Project Title

Managing EHP risk for sustainable shrimp farming through awareness, demonstration and dissemination of 'CIBA EHP Cura I'

Principal Investigator – Dr T. Sathish kumar

Co-principal investigators- Mrs R. Mary Lini, Mr Jose Antony, Dr Riteshkumar Tandal, Dr M Poornima, Dr K Ambasankar

Project coordinator – Dr Kuldeep K Lal

ICAR-Central Institute of Brackishwater Aquacutlure,
75, Santhome High Road, MRC Nagar,
Chennai 600028.

1. Project Title

Managing EHP risk for sustainable shrimp farming through awareness, demonstration and dissemination of 'CIBA EHP Cura I'.

2. Project summary

Shrimp aquaculture is the fastest growing food producing sector making major contribution in nation's foreign exchange revenue. Diseases are the major concern in the sustainable growth of shrimp aquaculture. EHP (*Enterocytozzon hepatopenaei*) the shrimp microsporidian poses a significant threat to the global shrimp aquaculture. EHP is the causative agent of the disease hepatopancreatic microsporidiasis (HPM). EHP is reported to be associated with size variation/ growth retardation and white feces syndrome (WFS). The production loss due to EHP infection in Indian shrimp farms were estimated to be 0.77 M tonnes with a revenue loss of around Rs 4000 crores. In this context, developing treatment and therapeutics protocol against EHP is crucial for the effective management of EHP infection in shrimp farms. However, the studies on therapeutics and treatment against EHP are very limited. Recently, ICAR-CIBA developed a natural plant-based therepeutic 'CIBA EHP cura I' for the treatment of EHP. 'CIBA EHP cura I' is found to be promising in experimental laboratory studies and field validation. 'CIBA EHP cura I' had been commercialized to three private partners and the technology had been released during 96th ICAR foundation day. A large-scale demonstration of this product is necessary to create widespread awareness and adoption among the shrimp farmers which may aid in the effective management of EHP and reducing disease related losses.

Key words: *Enterocytozoon hepatopenaei* (EHP), Hepatopancreatic microsporidiasis (HPM), *P. vannamei*, 'CIBA EHP cura I', Therepeutic.

3. Rationale

Diseases and disease-related losses are the major challenges to aquaculture productivity and sustainability. An emerging microsporidian *Enterocytozoon hepatopenaei* (EHP), the causative agent of hepatopancreatic microspordiasis (HPM) has been reported to cause severe epizootics in shrimp farming nations (Rajendran et al., 2016). EHP falls under the family Enterocytozoonidae and the order Microsporidia. EHP spores are monokaryotic, oval-shaped, and measure 1.1 ± 0.2 μm × 0.6 ± 0.2 μm (Tourtip et al., 2009). The spores are the sole infective stage of the microsporidian. During spore germination the infective sporoplasm is ejected out through the polar tubule into the host (Sathish Kumar et al., 2022a; Vávra and Lukeš, 2013; Weiss et al., 2014). EHP was first reported in *P. monodon* in Thailand in 2009 (Tourtip et al., 2009). Subsequently, the spread and epizootics of EHP were reported in different shrimp farming nations, including Taiwan, Malaysia, Brunei, Vietnam, Venezuela, Korea, Australia, and India (Kim et al., 2022; Liu et al., 2018; Rajendran et al., 2016; Salachan et al., 2017; Tang et al., 2017, 2016, 2015; Tourtip et al., 2009). The infection causes severe necrosis in hepatopancreatic tubules, hepatopancreatic epithelial cells, and desquamation and sloughing of epithelial cells (Tourtip et al., 2009; Rajendran et al., 2016). In India, the probability of occurrence of the disease was estimated to be 17% during 2018-19. EHP infection did not cause

mass mortality, but reported to be associated with stunted growth and white feces syndrome (WFS) (Rajendran et al., 2016; Sathish Kumar et al., 2022b; Sriurairatana et al., 2014). EHP targets shrimp hepatopancreas. The infection in shrimp hepatopancreas may affect the metabolism and, in turn, the shrimp's growth. In India, the annual economic loss during 2018-19 due to EHP was estimated to be around Rs 4000 crores (Patil et al., 2021). However, the studies on prophylactics and therapeutics for the treatment and control of EHP are very limited.

For the control of microsporidiasis, many anti-protozoan drugs have been evaluated. Few chemical drugs such as fumagillin, albendazole, nikkomycin, orlistat, synthetic polyamines, and quinolones were found to be effective in controlling microsporidian proliferation (Wei et al., 2022). Recently albendazole has been reported to control the EHP proliferation (Subash et al., 2023). However, the usage of chemical drugs may cause adverse effects on shrimp and the environment. Various plant extracts, phytochemicals, and essential oils were identified with anti-microsporidian activity (Porrini et al 2011). Recently ICAR-CIBA developed a plant-based therapeutic called 'CIBA EHP cura I' for the control and treatment of EHP. 'CIBA EHP cura I' is a combination of natural phytochemical and nutritional supplements that significantly reduce the EHP load, and vibrio load, and enhance the immunity, growth, and survival of shrimp. The product has been field evaluated in few shrimp farms in Tamil Nadu, Andhra Pradesh, Gujarat, and West Bengal. The laboratory results and preliminary field evaluation revealed the promising outcomes on 'CIBA EHP cura I' in the management of EHP. Further this technology has been transferred to Meenam Aqua needs, Chennai Sai Aqua feeds, Bapatla and M/s Neomeds, Hyderabad for commercial production and supply. Also, the technology 'CIBA EHP cura I', has been released during 96th ICAR foundation day by honorable union Agricultural minister on 16.07.2024. As EHP is the significant challenge to the present shrimp industry, a large-scale field demonstration is very much necessary to create wide awareness and encourage the large-scale adoption among the farmers to save the losses due to HPM.

