RHEA natural

POND GUARD


Broodstock
Hatchery
Shrimp Farming
Processing Plant
Finish Product
POND GUARD

CONTENTS

Product Introduction

- Pondguard is an Immuno-modulator.
- It maintains the general health of shrimp by improving the immune system of the animal.
- It works directly against specific pathogens like, WSSV,IMNV and EMS/AHPND etc.

Pondguard consist of natural oils extracts:

Lavender Oil. Pine Oil and Eucalyptus oil.

Characteristics of Pondguard

Product Description: Liquid.

Characteristics:
It has physical properties similar to potable water,
such as pH 6.5-7.5, alkalinity 140-180 ppm, salinity 0 ppt.
It is non-hazardous, user-friendly and consumable.

Packaging: plastic bottles **1.2L, 5L** and **20L**

It efficiently works in both high and low saline water environment.

Indication

- Pondguard, containing Natural oils, possesses pronounced capability to stimulate the induction of humoral and cell-mediated immune response of lymphocytes to the antigens of pathogenic bacteria and alloantigen.
- It maintains the immunity level of the shrimp which helps to protect shrimp from infectious diseases.
- It maintains the basic metabolic function of animal body. It is able to reduce the stress level in shrimp.
- It reduces the load of harmful pathogen (virus and bacteria) in the culture environment.
- It acts directly against specific pathogens like WSSV, IMNV and EMS/AHPND etc.

Recommended Dose of Application

Application		Culture Pond	Reservoir
Pond Preparation		Day -7 & Day -3 (0.4 ppm) 4 L / ha	Day -7 & Day -3 (0.4 ppm) 4 L / ha
During Culture	Normal Case	2 times/week (0.2 ppm) 2 L / ha	2 times/week (0.2 ppm) 2 L / ha
	Special Case (Stress Condition i.e. disease, DO drop, plankton crash etc)	2-3 times/week (0.2 ppm) 2 L / ha	2-3 times/week (0.4 ppm) 4 L / ha

Recommended usage per Hectare

Culture pond (100 days of Culture)

Number of application = 30 times

Total usage = 56-60 L/cycle.

Reservoir pond (100 days of Culture)

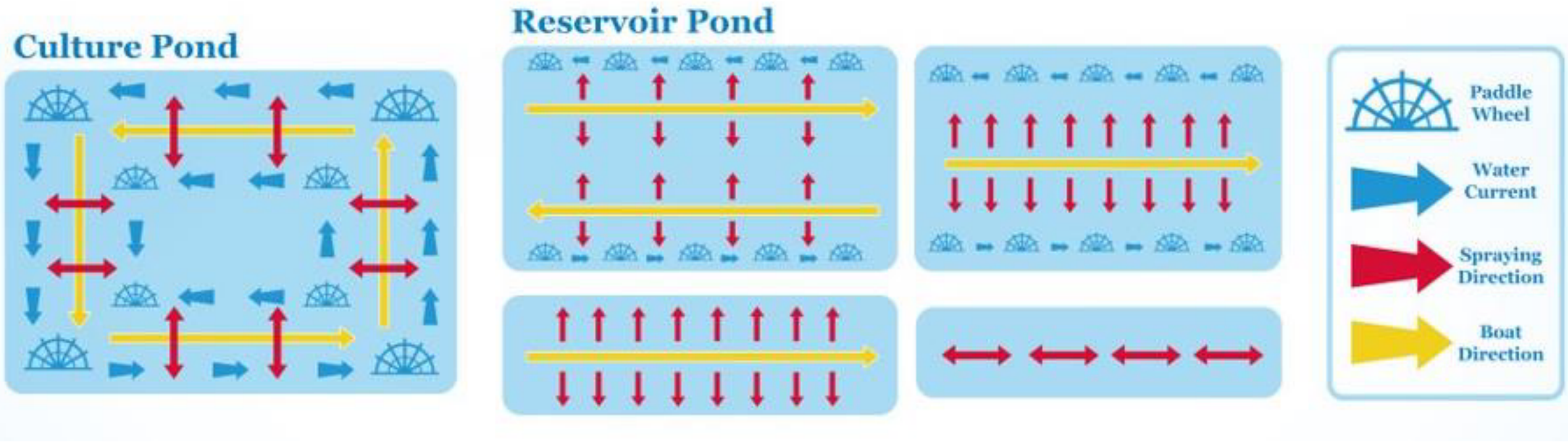
Number of application = 30 times

Total usage = 56-60 L/cycle.

Preparation Method

- Mix recommended dose of Pondguard in 10 liter of clean water.
- Mix well for 2 to 3 minutes.
- Apply over pond in maximum current area.
- Paddle wheel should be kept running for at least 1 hour, for homogenous and rapid mixing.

