrial farmed animal meat production. While the sector represented only 6 per cent of food fish production in 1970, its share increased to 12 per cent in 1985 and to 32 per cent in 2003. Asia accounts for over 91 per cent of global aquaculture production by weight and 82 per cent by value. Production increased from 14.3 million t in 1989 to over 42 million t in

2003, an annual growth rate of 11 per cent. Thus, it is the fastest growing food production sector in agriculture. Much of this growth has been in China, where the annual growth rate was 15 per cent. In the rest of Asia, the growth rate was only 3 per cent. Of the total aquaculture production of 42 million t in 2003, 60 per cent was from freshwater aquaculture and the rest from mariculture, indicating the importance of freshwater aquaculture to increased nutritional security (Lorenzen 2008). Further, most of the species cultured in brackish and marine waters are carnivores and require fishmeal in their feed, which puts more pressure on already depleted marine stocks. Nearly 80 per cent of aquaculture production comes from small-holder operated farms. Thus, small-holder farmers are not only consumers of fish but also the producers. To eliminate hunger and poverty in this segment of the population, the issue of their access to resources, lack of skills, vulnerability and aversion to risks have to be addressed. In the context of growing global demand for fish, the stringent requirements for environment and product quality, and the hope that aquaculture will bridge the gap between demand and supply.However, world per capita fish supply has outpaced population growth and reached a record high of 20 kg in 2014 (double the level of the 1960s), due to vigorous growth in aquaculture. In the last two decades, dramatic growth in aquaculture production has boosted average consumption of fish and fishery products worldwide (FAO 2010).

b) Status of Aquaculture in India

India offers a huge potential for aquaculture development. The country has a coastline of 7,517 km and an extensive river and canal system of about 195.210 km, consisting of 14 major rivers, 44 medium rivers and numerous small rivers and streams. In addition, pond and tank resources are estimated at 2.36 million ha.1 India experienced an eleven fold increase of fish production in the past six decades.In India, the annual fisheries and aquaculture production increased from 0.75 million tonnes in 1950-51 to 9.6 million tonnes in 2013-2014. Globally the country now takes the second position, after China, with regard to annual fisheries and aquaculture production. 2 According to the FAO, the total aquaculture production in 2012-2013 was 4.21 million tonnes. This constituted over a third of the country's total fish production. This quantity is almost fully consumed on the domestic market, except for shrimps and freshwater prawns, which are mainly exported (Lorenzen 2005). India is the largest exporter of shrimps to the Netherlands. Specifically freshwater aquaculture experienced over a tenfold growth in the past three decades, 0.37 million tonnes in 1980 to 4.03 million tonnes in 2010.5 Over ten percent of the global fish diversity can be found on or near the Indian subcontinent and more than 14.5 million people depend on fisheries activities. Nevertheless, the national average annual consumption of fish and fish products in 2010 was 2.85 kg/capita. In the coastal state of Kerala, fish is consumed the most, with 22.7 kg/per capita and in the mountainous state of Himachal Pradesh consumption is with 0.03 kg/capita relatively low. 6 About 40% of the Indian population does not eat fish since they are vegetarian and the remaining 60% only occasionally consumes fish.7 Lower income and rural families consume less fish than higher incomes or urban families (Verdegem *et al*, 2009).

