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ANNEX 2. Chemicals and treatments used in shrimp aquaculture in India

Part A. Summary of chemicals and their uses mentioned in this document

Use in hatchery	Chemical	Recommended concentration (parts active ingredient)
Disinfection of inflow seawater	Sodium hypochlorite [1]	20 ppm for not less than 30 min (or 10 ppm for not less than 30 min)
Chelation of heavy metals in inflow seawater	EDTA, versene	5–30 ppm, depending on concentration of heavy metals in water
Disinfection of discharge water	Sodium hypochlorite	>20 ppm for not less than 60 min
Determination of presence of chlorine in water	Ortho-toluidine	5 drops in 5 ml water sample [2]
Neutralization of chlorine in treated water	Sodium thiosulfate	1 ppm for every 1ppm residual chlorine
Chelation of heavy metals in broodstock tank water & hatching tank water	EDTA	Must be determined based on heavy metal loading at location up to 20 ppm or both at 20–40 ppm
Disinfection of broodstock upon entry to quarantine	Povidone iodine Formalin	20 ppm 50–100 ppm
Disinfection of broodstock following spawning	Povidone iodine	20 ppm for 15 sec (dip)
Washing and disinfecting eggs	Povidone iodine or Formalin, Chloramine-T & Treflan	50–100 ppm for 1–3 min, (or for 10–60 s) 100 ppm for 30 sec 60 ppm for 1 min (for nauplii) 0.05–0.1 ppm (to reduce fungal infections)
Disposal of discarded larvae	Sodium hypochlorite	20 ppm
Removal of epibiont fouling from PL	Formalin	up to 20–30 ppm for 1 h with full aeration
Stress testing of PL	Formalin [3]	30 min
Decapsulation of *Artemia* cysts	Caustic soda (NAOH) & chlorine liquid [4]	40 g in 4 litre (8–10% active ingredient)
Disinfection of *Artemia* nauplii	Sodium hypochlorite solution or Chloramine-T or both	20 ppm Wash with 60–100 ppm for 3 min, or use 30–60 ppm during hatch-out)
Treatment of water in spawning & hatching tanks	Treflan	0.05–0.1 ppm
Footbath	Sodium (calcium) hypochlorite solution	>50 ppm (or >100 ppm)
Disinfection of equipment (containers, hoses, nets etc.)	Sodium hypochlorite or Muriatic acid	20 ppm (or 30 ppm) 10% solution
Disinfection of hands	Povidone iodine or Alcohol	20 ppm 70% solution
Cleaning & disinfection of tanks used for broodstock spawning, egg hatching holding for nauplii & PL, hatching of *Artemia*	Sodium hypochlorite and/or Muriatic acid [5]	30 ppm (or 20–30 ppm) 10% solution (pH 2–3)
Disinfection of previously cleaned & disinfected tanks prior to starting a new cycle	Muriatic acid	10% solution
Disinfection of algal culture tanks	Sodium hypochlorite followed by Muriatic acid	10 ppm 10% solution
Disinfection of sand filters	Sodium hypochlorite or Muriatic acid	20 ppm 10% solution (pH 2–3)
Disinfection of cartridge filters	Sodium hypochlorite or Muriatic acid	10 ppm 10% solution (pH 2–3) for 1 h
Washing of feed preparation equipment (knives, tables, mixers, pelletisers etc.)	Povidone iodine	20 ppm

[1] or calcium hypochlorite
[2] Presence of chlorine is indicated by a yellow color
[3] Salinity change can also be used.
[4] See Section 4.3.2 for details.
[5] In the past, muriatic acid referred to 3:1 HCl and HNO_3, but currently it refers to 34–37% HCl.