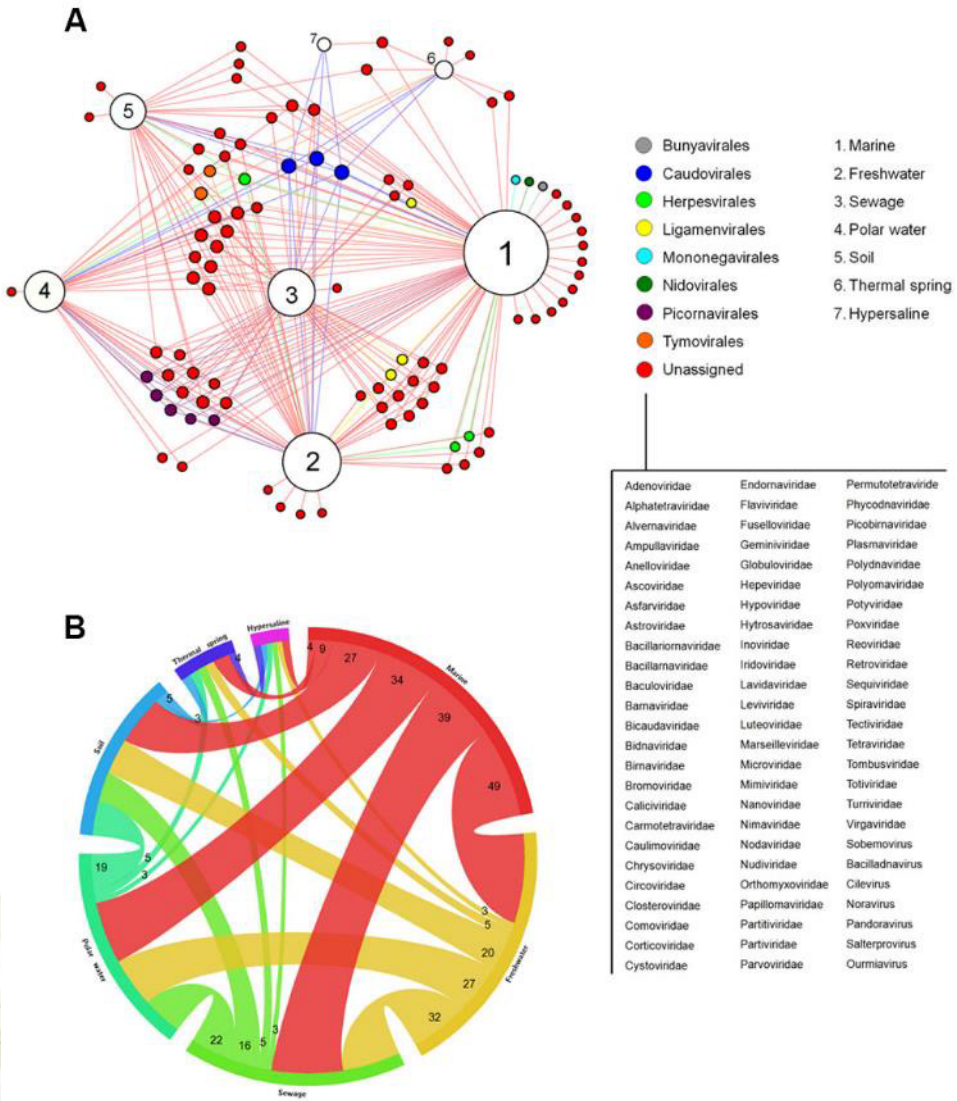
Application Method



Time of application: Suitable time of application 9.00 to 16.00 hrs.

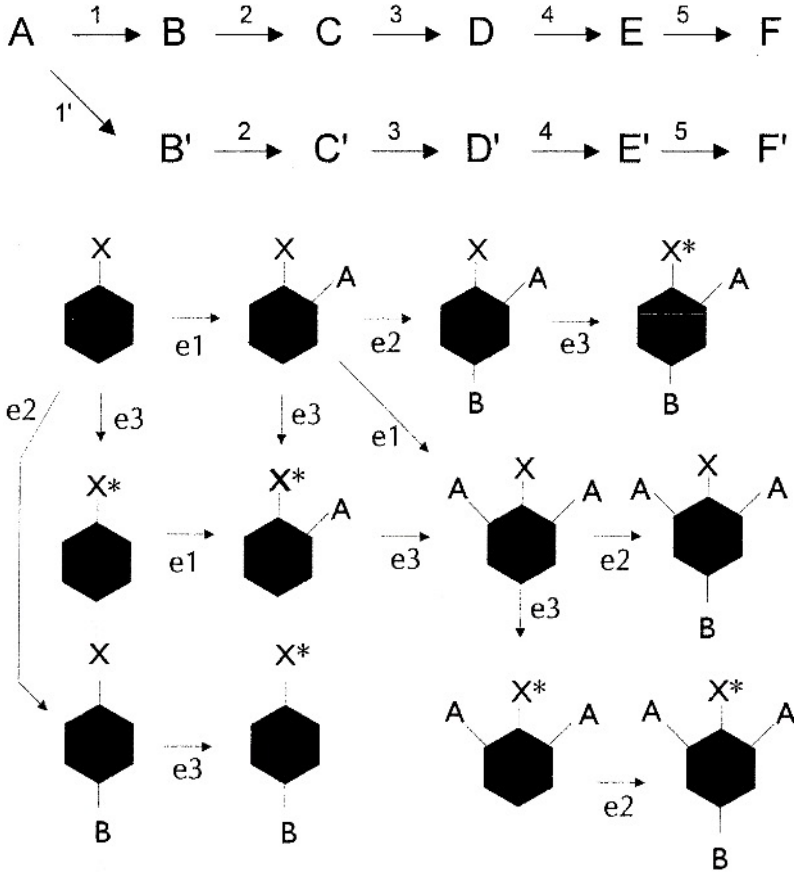
Storage and handling: stored at room temperature

CONCEPT 1



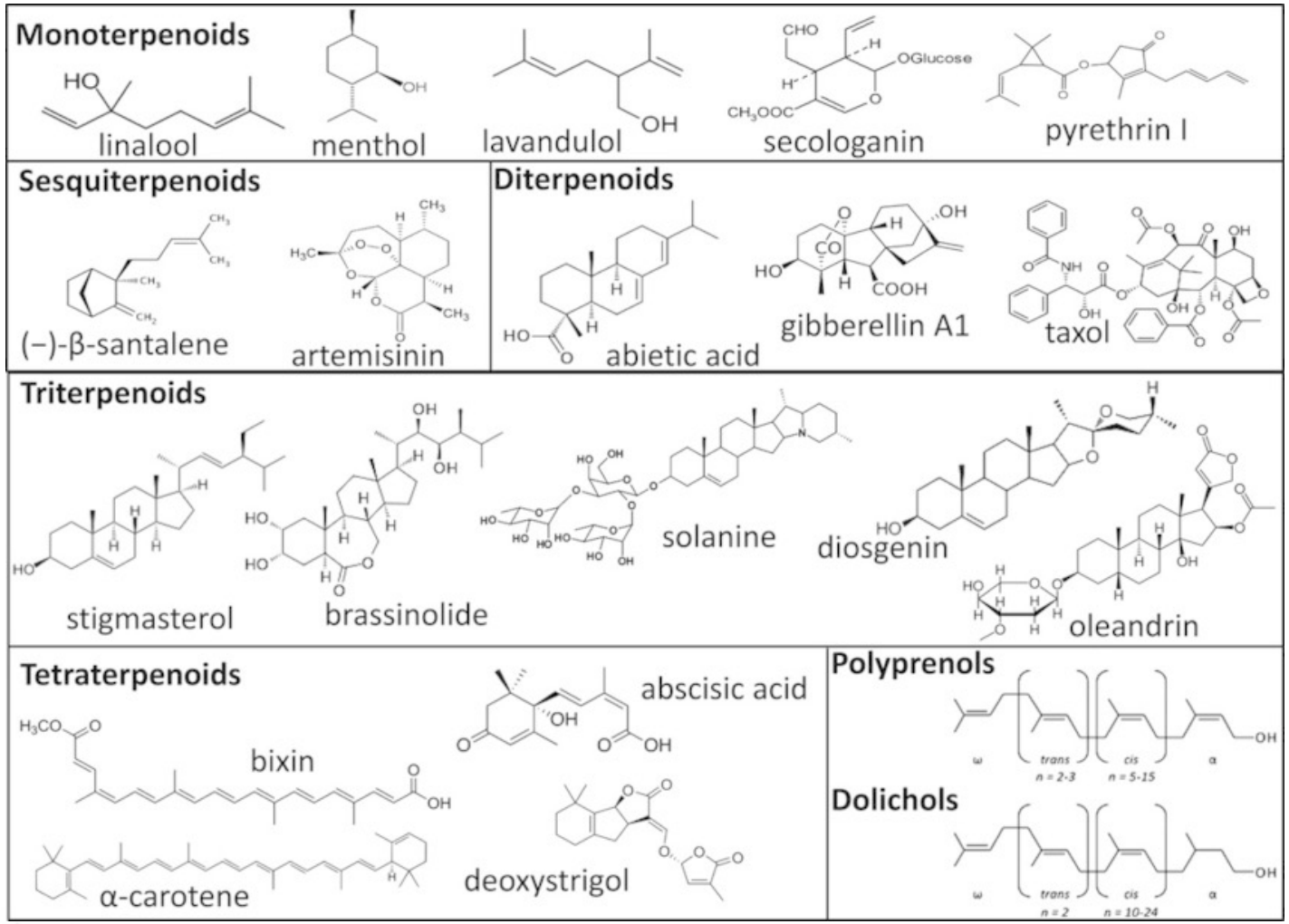
- The ever-present virosphere
- Dark matter of the virosphere: persistence
- Since virus persistence is exceedingly common but usually a silent state, it represents a large but mostly unnoticed force in **evolution—the dark matter of biology**.
- Jonathan R. Goodman 2020