c) Status of Aquaculture in Nepal

Nepal is a land-linked federal democratic republic country situated between China to the north and India to the south, east and west. It covers an approximate area of 147,181 square kilometers and stretches 885 kilometers East to West and about 193 kilometers North to South. In the south the altitude is about 60 m above sea level, while at north elevation goes up to 8,848 m (Mt. Everest), the highest peak of the world (Nargis *et al*, 2011). High geographical variations, abundant freshwater resources and low labor cost are positive benefits to develop aquaculture in Nepal. There are a lot of water resources in Nepal with about 6,000 rivers flowing north to south. These rivers are characterized by low water temperature, high dissolved oxygen, high turbulent fast current in higher mountainous and hilly region. On the other hand, high water temperature, low dissolved oxygen, low turbulence is normal for the river in Terai (low land) region (Paudel 2003). In Nepal, aquaculture contributes 2.68% to Agricultural Gross Domestic Production (AGDP). Rivers and streams are a major source of capture fishery covering 395000 ha of natural water resources in the country. Around 5, 83, 467 people are directly engaged in aquaculture and capture fishery with net fish production of 83,897 MT. (Culture fisheries 62,924 MT and capture fisheries 20,973 MT) in the year 2016 (DoFD 2017). Approximately 5% of Nepal is occupied by different freshwater aquatic habitats where 232 fish species are reported to thrive (Shrestha 2008). Among the 232 fish species 217 are indigenous and 15 are exotic. The aquatic habitats and varied fish species are the prospects for fisheries and aquaculture development in the country (Dahal *et al*, 2013). The present aquaculture status of Nepal can be improved by adopting long-term sustainable plan, scientific and technological studies on indigenous fish species, proper hygiene management and better disease control (Gautam 1997). Total fisheries production (metric tons) in Nepal was reported at 69500 in 2015, according to the World Bank collection of development indicators, compiled from officially recognized sources.

2. Challenges in Fish Production During Aquaculture

Today we live in a world where poverty, hunger and malnutrition are prevalent. It has been estimated that 14 per cent of the global population or 852 million people are going hungry and of these 690 million are in the Asia-Pacific region. Micronutrient deficiencies or hidden hunger in one form or the other are affecting more than 2 billion people worldwide. Each year, 5.5 million children are dying of causes related to hunger and malnutrition. Over 1.1 billion people live on less than US$1 a day, of which 700 million are in Asia. Fifty per cent of the hungry are in smallholder farming households and 20 per cent among the landless poor. These statistics reveal the unpalatable truth that in a world that has resources and knowledge, the situation remains a continuing travesty of the recognized fundamental human right to adequate food and freedom from hunger and malnutrition. The Millennium Development Goals adopted by the world's governments in 2000 set a target of halving the hungry and malnourished population by 2015 (Labh *et al.*, 2015).

Fish and fisheries can play an important role in addressing hunger and poverty in Asia. Fish are a rich source of protein, essential fatty acids, vitamins and minerals. The fats and fatty acids in fish, particularly the long chain n-3 fatty acids (n-3 PUFA), are highly beneficial and difficult to obtain from other food sources. Fish contributes over 20 per cent of the animal protein intake for more than 2.6 billion people around the world. For example, the contribution of fish to animal protein intake is estimated at 34.5 per cent in Malaysia, over 53 per cent in Indonesia and Sri Lanka, and 84.4 per cent in the Maldives. However, these national averages hide the importance of fish to the rural poor and coastal communities. In many countries of the Asia-Pacific region, rural poor household depend on fish for as much as 60-80 per cent of their animal protein intake. In addition to contributing to nutritional security, the sector has been providing employment to over 35 million people in Asia directly and many more when the support sector is included (Dabrowski 1984).

3. Use of Medicinal Plants as Immunostimulants

Immunomodulators are natural or synthetic materials that regulate the immune system and induce innate and adaptive defense mechanisms. These substances are classified into two types, immunostimulants and immunosuppressants. Immunostimulants can enhance body's resistance against various infections through increasing the basal levels of immune response (Jian 2004). These agents could increase the oxidative activity of neutrophils, augment engulfment activity of phagocytic cells, and stimulate cytotoxic cells as necessary defense mechanisms. Many disorders could be treated using some immunostimulants such as autoimmune diseases, viral infections, and cancer (Yin *et al*, 2009).