Part B. Characteristics of some chemicals and treatments commonly used in shrimp hatcheries

Chlorine

Chlorine is available as hypochlorites (sodium hypochlorite, NaClO and calcium hypochorite $Ca(OCl)_2$). A variety of micro-organisms such as bacteria, fungi, protozoans and viruses are killed by chlorine at various concentrations. Hypochlorites act by releasing hypochlorous acid, which is the primary active ingredient, a potential germicide. They are particularly effective in acidic conditions. The bactericidal effect of hypochlorite is 10 times greater at pH 6 than at pH 9. Shelf life is very short. Sodium hypochlorite will remain stable only at 4 °C, and the entire contents of the package should be used quickly once the container has been opened. Calcium hypochlorite must be maintained in properly sealed polythene bags protected from sunlight in a cool dry place. The effectiveness of chlorine is also affected by the amount of organic matter, reduced compounds and turbidity present in the water to be treated. If chlorine is used in water with high organic matter content, the rate of application should be higher. The dosage depends on the active ingredient of the residual chlorine.

Hypochlorites are not consumed by the animals nor do they penetrate their tissues. It is an active oxidizing agent and its activity is limited to the animal's surface. WSSV is inactivated by contact for 10 min at room temperature with a final concentration of 100 ppm sodium hypochlorite.

Chloramine–T

Chloramine–T is one of the most useful chemicals available for use in aquaculture. It is used as a disinfectant and also as an antimicrobial and anti-protozoan. In comparison with formalin, chloramine–T has greater efficacy against bacteria but much lower efficacy against protozoans. Chloramine–T is considered to be a safer disinfectant than chlorine because chlorine combines with organic matter to form carcinogenic trichloromethane.

Formalin

Formalin is a generic term that describes a solution of 37 percent formaldehyde gas dissolved in water. At 1 percent it is effective in killing spores of protozoans. Formalin effectively kills external parasites. It is not the preferred treatment for external bacterial or fungal infections. Formalin is not effective against internal infections of any type. It acts as a disinfectant, antiseptic and astringent. For application, dilution is necessary in order to insure that therapeutic dosages may be safely discharged to receiving waters. In most current applications, this dilution will occur before discharge. Treatment concentrations are typically 100 ppm for 30 seconds for control of MBV in eggs and nauplii and 10–30 ppm for 1 h for treating external parasites of larvae and PL. Prolonged bath treatment is not safe at any larval stage. Water quality parameters such as DO, CO_2, pH, total ammonia and nitrite are influenced when used at recommended rates, as it is toxic to many aquatic plants, especially phytoplankton and algae. Formalin is toxic to aquatic life at low concentrations with 96-h LD_{50} values ranging from 1 to 1 000 ppm depending on species. Formalin toxicity is increased at high water temperatures. If water temperatures exceed 21 °C, the concentration used should be reduced. Formalin is a potential carcinogen and should be handled very carefully to avoid skin contact, eye irritation and inhalation.

EDTA

EDTA (di-sodium ethylene diamine tetraacetic acid [$(C_{10}H_{14}N_2O_8Na_2.2H_2O)$] is used to treat ectocommensal fouling by stimulating juvenile molting. Added to larval-rearing water in shrimp hatcheries it chelates divalent and trivalent metal cations,

reducing the bioavailability of heavy metals by complexation. It is used to improve water quality by reducing heavy metal concentrations. EDTA may also help reduce bacterial contamination of the eggs, allowing better oxygen transfer into the egg and enhancing hatching rate. EDTA can inhibit the capacity for agglutination and adhesion in some bacteria and the production of extracellular proteases by some pathogenic vibrios, including *Vibrio harveyi*.

In shrimp larval rearing, it is applied at 5–30 ppm for spawning and hatching and at 5–10 ppm prior to stocking of nauplii. It may also be applied at 1–5 ppm to remove organic substances in the water.