4. Hypothesis

- The application of 'CIBA EHP cura I' reduces the EHP disease outbreaks
- 'CIBA EHP cura I' can effectively improve the shrimp health and enhance the growth of shrimp compared to untreated ponds.
- 'CIBA EHP cura I' application will increase the survival and yield.
- The usage of 'CIBA EHP cura I' can reduce the usage of antibiotics and harmful chemicals

5. Objectives

- To assess the efficacy of 'CIBA EHP cura I' in the treatment of EHP in shrimp culture.
- To evaluate the impact of 'CIBA EHP cura I' in shrimp growth, health and productivity.
- To promote capacity building among shrimp farmers on sustainable shrimp farming practices by reducing the disease outbreaks and the usage of chemicals.
- To disseminate success stories and promote technology adoption of 'CIBA EHP cura I' among stakeholders.

6. Objective wise activity and Deliverables

Objective wise	***Activity***	***Month & Year of***	***Output monitorable target(s)***
		Start Completion	
To understand the farmers awareness on EHP impact and its management.	Conduct baseline surveys to evaluate the awareness of farmers on disease, management practices and knowledge gaps in the shrimp farms of three states such as Tamil Nadu, Andhra Pradesh and Punjab	Months 1-2	
To assess the efficacy of 'CIBA EHP Cura I' (CURA) in the treatment of EHP in shrimp culture	Selection of EHP prevalent farms based on baseline data and lab results	Months 1- 2	Identified shrimp farms for CURA application
	Application of CURA in the identified treatment ponds and while the control ponds are without any treatment.	Months 3- 20	Implementation of treatment protocol
	Periodic sampling and monitoring EHP prevalence and load through microscopy, PCR and qPCR at regular intervals.	Months 4- 22	Data on spore count, EHP prevalence.
	Evaluation of efficiency variation of CURA by comparing four culture cycles.	Months 4- 22	Efficiency of CURA in different seasons
To evaluate the impact of CURA on shrimp growth, health, and productivity.	Collection of growth parameters (Weight gain, survival, FCR)	Month 6- 22	Shrimp growth parameters data
	Monitoring any improvement in shrimp health by clinically, microscopically especially through histopathological investigations and water quality.	Months 4- 22	Effect of CURA on shrimp health and water quality
	Conduct economic		

	evaluation (Cost benefit analysis) of CURA treated ponds.	Month 20- 22	Demosntration of economic vaiblity of CURA
To promote capacity building among shrimp farmers on sustainable shrimp farming practices by reducing disease outbreaks and chemical usage.	To conduct pre training sessions on the awareness of application of CURA and aquaculture practices	Month 3-4, 7-8, 15-16, 18-19	Promoting capacity building of farmers on the usage of CURA.
	Organizing periodic on-site demonstrations and monitoring of the usage of CURA.	Month 6- 22	Proper usage of CURA and awareness of improved practices
	Developing training materials (guidelines, manuals, videos, e-content)	Month 6- 22	Development of learning content
	Conducting mid programme review and feedback sessions with farmers.	Month 12 - 14	Enhanced farmers involvement
To disseminate success stories and promote technology adoption of 'CIBA EHP Cura I' among stakeholders.	Analyze and compiling of post-harvest results of all culture cycle to identify success stories and key findings (videos, case studies)	Month 14-22	Documenting success stories and key findings
	Conducting and organizing outreach programs/meeting with stakeholders end of the project to present the key findings and adoption strategies.	Month 21-24	Awareness among stakeholders
	Publish findings in extension bulletin, scientific journal, online platform	Month 22- 24	Widespread dissemination of CURA benefits, advantages and application.
	Distribute findings to farmers and stakeholders through interactive sessions and digital platforms.	Month 20- 24	Enhanced adoption of CURA among Stakeholders.

7. Methodology

Baseline survey

A survey will be conducted in 100 farms, twenty farms each of two districts of Tamil Nadu, and two districts of Andhra Pradesh and one district of Punjab to evaluate the awareness of farmers on EHP disease, management practices and knowledge gaps and to understand the impact of EHP in these districts. A standard questionnaire will be developed for the survey.