The immunomodulation of larval fish has been proposed as a potential method for improving larval survival by increasing the innate responses until its adaptive immune response is sufficiently developed for effective response against the pathogen. To this end, it has been proposed that the use of immunostimulants as a dietary supplement to larval fish could be of considerable benefit in boosting the animal's innate defences with little detriment to the developing animal (Bricknell *et al.*, 1997). Galeotti (1998) suggested that *in vitro* screening methods should be used to elucidate the mechanisms of immune-stimulation and then *in vivo* methods should be used to establish whether the benefits occur in live fish. The addition of various food additives such as vitamins, carotenoids and herbal remedies to the fish feed has been tested in aquaculture. Reducing the stress response, increasing the activity of innate parameters and improving disease resistance (Amar *et al.*, 2004) are the overall beneficial effects.

Immuno-modulation is a procedure that can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as immune-stimulatory drug which primarily implies stimulation of non-specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immuno-suppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immuno-stimulation and immune-suppression both need to be tackled in order to regulate the normal immunological functioning. Hence both immune-stimulating agents and immune-suppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world (Ardó *et al*, 2008).

Medicinal plants have been known as immunostimulants, growth promoters, immune enhancers, where they act as antibacterial and antiviral agents to the host immune system. Natural plant product promote various activities such as anti-stress, growth promotion, appetite stimulation, immune-stimulation, aphrodisiac and antimicrobial properties, due to the active substances such as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids, and essential oils.Herbal extracts are used as immunostimulants to culture fish because they are easily obtained and act against a broad spectrum of pathogensdue to the presence of various active components such as alkaloids, terpenoids, pigments, phenolics, tannins, steroids flavonoids and essential oils (Citarasu 2010).

Most herbs and herbal extracts are administered orally. In fish, medicinal fruits as an immunostimulant enhance the phagocytic capacity of neutrophils and lymphocytes, stimulate secretion of cytokines from lymphocytes, coordinate cellular and humoral immunity and evoke antibody and complement responses (Jeney *et al*, 1993). Growth rates, survival rates and disease resistance have significantly improved by supplementation of immuno-stimulants in fish diet. Some medicinal plants were described as following:

a) **Tulsi (*Ocimum sanctum*):** Leaves of O. sanctum containing water-soluble phenolic compounds and various other constituents may act as an immunostimulant. Leaves extract of O. sanctum affected both specific and nonspecific immune responses. It stimulated both antibody response and neutrophil activity (Kayastha 2014).

b) **Amla (*Phyllanthus emblica*):** *P. emblica* has antioxidant, anti-fungal, anti-microbial, and antiinflammatory activities. Amla fruit pulp contains a large amount of vitamin C as an immuno-stimulant (Liu *et al*, 2008)..

c) **Neem (*Azadirachta indica*):** A. indica possesses antihuman immunodeficiency virus, anti-tumor, and antimicrobial activities. *Azadirachtin*, a triterpenoid derived from *A. indica*, enhanced respiratory burst activities, the leukocyte count and the primary and secondary antibody responses against SRBC (sheep erythrocytes) in tilapia (Das *et al*, 2002).

d) **Pea Eggplant (*Solanum trilobatum*):** The herbal extract of *S. trilobatum* possesses a broad spectrum of antibiotic, antibacterial and anticancer activities. A study showed that the water-soluble fraction of *S. trilobatum* significantly enhanced the production of reactive oxygen and decreased the percentage of mortality following a challenge with *Aeromonas hydrophila* (*Christybapita et al*, 2007).

e) **Bhringraj (*Eclipta alba*):** *E. alba* possesses several medicinal properties. The methanol extracts of E. alba significantly increased the phagocytic index, antibody titer and WBC count in mice (Christybapita *et al*, 2007)..

f) **Ginger (*Zingiber officinale*):** The extracts of *Z. officinale* contain polyphenol compounds which have a high antioxidant activity. Moreover, it showed a significant increase in proliferation of neutrophils, macrophages, and lymphocytes, as well as it enhanced phagocytic,

g) **Garlic (*Allium sativum*):** Echinacea and *A. sativum* improved the gain in body weight, survival rate and resistance against challenge infection of Aeromonas hydrophila. Both compounds developed resistance to cold stress during the winter season (Nya *et al*, 2009).

h) **Green tea (*Camellia sinensis*):** Green tea extracts possess biological activity including antioxidant, antiangiogenesis, and anti-proliferative activities that are related to the prevention and treatment of various forms of cancer (Abdel Tawwab *et al*, 2010).