Iodine

Iodine (I_2) is available as polyvinyl pyrrolidone iodine (PVPI)/iodophor compounds (povidone iodine). These preparations are complexes of iodine with a solvent or carrier that liberates free iodine into solution slowly. They can prevent and control diseases caused by *Aeromonas*, *Pseudomonas*, *Vibrio*, fungi and several viruses. These compounds are particularly used to treat eggs and larvae and to disinfect equipment. They are lethal to viruses, which are killed within 15 min in a 50 ppm solution. Iodine is an oxidizing agent that can oxidize and inactivate proteins with sulfhydril groups (-SH group). The action involves halogenation of tyrosine units of enzymes and other cellular proteins requiring tyrosine for activation. The preparation is diluted in water and added as required. The iodine compounds should be maintained in airtight dark bottles in a cool dry place. In such situations they can remain stable for long periods. Shrimp eggs and nauplii are dipped in iodine solution for 30 seconds at concentrations of 50 ppm and 100 ppm, respectively, to avoid the transmission of MBV and other viruses from broodstock. The shrimp body does not consume iodine and no residual effects are reported.

Treflan

Treflan is a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine having the empirical formula $C_{13}H_{16}F_3N_3O_4$. Trade names include Flurene SE, Treflan, Tri-4, Trust, M.T.F., Trifluralina 600, Elancolan, Su Seguro Carpidor, Trefanocide, Treficon, Trim, L-36352, Crisalin, TR-10, Triflurex and Ipersan. Treflan is commonly used as a prophylactic chemical against fungal infections such as larval mycosis in shrimp caused by *Lagenidium callinectes*, *Haliphthoros* sp. etc. It inhibits cell wall synthesis in fungi. The chemical has to be diluted in water and applied to the rearing water at appropriate concentrations. When refrigerated it remains stable for more than two years. Dose rates of 0.2–1.0 ppm have no inhibitory effect on hatching rate of eggs of *Penaeus monodon*. However, survival rate of hatched nauplii subsequent to treatment will be significantly reduced in most cases. Treflan at 0.05–0.1 ppm is recommended to treat *Lagenidium* sp. infections in nauplii or larvae. Treflan is not known to be absorbed by animal bodies, and residual effect in host species is not recorded. Exposure of non-target species such as *Teraselmis cheuii*, a phytoplankton used as food for penaeid larvae, to 0.1ppm trifluralin delays its growth and reduces protein content. Treflan is rapidly degraded in soil-water systems.

Ascorbic acid (Vitamin C)

Known as ascorbic acid, the L-isomer is the physiologically active form of vitamin C. The R-isomer, which is called erythorbic acid (or occasionally iso-ascorbic acid), has no vitamin value, although it does function as an *in vitro* antioxidant. The ingredients used for the manufacture of compounded feeds are unlikely to contain any measurable amounts. Vitamin C is usually measured in weight units of pure crystalline L-ascorbic acid. Diets supplemented with vitamin C have been reported to give protection against bacterial pathogens.

Ascorbic acid is transported to all living cells for use in important oxidation/reduction reactions in cell metabolism. It is essential for the formation and maintenance of function of the intercellular substances of skeletal tissues, particularly collagen. It also exerts a stimulant action on defensive mechanisms. According to recent research, it plays an essential role in transporting iron from plasma to storage sites. It is an important intercellular antioxidant and is involved in quenching highly reactive free radicals. In addition to reacting directly with aqueous free radicals, ascorbic acid indirectly affects the balance between oxidative products and antioxidant defense mechanisms. Accordingly ascorbic acid can donate an electron to the tocopheryl free radical, regenerating the active tocopherol. The concentration of ascorbic acid in phagocytes in blood is many times higher than in the erythrocytes and is approximately 150 times the concentration in plasma. These phagocytes use free radicals and other highly reactive oxygen-containing molecules to help kill invading pathogens. The antioxidant action of ascorbic acid helps protect these cells from oxidative damage.

Crystalline ascorbic acid is relatively stable in air if moisture is completely absent. In the presence of even small amounts of moisture there is rapid oxidation, first to dehydroascorbic acid, and then to other, non-vitamin-active products. To optimize stability of vitamin C during feed production and storage as well as bioavailability in fish/shrimp, the use of vitamin C in its phosphorylated form is recommended. Active uptake of vitamin C seems to be very important at low doses while at high doses, uptake by passive diffusion also occurs.