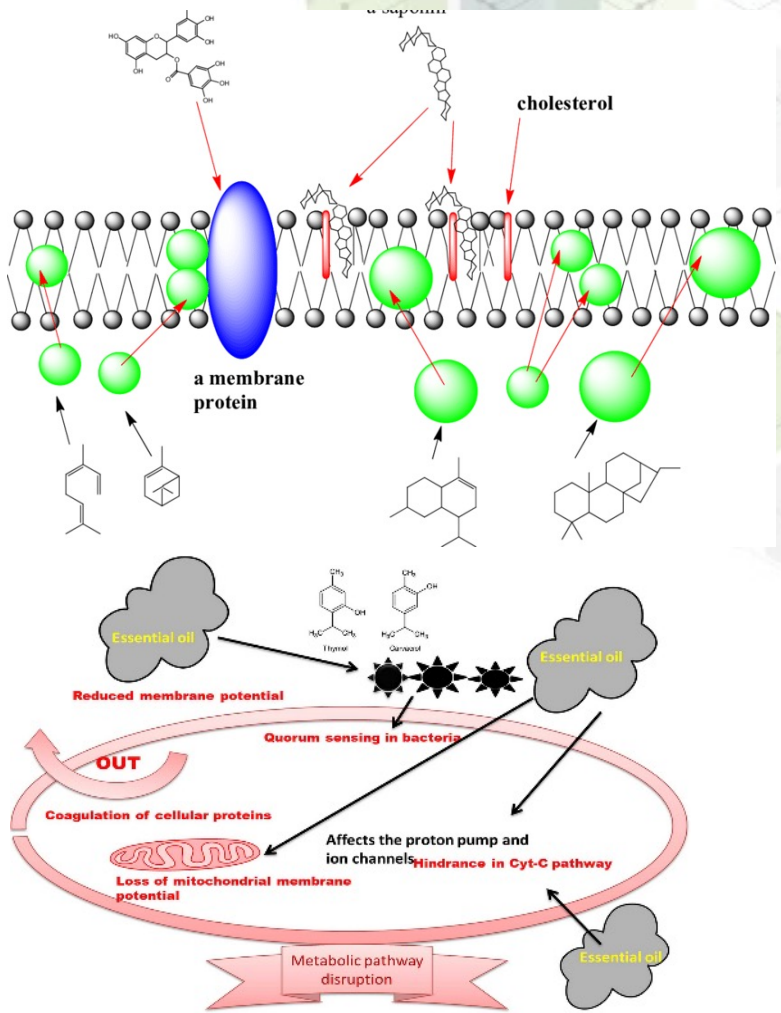
Concept 2



- Evolution and natural product diversity. **The Screening Hypothesis – the basic concepts**
- *using enzymes with broad substrate specificity*
- *exploiting the fact that many chemical reactions give multiple products.*
- *Branched and matrix pathways*
- **Richard D. Firn and Clive G. Jones 2003**
- Phytochemical diversity: **The sounds of silent metabolism**
- **Efraim Lewinsohn, Mark Gijzen 2009**

Concept 3


Monoterpenoids
linalool
menthol
lavandulol
secologanin
pyrethrin I
Sesquiterpenoids
(−)-β-santalene
artemisinin
Diterpenoids
abietic acid
gibberellin A1
taxol
Triterpenoids
stigmasterol
brassinolide
solanine
diosgenin
oleandrin
Tetraterpenoids
bixin
α-carotene
abscisic acid
deoxystrigol
Polyprenols
Dolichols

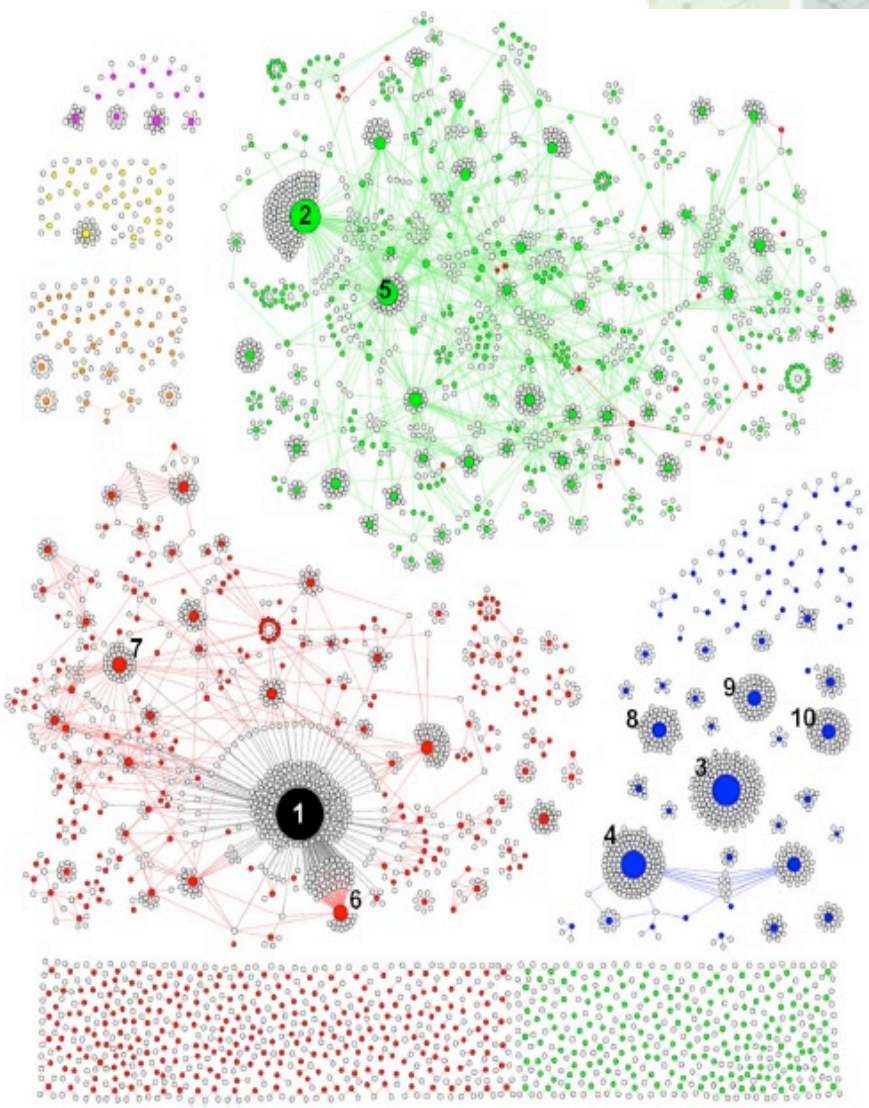

cholesterol
a membrane protein
Essential oil
Reduced membrane potential
Quorum sensing in bacteria
OUT
Coagulation of cellular proteins
Affects the proton pump and ion channels
Hindrance in Cyt-C pathway
Loss of mitochondrial membrane potential
Metabolic pathway disruption