I) **Ghiu kumari *(Aloe vera):*** Oral administration of A. vera could enhance the specific and non-specific immune responses and increase lysozyme activity, serum bactericidal potency, and the total protein and IgM levels (Alishahi *et al*, 2010).

j) **Bermuda Grass (*Cynodon dactylon*):** The antiviral activity of *C. dactylon* was confirmed to prevent white spot syndrome virus (WSSV) infection with no mortality and no signs of WSD (White spot disease) (Direkbusarakom 2004).

k) **Chirchita (*Achyranthes aspera*):** *A. aspera* showed both specific and non-specific immunity revealed by higher levels of serum antibody and also serum antiproteases in fish. Moreover, the level of serum globulin and RNA/DNA ratio of the spleen were also significantly enhanced in the fish fed with *A. aspera* (Vasudeva Rao *et al*, 2005).

Many parts of the plant materials possess medicinal properties and numerous plant materials are widely used in aquaculture for preventing diseases by controlling the pathogenic microbes and enhancing the immunity. *A. hydrophila* infection in rainbow trout (*Oncorhyncus mykiss*) was controlled by garlic (Corzo-Martinez *et al.*, 2007). Garlic can help in the control of bacteria and fungi and increase the welfare of fish (Logambal *et al.*, 2000). Nya and Austin (2009) used ginger to control an experimental infection of A. hydrophila in rainbow trout, and mortality was reduced to zero compared with the control group. Achyranthes aspera seed was incorporated into the diet of *Labeo rohita*, rohu fingerlings, and the results indicated that *A. aspera* seed stimulated immunity and increased resistance to *A. hydrophila* infection in fish (Logambal *et al.*, 2000). Dorucu *et al.*, (2009) reported that black cumin seed extract enhances the total immunoglobulin level in *Oncorhynchus mykiss* after 3 weeks feeding period. Rainbow trout fed with *Z. officinale* (ginger) extract had significantly higher extracellular activity of phagocytic cells in blood and in trout fed with nettle, and mistletoe extracts increased the production of extracellular superoxide anion (Dugenci *et al.*, 2003).

4. Use of Lapsi (*Choerospondias axillaris*) fruits as an Immunostimulant

Lapsi (*Choerospondias axillaris*) is a large, deciduous fruit-bearing tree of the family Anacardiaceae. A native of the hilly regions of Nepal (850–1900 m above sea level), but is also found in India, China, Thailand, Japan and Vietnam. Lapsi wood is used as light construction timber and fuelwood; seed stones are used as fuel in brick kilns and the bark has medicinal value (Nguyen *et al*, 1996). Lapsi is rich in vitamin C content (Shah 1978). It is generally consumed fresh and processed to prepare a variety of sweet and sour, tasty food products called Mada and Achar locally (Labh *et al*, 2015). Nowadays, other products such as candy, jam, squash and powder are also prepared from Lapsi and its fruits are used in rituals as an offering to the Gods and Goddesses.

Lapsi is cultivated in 301 Village Development Committees (VDCs) in 29 hill districts of Nepal (Paudel 2003). Lapsi trees are found in small patches in forests in mid hills of central Nepal and scattered in private farmlands and at different religious sites. It is a potential agro-forestry tree species to generate income and to provide nutrients in the mid hills of Nepal. With increase in demand for lapsi fruit, popularity of lapsi tree has also increased. It has become a commercially important tree mainly in districts surrounding Kathmandu valley. The increased demand for lapsi as a fruit in Kathmandu led the forestry program to expand the tree plantation. Since then, lapsi farming has become attractive for cultivation (Chakraborty *et al*, 1998).