Site selection and sample design

Based on the baseline survey, farms which had earlier history of EHP and those tested PCR positive for EHP will be selected for this demonstration. The product will be evaluated over four crop cycles in two districts each from Tamil Nadu and Andhra Pradesh and two crops from one district of Punjab. For each crop, four farms will be selected per district resulting in a total of 72 farms included in the study. Each farm will have designated treatment ponds for CURA application with control non treated ponds. This design allows for robust evaluation of CURA in treatment and control ponds under similar farming conditions.

Awareness and capacity building

Conducting pre-project awareness and training sessions for farmers, disease identification and biosecurity measures, sustainable shrimp farming practices and usage and application of CURA across the selected districts of Tamil Nadu, Andhra Pradesh and Punjab. Organizing onsite demonstration during the treatment application of CURA. Developing training materials, guidelines, manuals and videos.

Demonstration

CURA will be administered to the identified shrimp farms during the culture as per the ICAR-CIBA recommended guidelines. The product will be top coated onto feed at the specified dosage to ensure uniform intake by the shrimp. The application will be carried out for a prescribed duration and monitored continuously. The demonstration will be conducted for two culture cycles to evaluate efficacy across seasons.

Sample collection

Shrimp tissues samples will be dissected aseptically and preserved in dry ice at the field site for microscopy, and collected in 95% ethanol for PCR, qPCR and collected in Davidson fixative for histology. For water quality parameters water will be collected and brought to the laboratory.

Monitoring, analysis and Data collection

Data collection will involve regular sampling from both control and treatment ponds. Shrimp samples will be collected and tested periodically for the presence of EHP by microscopy (Sathish Kumar et al., 2022b), and PCR (Jaroenlak et al., 2016) after the administration of

therapeutics. Also, EHP load will be quantified using real-time PCR (qPCR) following standard protocols (Sathish Kumar et al., 2022c). Growth parameters such as average daily growth, feed conversion ratio (FCR), survival rate and overall biomass will be measured 15 days once. Water quality parameters such as Do, pH, salinity, ammonia, nitrite and microbial load will also be monitored 15 days once. Collecting post-harvest data of both and control and treatment to estimate the profitability of CURA like total production - Shrimp production per pond, total expenditure - including feed, CURA, inputs, etc, analysis of data – comparative revenue analysis of both control and treatment.

Economic analysis

The cost effectiveness of application of CURA will be evaluated by involving total production Shrimp production per pond, total expenditure -including feed, CURA, inputs, etc. A structured questionnaire developed with expert input, will include all farming cost parameters including feed, seed, input, treatment and also income from harvest. A comparative analysis will be done between treatment and control ponds.

Harvest Mela and Dissemination.

Harvest mela and mass awareness programmes will be organized at the conclusion of the trial offering a platform for the farmers to share their feedback and success. The findings, success stories and the technology will be disseminated to encourage broader adoption of the product by different stakeholders. Pamphlets, e-content, success stories will be prepared in vernacular languages and popularized.

8. Expected Output

- Increased farmer understanding about EHP and its control
- Reduced EHP infection level in treatment ponds.
- Improved shrimp health, growth, survival and overall productivity
- Increased awareness among the farmers for the adoption of CURA and sustainable, health management practices.
- Dissemination of success stories to encourage wider adoption of the technology.

9. Budget (duration 2 years)

Sl. No	*Items of Expenditure*	*Year 1*	*Year 2*	*Total (Rs. in lakh)*
A	*Non- recurring cost*	-		
1	*Works*	-		
2	*Equipments*	-	-	-
	Sub Total of A (1-2)	0	0	0
B	*Recurring cost*			
3	*Travelling Allowance*	6	6	12
4	*Research & Operational Expenses*			
	i) Research Expenses	10	10	20
	ii) Operational Expenses	5	10	15
5	*HRD (2 YP I)*	7.2	7.2	14.4
	Sub Total B (3 to 5)	28.2	33.2	61.4
	Grand Total (A+B)	28.2	33.2	61.4

10. Scientific team

S. No.	*Consortium Partners (Instt.)*	*Name & Status (PI/Co-PI)*	*Designation*	*Full address with Phone, and E-mail*
1.	Kuldeep K lal	Project coordinator	Director	ICAR-CIBA, Chennai-28, 9415102037, director.ciba@icar.gov.in
2.	T Sathish Kumar	PI	Scientist	ICAR-CIBA, Chennai-28, 8760619879, sathishkumar.t@icar.gov.in
3.	R Mary Lini	Co-PI	Scientist	ICAR-CIBA, Chennai-28, 9820596026, mary.r@icar.gov.in
4.	Jose Antony	Co-PI	Scientist	ICAR-CIBA, Chennai-28, 9514946556, jose.antony@icar.gov.in
5.	Ritesh Tandal	Co-PI	Scientist	ICAR-CIBA, Chennai-28, 7300844288, ritesh.tandel@icar.gov.in
6.	M Poornima	Co-PI	Principal Scientist	ICAR-CIBA, Chennai-28, 9444749519, m.poornima@icar.gov.in
7.	K Ambasankar	Co-PI	Principal Scientist	ICAR-CIBA, Chennai-28, 9444234498, k.ambasankar@icar.gov.in