Concept 4

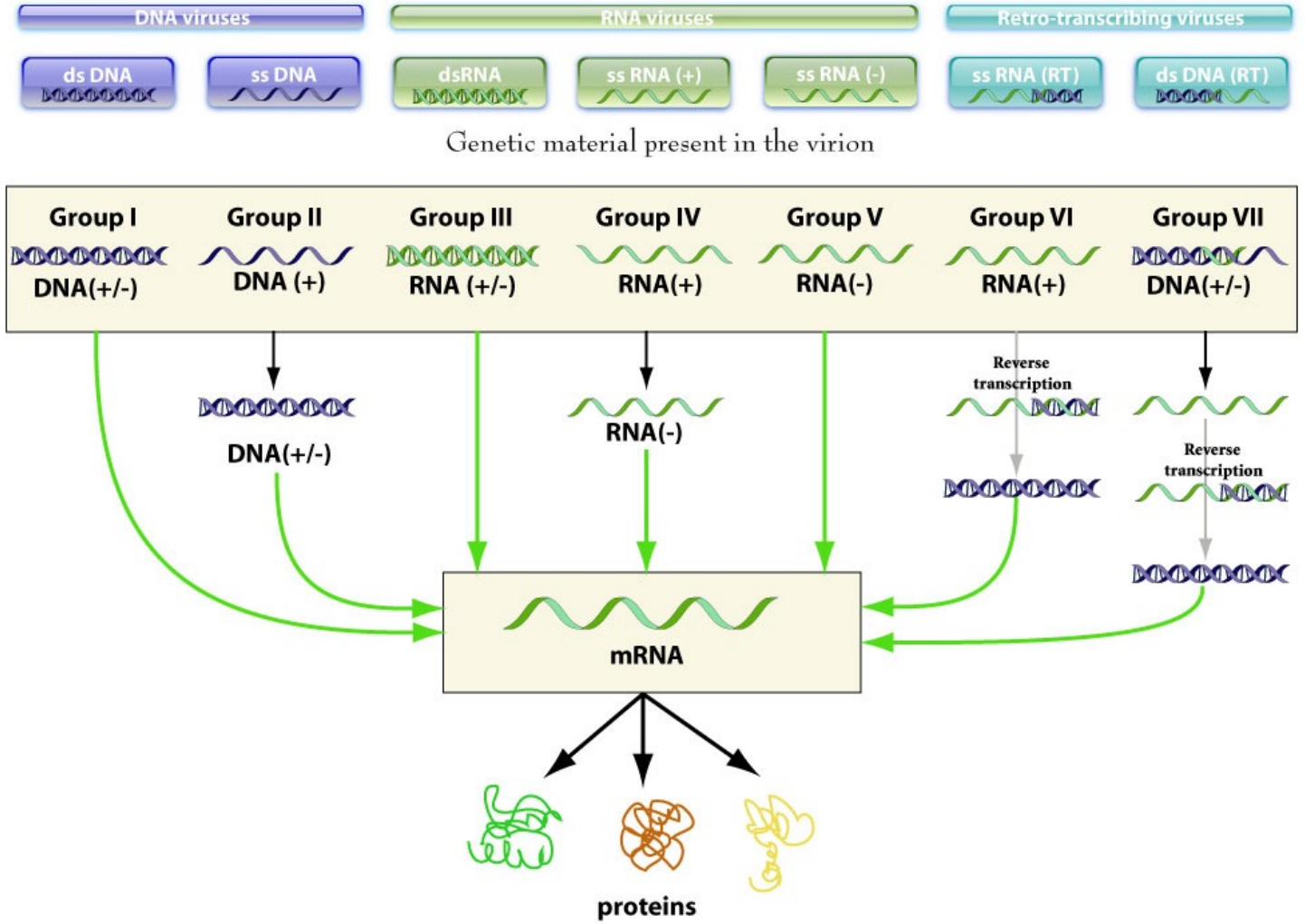
Definitions of One Health

One Health Commission

One Health is a collaborative, multisectoral, and trans-disciplinary approach - working at local, regional, national, and global levels - to achieve optimal health and well-being outcomes recognizing the interconnections between people, animals, plants and their shared environment.