The annual transaction of lapsi fruit in Kathmandu alone is estimated to be worth over 50 million Nepalese Rupees (approximately US$0.65 million (BM, Hot and spicy business 1999). Lapsi has great potential as a cash-generating tree for hill farming communities in Nepal (Gautam 1997), thus reducing farmers' reliance on subsistence food production and improving their welfare (Tomich *et al*, 1994). In Mongolia, fruits of *Choerospondias axillaris* are used to treat myocardial ischemia, to calm nerves, to ameliorate blood circulation and to improve microcirculation (Dai *et al*, 1992). Fruits of lapsi contain phenolic and flavonoid compounds that possess antioxidants properties (Wang *et al*, 2008).

Fig.1: Lapsi *Choerospondias axillaris* fruits in the tree (photo from Amrit Campus, Kathmandu).

CONCLUSION

Lapsi fruits are rich in essential amino acids, minerals and vitamin C. Lapsi is processed to make candies of various compositions and taste. There is a tremendous market opportunity for processed lapsi, both in domestic and international market. The participatory approach used in the occurrence and distribution of Lapsi in Nepal has been effective to draw a distribution map of a single species of farmers' interest. Lapsi has been growing in 301 VDCs in 29 hill districts of Nepal and is further expanding. Information on distribution of lapsi would help to identify better stands, individual trees for tree improvement. In aquaculture, ethanol extracts of lapsi has been used in fish diets for growth and immunity in fishes. Experiments on tilapia, common carp, major carp, pangasius and rainbow trout have been done successfully. Use of lapsi in the fish diets are the alternate of vaccine and suitable for aquaculture.

ACKNOWLEDGEMENT

Authors are grateful to indigenous people of Kavrepalanchok, Kirtipur and Bhaktapur of Kathmandu Valley for provided information regarding the culture and use of lapsi in the society.