Benzalkonium chloride (BKC)

Benzalkonium chloride is a quaternary ammonium compound containing four carbon-containing groups and a negatively charged ion such as bromine or chlorine. A large number of different quaternary ammonium compounds have been synthesized and evaluated for their antimicrobial action. Bactericidal concentration ranges from 1 part in a few thousand to 1 part in a million under dilution. The combined properties of germicidal and detergent action, low toxicity and high solubility in water, stability in solution and noncorrosiveness are its important characteristics.

The mode of action includes denaturation of proteins and interference with glycolysis and membrane damage. The most likely site of damage to the cell is the cytoplasmic membrane where the compound alters the vital permeability feature of the cell structure.

Benzalkonium chloride is targeted at a variety of microorganisms including *Lagenidium* and *Haliphthoros* species. Shrimp farmers use it to reduce the concentration of plankton and dinoflagellates in closed pond systems. In shrimp hatcheries and grow-out systems it is used in low concentrations of 0.1–0.5 ppm. At 10 ppm no inhibitory effect on the hatching rate of eggs of *Penaeus monodon* has been noted, but survival rate of hatched nauplii was significantly reduced. At 0.01 ppm, BKC has immunostimulatory properties and at 1.0 ppm it can induce moulting. If applied in large amounts, the resulting decomposition of organic matter will have an effect on animal health.

β-1-3 glucan

β-glucans are the cell wall component of mushroom and yeasts, which appear to be the most promising of all immunostimulants so far examined in fish and shrimp. β-glucans are poly-glucose molecules linked through β-1,3 bonds in a long chain with β-1,6 branches consisting of single or multiple glucose molecules. Such glucans can exist in various structural forms and may be in the form of water-soluble oligomers, water-soluble or insoluble macromolecules or particulates. Glucans extracted from *Saccharomyces cerevisiae* (baker's yeast) is one such type and is an important structural element of the yeast cell wall. Yeast glucans are polysaccharides composed of smaller

units linked together by β-1,3 bonds. These bonds hold the glucan molecule together, hence the name, β-1,3 glucan.

The mode of action of β-1,3 glucan is that there is a specific receptor for β-1,3 glucan on the surface of macrophages that when activated, stimulates a cascade of events turning the body into "an arsenal of defense". There is now evidence to show that glucan is, from an evolutionary point of view, the most widely and commonly observed macrophage activator in nature and is proven to overcome the negative effects of immunosuppression.

The phenol oxidase system is an important element of the disease resistance of crustaceans. Crustaceans use lipopolysaccharide and the β-1,3 glucan structure as specific signals to activate the prophenol oxidase system. The crustacean blood contains proteins that specifically bind β-1,3 glucans. Activation of this protein on the hemocytes by β-1,3 glucan induces degranulation and release of the prophenol oxidase, which can be converted from its proform into an active enzyme by serine proteases. Phenol oxidase then oxidizes the phenolic group containing amino acids (thyrosine) into semiquinones, which have microbicidal action, and these semiquinones are polymerized into melanin.

Commercially available forms of β-glucan are Macrogard, Betafectin, Lentinan, Schizophyllan and Scleroglucan. They are normally added to the feed and fed directly to the shrimp at concentrations recommended by the manufacturers.

Ozone

Ozone is a powerful oxidizing agent with numerous beneficial uses in aquaculture. The quality of aquaculture production water can be improved by ozone treatment, including improvement in solids settling and reductions in nitrite-nitrogen (NO_2-N), colour, fine particulate matter and microbial activity. Ozone shows excellent potential for many aquaculture systems because of its rapid reaction rate, few harmful reaction by-products and the oxygen produced as a reaction end product.