CONCEPT PROVING

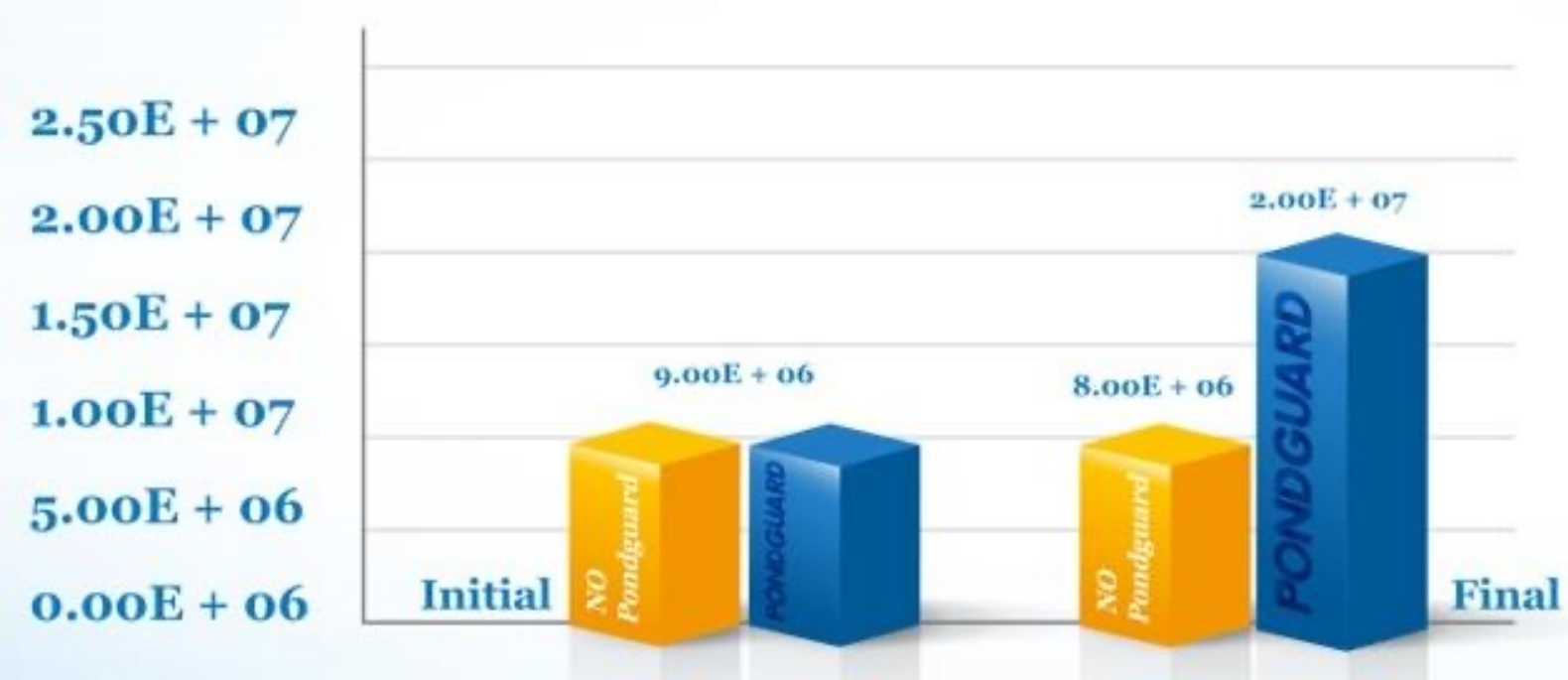


Pondguard as an Immunomodulator

Pondguard Trial at Lab Scale: IMMUNITY

Total Hemocyte Count:

Shrimp Total Hemocyte Count increased after 3-4 weeks of PG application.



- This trial was designed to observe the efficacy of Pondguard as immunomodulator.
- A hemolymph count of a normal healthy shrimp should be about log 7.
- A 3 weeks controlled small scale trial was conducted.
- The initial and final hemolytic count of shrimps were analyzed from Pondguard applied tanks and not applied tanks.
- The hemolytic count of shrimp in Pondguard applied tanks were found in optimum level i.e. log 7 after 3 weeks of application.
- The trial shows that Pondguard is acting as immunomodulator.

Lab Trial

Trial 1

Efficacy of Pondguard against AHPND/EMS pathogen in Shrimp

Disease Challenge lab,

Center for Research in Agriculture and Fisheries, Hanoi, Vietnam

POND GUARD

Objectives

- To determine the antimicrobial activity of Pondguard against *Vibrio parahaemolyticus* (AHPND) bacteria
- To recommend for application on shrimp farms in Vietnam based on trial results

Materials and Methods

Group	R	Shrimps		Challenge Methods		Product Application	Water Exchange	
		MBW	Number of shrimps	Volume of Bacteria/TSB	Immersion Time		Rate	Frequency
Negative Control	3	0.6 - 0.8	20	30 mL of TSB	15 minutes		20%	Every day started at dpi 3
Positive Control				30 mL of bacteria				
Treatment 1				30 mL of bacteria		0.2%		
Treatment 2				30 mL of bacteria		0.3%		

Challenge Preparation Procedure

Re-culture the *Vibrio parahaemolyticus* into chrom agar Vibrio (CAV).

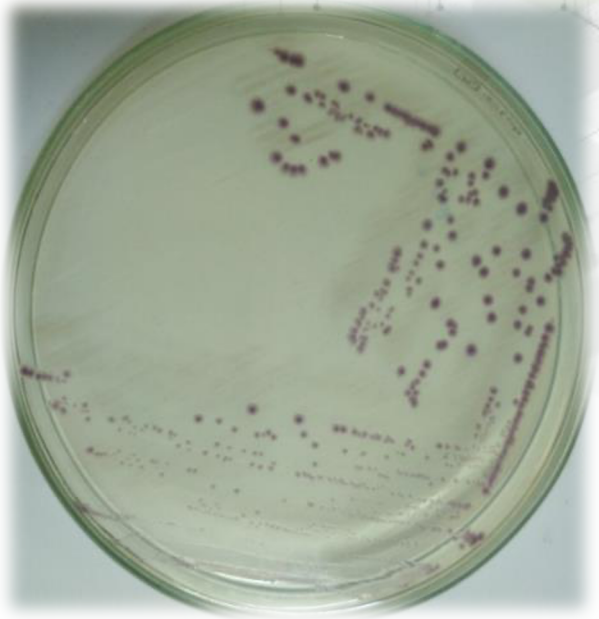
↓

Take 6 bacteria colonies and put into 10 mL of TSB +; incubate for 18 hours at 28°C with shaker

↓

Treatment 1 : inoculate 30 uL of bacteria into 30 mL and add 60 uL of **PG (0.2%).** incubate for 18 hours at 28°C with shaker

Treatment 2 : inoculate 30 uL of bacteria into 30 mL incubate for 18 hours at 28°C with shaker and add 90 uL of **PG (0.3%)**



Immersion Challenge Method

Put 300 mL of sea water and 20 shrimps into a container

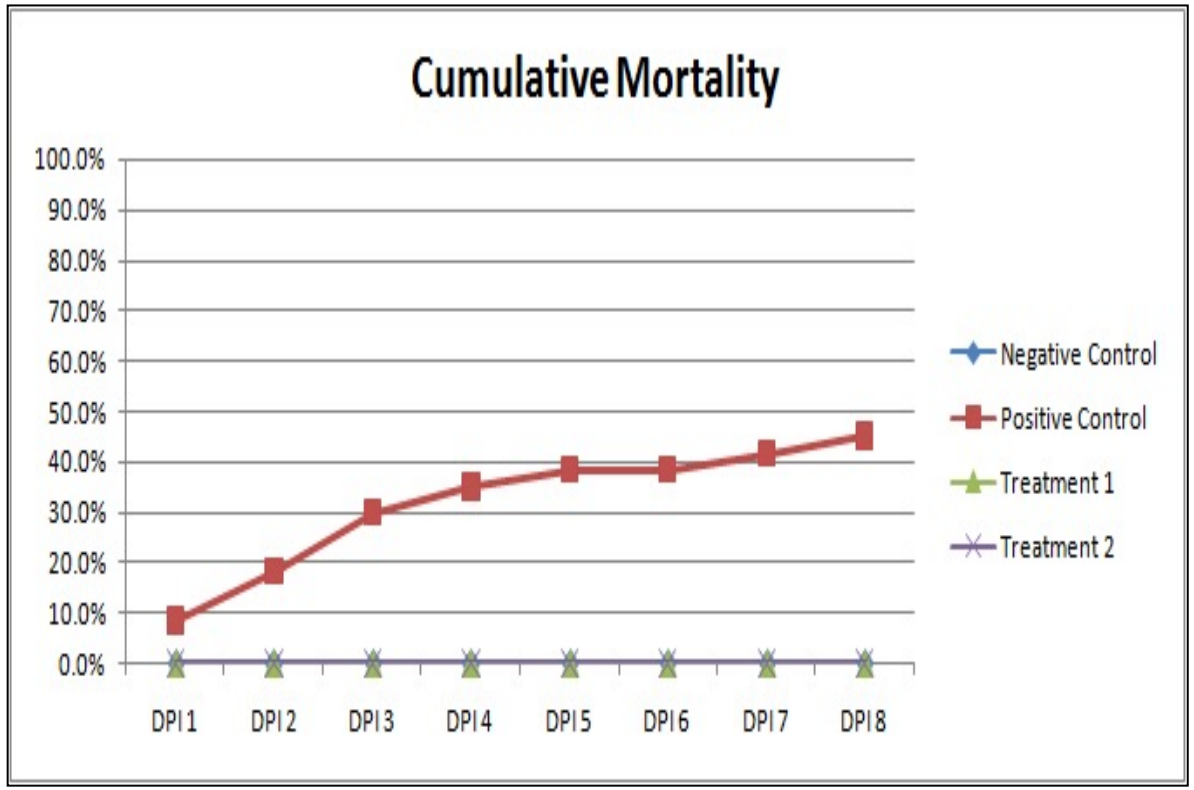
↓

Pour the bacteria solution (treated or not treated) into separate containers and keep it for 15 minutes with continuous aeration