REFERENCES

1. **Abdel Tawwab M., Ahmad M.H. Seden M.E. and Sakr S.F.** (2010). Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against Aeromonas hydrophila infection. *J. World Aquacult. Soc*. 41 (s2): 203-213.
2. **Alishahi M., Ranjbar M.M., Ghorbanpour M., Mesbah M. and Razi Jalali M.** (2010). Effects of dietary Aloe vera on some specific and nonspecific immunity in the common carp (*Cyprinus carpio*). *Int. J. Vet. Res*. 4: 189-195.
3. **Allison E.H.** (2011). Aquaculture, Fisheries, Poverty and Food Security. The World Fish Center.
4. **Amar E.C., Kiron V., Satoh S. and Watanabe T.** (2004). Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum) associated with dietary intake of carotenoids from natural products. *Fish Shellfish Immunol*. 16 (4):527–537.
5. **Ardó L., Yin G., Xu P., Váradi L., Szigeti G., Jeney Z. and Jeney G.** (2008). Chinese herbs (Astragalus membranaceus and Lonicera japonica) and boron enhance the nonspecific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture*. 275 (1-4): 26-33.
6. BM, Hot and spicy business (1999). LAPSI, In: Business manager for managers, special report, (Kathmandu, Nepal) 38.
7. **Bricknell I.R., Bowden T.J., Lomax J. and Ellis A.E.** (1997). Antibody response and protection of Atlantic salmon (*Salmo salar*) immunized with an extracellular polysaccharide of *Aeromonas salmonicida*. *Fish Shellfish Immunol*. 7(1):1-16.
8. **Chakraborty C. and Chattopadhya A.K.** (1998). Turmeric and neem leaf extract in the management of bacterial infection in the African catfish. *Fishing Chimes*. 18 (8):17-18.
9. **Christybapita D., Divyagnaneswari M. and Michael R.D.** (2007). Oral administration of Eclipta alba leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. *Fish Shellfish Immunol*. 23(4): 840-852.
10. **Citarasu T.** (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*. 18(3): 403–414.
11. **Corzo-Martinez M., Corzo N. and Villamiel M.** (2007). Biological properties of onions and garlic. *Trends Food Sci. Technol*. 18(12): 609-625.
12. **Dabrowski K.** (1984). The feeding of fish larvae: present state of the art and perspectives, Reproduction, nutrition and development, Special publication, European Mariculture Society. 24(6): 807-833.
13. **Dahal K.R., Sharma C.M. and Gupta R.K.** (2013). 'Threats on fishery resources and fishers' livelihood due to riverbed extraction in Tinau River, Nepal. *Journal of Sustainable Environmental Research*. 2(1): 1-11.
14. **Dai H. Y., Li Q. A., Chen L. F. and Deng H. W.** (1992). Protective effect of extract from Choerospondias axillaris fruit on myocardial ischemia of rats. *Chinese Traditional and Herbal Drugs*. 23: 641-643.
15. **Das B. K., Mukherjee S. C. and Murjani O.** (2002). Acute toxicity of neem (*Azadiractha indica*) in Indian major carps. *J. Aquac. Trop*. 17: 23–33.
16. **Direkbusarakom S.** (2004). Application of medicinal herbs to aquaculture in Asia. *Walailak J. Sci. Technol*. 1(1): 7-14.
17. **DoFD**. (2017). Fisheries Statistics and Annual Progress Report (Fiscal Year 2016/017), Government of Nepal, Ministry of Agriculture, Land management and Co-operatives.
18. **Dorucu M., Ozesen Colak S., Ispir U., Altinterim B. and Celayir Y.** (2009). The Effect of Black Cumin Seeds, Nigella sativa, on the Immune Response of Rainbow Trout, *Oncorhynchus mykiss*. *Mediterranean Aquaculture Journal*. 2(2): 1-7.
19. **Dugenci S. K., Arda N., and Candan A.** (2003). Some medicinal plants as immunostimulant for fish. *J. Ethnopharmacol*. 88 (1): 99-106.
20. **FAO** (2010). Fish Stat fishery statistical collections: aquaculture production (1950–2008; released March 2010). Rome, Italy: Food and Agriculture Organization of the United Nations.
21. **FAO** (2018). The state of world fisheries and aquaculture. Rome, Italy: Food and Agriculture Organization of the United Nations. See http://www.fao.org/fishery/sofia/en.
22. **Galeotti M.** (1998). Some aspects of the application of immunostimulants and a critical review of methods for their evaluation. *J Appl Ichthyol*. 14(3-4):189-199.
23. **Gautam K.H.** (1997). The sweet and sour tale of Lapsi domesticating and commercializing *Choerospondias axillaris*. *Agroforestry Today*. 9(3): 13-16.
24. **Hemapriya V.S.** (1997). Immunostimulatory effect of leaf extracts of few medicinal plants in Oreochromis mossambicus (Peters). M.Sc. Thesis. The American College, Madurai, India.
25. **Jeney G. and Anderson D.P.** (1993). Enhanced immune response and protection in rainbow trout to Aeromonas salmonicida bacterin following prior immersion in immunostimulants. *Fish & Shellfish Immunology*. 3(1): 51-58.
26. **Jian J. and Wu Z**. (2004). Influences of traditional Chinese medicine on non-specific immunity of Jian

Carp (*Cyprinus carpio* var, Jian). *Fish & Shellfish Immunology*. 16 (2): 185-191.

27. **Kayastha B. L.** (2014). Queen of herbs tulsi (*Ocimum sanctum*) removes impurities from water and plays disinfectant role. *Journal of Medicinal Plants Studies*. 2(2): 01-08.

28. **Labh S.N., Shakya S.R. and Kayasta B.L.** (2015). Extract of Medicinal lapsi *Choerospondias axillaris* (Roxb.) exhibit antioxidant activities during in vitro studies. *Journal of Pharmacognosy and Phytochemistry*. 4(3):194-197.