Ozone use for aquaculture began in the mid-1970s and was initially focused on disinfection and colour reduction of aquarium water with low fish densities and low feed loading. Since then ozone has been used to improve water quality in various types of aquaculture systems, ranging from flow-through raceway systems to indoor recirculating systems. Although ozone has proven effective in the reduction and control of certain water quality characteristics, it is not a one-step water treatment technology. Because less ozone is needed when supporting water treatment technologies are also used, the use of ozone as part of a larger water treatment system maximizes its efficacy and cost-effectiveness. The effective concentration for ozone to reduce WSSV infectivity to zero is 0.5 µg/ml as a total residual oxidant for 10 minutes at room temperature.

Ultra-violet (UV) radiation

UV rays are non-ionizing radiations that can cause permanent damage to DNA and consequent death of cells, if doses are high enough. It has been calculated that WSSV becomes inactivated by 60 min UV irradiation at 900–000 mws/cm^2. However, most commercial UV systems are only rated at 15 000–30 000 mws/cm^2, which is still sufficient to kill most bacteria, fungi and protozoans, but will have little effect on most viruses. Therefore appropriate standardization and calibration has to be made for effective treatment.

ANNEX 3 - List of antibiotics and pharmacologically active substances banned for use in aquaculture in India[1]

1. Chloramphenicol
2. Nitrofurans including Furazolidone, Nitrofurazone, Furaltadone, Nitrofurantoin, Furylfuramide, Nituratel, Nifursoxime, Nifurprazine and all their derivatives
3. Neomycin
4. Nalidixic acid
5. Sulphamethoxazole
6. *Aristolochia* spp. and preparations thereof
7. Chloroform
8. Chlorpromazine
9. Colchicine
10. Dapsone
11. Dimetridazole
12. Metronidazole
13. Ronidazole
14. Ipronidazole
15. Other Nitroimidazoles
16. Clenbuterol
17. Diethylstilbestrol (DES)
18. Sulfonamide (except approved sulfadimethoxine, sulfabromomethazine and sulfaethoxyrpyidazine)
19. Floroquinolones
20. Glycopeptides

[1] Source: Coastal Aquaculture Authority (2006).

ANNEX 4 - Quarantine/maturation tank daily data sheet

Tank No: Sex: Date stocked: Source: Condition: Gravid: Weight (g): Length (cm): Date of ablation:

Date	Time	Volume	No. males	No. females	Feed type	Feed Amount (g)	Feed Left Over	Water exchange (%)	Temperature (°C)	Salinity (ppt)	Notes

ANNEX 5 - Spawning/hatching tank daily data sheet

Tank No:

Date	Volume	No. females	Ovary stage	No. Source tank (ST or tonnes)	No. Spawned	No. Eggs	No. Nauplii	Temperature (°C)	Salinity (ppt)	Transferred to LRT No.	Notes

ANNEX 6 - Larval-rearing tank daily data sheet

Tank No: **Date stocked:** **Nauplii stocked:** **Female No.:** **PL harvested:** **Survival (%):**

Day	Date	Larval stage	Larval number	Larval health	Tank volume (Tonnes)	Water exchange (%)	Temp (°C)	Algae			*Artemia*		Artificial feed		Notes
								Species	Cells/ml in tank	Cells/ml fed	No./ml in tank	No./ml Fed	Type	g. fed	
1		N6/Z1													
2		Z1													
3		Z1/Z2													
4		Z2													
5		Z3													
6		M1													
7		M2													
8		M3													
9		M3/PL													
10		PL1													
11		PL2													
12		PL3													
13		PL4													
14		PL5													
15		PL6													
16		PL7													
17		PL8....													
18		PL15													

ANNEX 7 - Level 1 larval health data sheet

Tank No: **Date:** **Average Score: (>60 good, 40–60 medium, <40 poor):**

Larval sample	Swimming activity	Phototaxism	Faecal string	Luminescence	White-body disease	Homogenous stage	Intestinal contents	Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
Total								
Notes: Average								

Scores: 10 Good, highest, most; 5 Medium; 0 Poor, lowest, least.