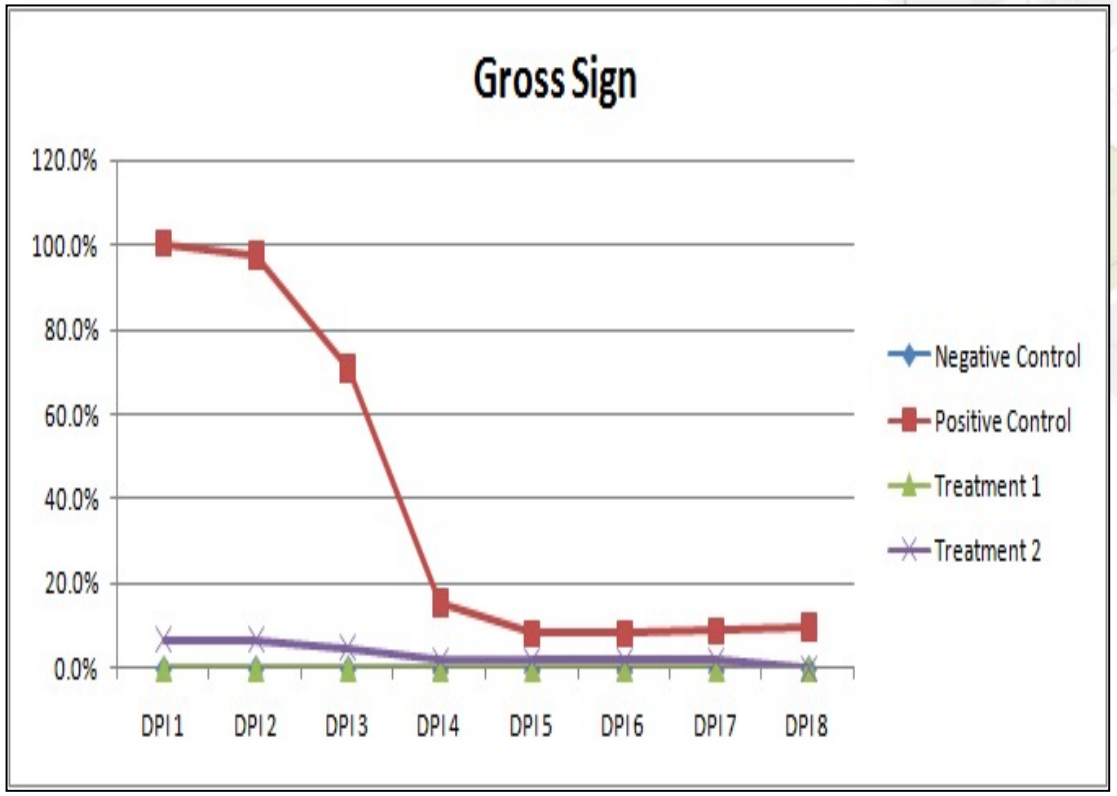
↓

Transfer the bacterial solution and shrimps into aquarium with 30 L of sea water. Water temperature was maintained at 29^{o}C – 30^{o}C