29. **Liu X., Zhao M., Wang J., Yang B. and Jiang Y.** (2008). Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. *Journal of Food Composition and Analysis*. 21 (3):219–228.

30. **Logambal S.M. and Michael R.D.** (2000). Immunostimulatory effect of azadirachtin in *Oreochromis mossambicus* (Peters). *Indian J. Exp. Biol*. 38(11):1092-1096.

31. **Lorenzen K.** (2005). Population dynamics and potential offisheries stock enhancement: practical theory for assessment and policy analysis. *Philos Trans R Soc Lond B Biol Sci*. 360(1453): 171–189.

32. **Lorenzen K.** (2008). Understanding and managing enhancement fisheries systems. *Rev. Fish. Sci*. 16(1-3): 10-23.

33. **Nargis A., Khatun M. and Talukder D.** (2011). Use of medicinal plants in the remedy of fish diseases. *Bangladesh Res. Publ. J*. 5(3): 192-195.

34. **Nguyen D. D., Nguyen N. H., Nguyen T. T., Phan T. S., Nguyen V. D., Grabe M., Johansson R., Lindgren G., Stjernstrom N. E. and Soderberg T. A.** (1996). The use of water extracts from the bark of *Choerospondias axillaris* in the treatment of second degree burns. Scandinavian. *Journal of Plastic and Reconstructive Surgery and Hand Surgery*. 30(3): 139–144.

35. **Nya E.J. and Austin B.** (2009). Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis*. 32(11): 963-970.

36. **Paudel K. C.** (2003). Domesticating Lapsi, *Choerospondias axillaries* Roxb. (B.L. Burtt & A. W. Hill) for fruit production in the middle mountain agroforesty systems in Nepal. *Himalayan Journal of Sciences*. 1(1): 55-58.

37. **Shah D. J.** (1978). Ascorbic acid (Vitamin C) content of Lapsi (*Spondias axillaris*) pulp and peel at different stage of maturation (tropical fruit tree, Nepal). Food Research Section Research Bulletin. Dept. of Food and Agricultural Marketing Services, Kathmandu.

38. **Shrestha T. K.** (2008). Ichthyology of Nepal. A study of Fishes of the Himalayan waters. Kathmandu (Nepal) Himalayan Ecosphere. 388 p.

39. **Subeenabegum S. and Navaraj P.S.** (2016). Studies on the immunostimulatory effect of extract of *Solanum trilobatum* and *Ocimum sanctum* in *Mystus keletius*. *International Journal of Fisheries and Aquatic Studies*. 4(2): 376-381.

40. **Tomich T.P., Roemer M. and Vincent J.** (1994). Development from a participatory export base in Asia and Africa: legacies and opportunities in development, (ICS Press, San Francisco, USA). 151p.

41. **Troell M., Kautsky N., Beveridge M., Henriksson P., Primavera J., Ronnb ack P., Folke C.** (2013). Aquaculture. In: Levin, S.A. (Ed.), Encyclopedia of Biodiversity. Academic Press, Waltham, MA. 189-201p.

42. **Vasudeva Rao Y. and Chakrabarti R.** (2005). Stimulation of immunity in Indian major carp Catla catla with herbal feed ingredients. *Fish Shellfish Immunol*. 18(4): 327-334.

43. **Verdegem M. C. J. and Bosma R. H.** (2009). Water withdrawal for brackish and inland aquaculture, and options to produce more fish in ponds with present water use. *Water Policy*. 11(51): 52–68.

44. **Wang H., Gao X.D., Zhou G.C., Cai L. and Yao W.B.** (2008). In vitro and in vivo antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. *Food Chemistry*. 106(3): 888–895.

45. **Yin G., Ardo L., Jeney Z., Xu P. and Jeney G.** (2009). Chinese herbs (*Lonicera japonica* and *Ganoderma lucidum*) enhance non-specific immune response of tilapia, *Oreochromis niloticus* and protection against *Aeromonas hydrophila*. *Fish Shellfish Immunol*. 26 (1): 140-145.