ANNEX 8 - Level 2 larval health data sheet

Tank No: **Date:** **Average Score: (>60 good, 40–60 medium, <40 poor):**

Larval sample	Hepatopancreas (Lipid Vacuoles)	Intestinal Contents	Necrosis	Deformities	Epibiont fouling	Bolitas	Baculovirus	Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
Total								
Notes: Average								

Scores: 10 Good, highest, most; 5 Medium; 0 Poor, lowest, least.

ANNEX 9 - PL quality testing results sheet

Tank No.: Date: PL Stage: Female No.: Score (>50 pass):

1. Gross examination			
Characteristic	Comment		
Size distribution			
Size (more than 12 mm)			
Swimming activity			
Feeding			
Colour			
2. Microscopic examination			
Hepatopancreas	Full, dark (10)	Medium (5)	Empty, pale (0)
Gut condition	Full, vacuoles (10)	Medium (5)	Empty, SHG (0)
Fouling	None (10)	Medium (5)	Heavy (0)
Deformity	None (10)	Medium (5)	Heavy (0)
Muscle:gut ratio	75% (10)	50-75% (5)	<50% (0)
MBV	None (10)	Few (5)	Many (0)
3. Stress test			
Freshwater (1:1)	>75% (pass)	<75% (fail)	
Formalin (100ppm)	>75% (pass)	<75% (fail)	
4. V*ibrio* testing			
Green	<60/plate (pass)	>60/plate (fail)	
Yellow	<80/plate (pass)	>80/plate (fail)	
Luminescence	Absent (pass)	Present (fail)	
5. PCR testing			
WSSV	Negative (pass)	Positive (fail)	

ANNEX 10 - Research and development and extension requirements

Broodstock:

- Reasons and solutions for the seasonal variations in quality/quantity of broodstock caught at each landing centre
- Development and design of transportation containers for broodstock from the wild
- Comparison and evaluation of performance of induced matured spawners versus wild gravids
- Anaesthetics and transportation of broodstock
- Nutritional requirements and the role of live and artificial diets in promoting maturation and good quality larvae
- Genetic selection of families/domestication and captive production of SPF/SPR lines of *Penaeus monodon*
- Vertical disease transmission issues
- Cryopreservation of eggs and sperm of *P. monodon*
- Reasons for failure/success of induced maturation programmes in hatcheries
- Validation of re-circulation system with bioreactor technology for maturation and larval rearing systems

Larval culture:

- Nutritional requirements and the development of appropriate artificial diets for each larval stage
- Probiotics and their role in nutrition and health management
- Larval diseases and methods for their prevention
- Research on replacement of live feeds to minimize use of *Artemia* and reduce feed cost
- Development of reliable algal culture laboratories to supply algal stock to hatcheries
- Analysis of the options for nursery rearing of PL

Others:

- Bioremediation of wastewater

Extension requirements:

- Harmonization and intercalibration of PCR methods used in diagnostic centres and hatcheries
- Suggested annual closure of all hatcheries on the east coast during November, December
- Training on broodstock capture/handling and transport on-board vessels, at landing centres/nauplii centres etc.
- Awareness programmes for broodstock collectors, handlers and auctioneers
- Hands-on training for hatchery technicians for better management practices
- Awareness programmes on better management practices for commercial nursery owners
- Awareness programmes to eliminate chemicals, antibiotics and other drugs by adopting alternate approved methods such as probiotics
- Information dissemination to farmers using the cluster approach on seed selection, pond preparation, stocking and pond management
- Training of nontechnical and managerial staff
- Upgrading skill of technical staff
- Development of quarantine at landing centres

- Definition and utilization of biosecure procedures in hatcheries
- Government-run training of hatchery technicians for maturation, larval and PL rearing about phytosanitary measures and critical control points