Observation and Results

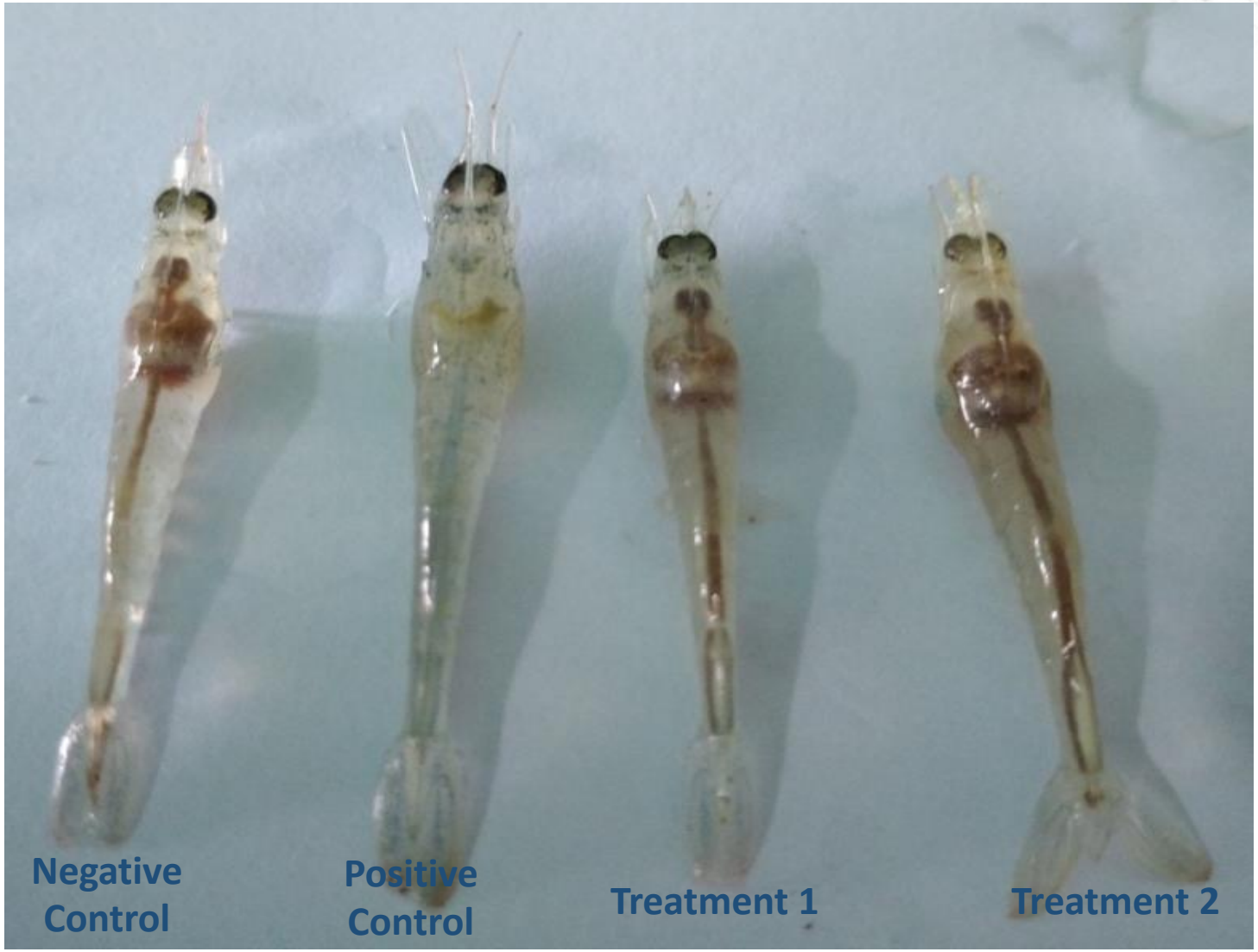


Cumulative Mortality



Cumulative Gross Sign Appearance

Observation and Results



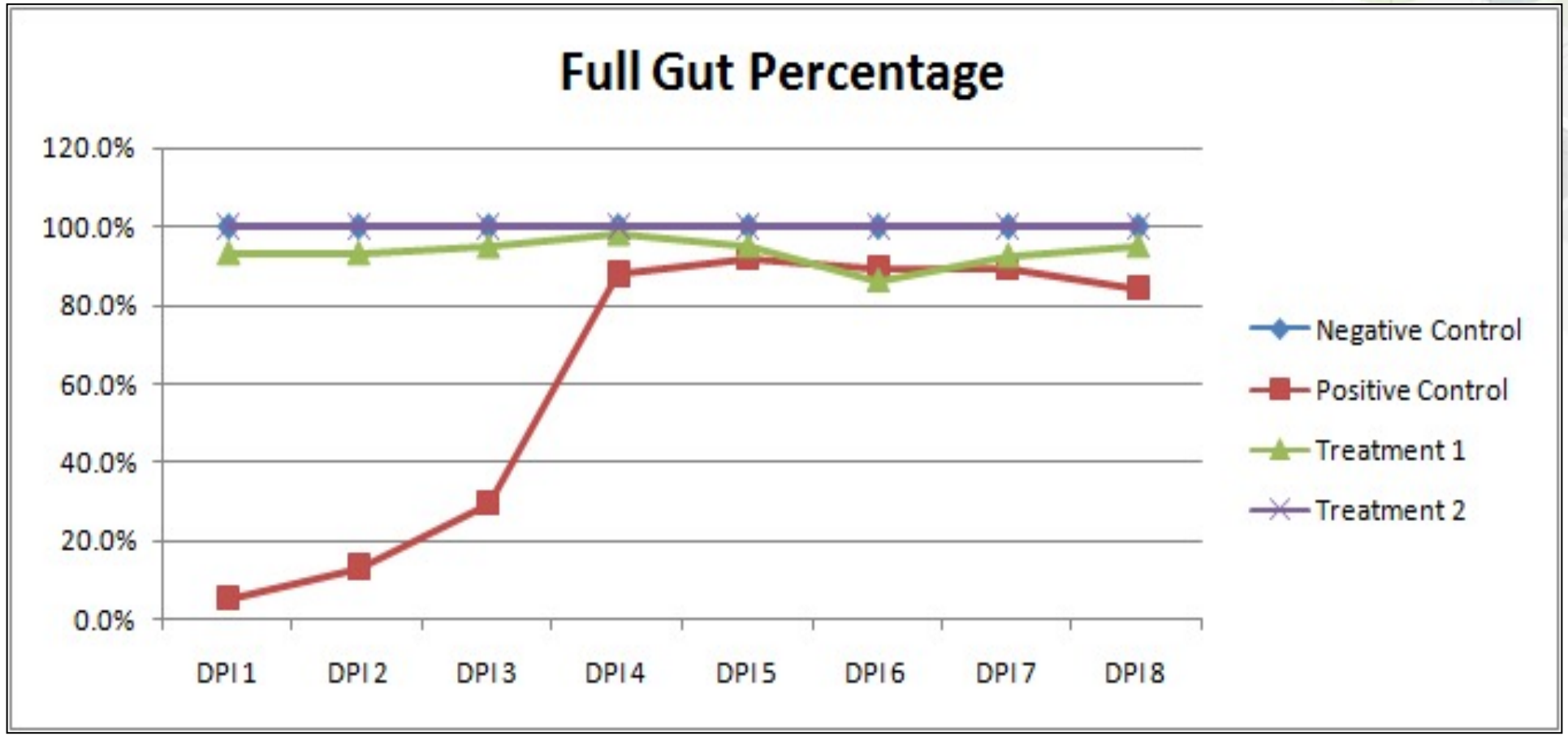
Observation and Results

Group	R	RT PCR Results (CT Value)									
		Bacteria after Incubation	Shrimps Samples								
			After Challenge	DPI 1	DPI 2	DPI 3	DPI 4	DPI 5	DPI 6	DPI 7	DPI 8
Negative Control	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Positive Control	1	24.3	33.4	28.4	27.1	23.2	21.8	33.6	0.0	32.7	0.0
	2	23.7	33.6								
	3	25.4	33.6								
Treatment 1	1	13.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	14.0	0.0								
	3	13.8	0.0								
Treatment 2	1	13.8	36.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	15.8	34.4								
	3	16.2	35.4								

Real time PCR tests

POND GUARD

Observation and Results



Percentage of Full Gut

Conclusions and Recommendations

Conclusions:

1. *Vibrio parahaemolyticus*-EMS strains performed the mortality in shrimps with typical gross signs of EMS/AHPND.
2. Pondguard has ability to inhibit the *Vibrio parahaemolyticus*-EMS growth, therefore, NO mortality and gross sign of EMS were appeared in the treated shrimps.

Recommendations:

1. Pondguard can be used as anti-EMS/AHPND treatment product.
2. Pondguard has proven to inhibit and segregate the growth of *Vibrio parahaemolyticus*-EMS. RT-PCR indicates that Pondguard have been able to eliminate the *Vibrio parahaemolyticus*-EMS from both tank water and from shrimp body.

Trial 2

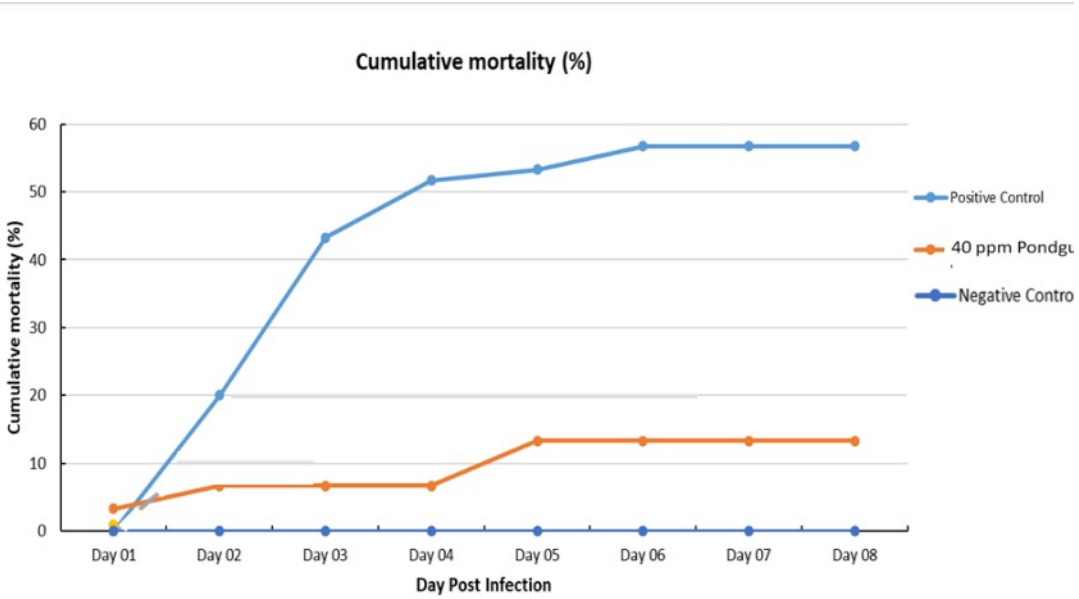
Efficacy of Pondguard against AHPND/EMS

Disease Challenge lab,

Can Tho University, Can Tho, Vietnam

Objectives

- **Trial was conducted to determine the effect of Pondguard on the survival rate (SR) in EMS/AHPND infected shrimp.**
- **The treatment group (Pondguard) showed protection against EMS/AHPND.**
- **Pondguard application was able to maintain SR 76.5% against EMS.**



RPS (Relative Percent Survival) on the basis of Cumulative Mortality (CM)

Formula = 1-(% mortality in Treatment Group/% mortality in Positive Control) x 100

No	Group	DPI 7
1	CM Positive Control	56.7%
2	CM (Regular Feed+40 ppm Pondguard+EMS)	13.3%
3	RPS (No BAV Feed+40 ppm BAV Liquid+EMS)	76.5%

- A small scale bioassay trial was conducted to determine the efficacy of Pondguard against AHPND-Vibrio parahemolyticus of shrimp.
  The Pondguard was applied for 2 weeks in the tanks prior to challenge.

The shrimp were challenged by sub-lethal dose of VP-AHPND.

The higher survival rate i.e. 76.5 % and RPS 76.5% was recorded in Pondguard applied tanks after 10 days of observation.

The trial results prove that Pondguard improved the immune system of shrimp to fight against of VP-AHPND.

Note: This study was conducted by Cantho University, Vietnam

Trial 3

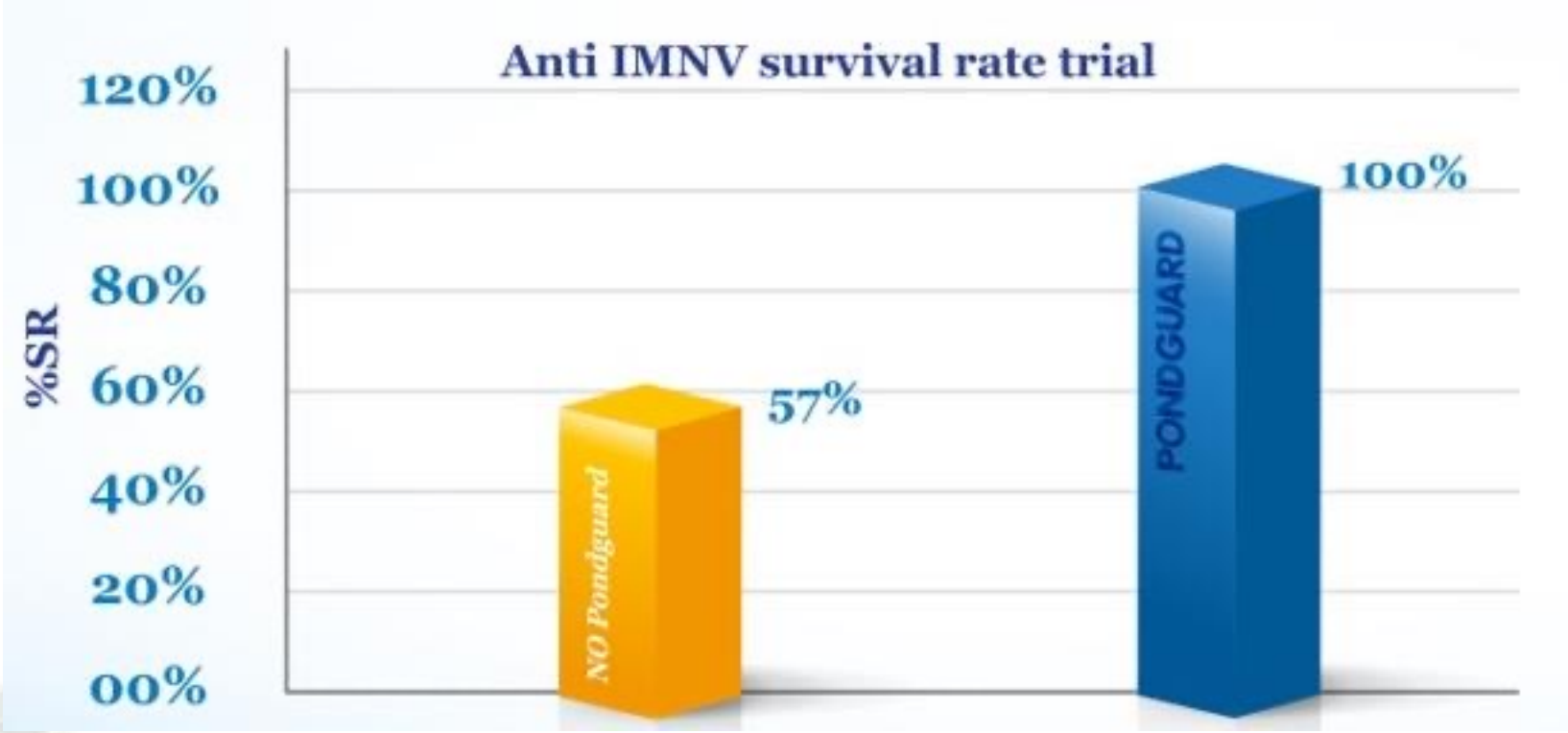
Efficacy of Pondguard against Infectious Myonecrosis Virus (IMNV) of Shrimp

Disease Research Challenge lab,

PT. Central Proteina Prima Tbk.

Pondguard against Infectious Myonecrosis Virus (IMNV)

Pondguard application was able to maintain SR 60-100% against Infectious Mionecrosis Virus (IMNV) in several trials conducted.



Note: This study was conducted by DRC Team, Lampung

- A small scale bioassay trial was conducted to determine the efficacy of Pondguard against IMN Virus of shrimp.
- The Pondguard was applied for 2 weeks in the tanks prior to challenge.
- The shrimp were challenged by sub-lethal dose of IMN Virus using *per os* method.
- The higher survival rate i.e. 100% was recorded in Pondguard applied tanks after 10 days of observation.
- The trial results prove that Pondguard improved the immune system of shrimp to fight against IMN Virus.

Trial 4

Efficacy of Pondguard against White Spot Syndrome Virus (WSSV) of Shrimp

CP PRIMA

Disease Research Challenge lab,

PT. Central Proteina Prima Tbk.

Pondguard against White Spot Syndrome Virus (WSSV)

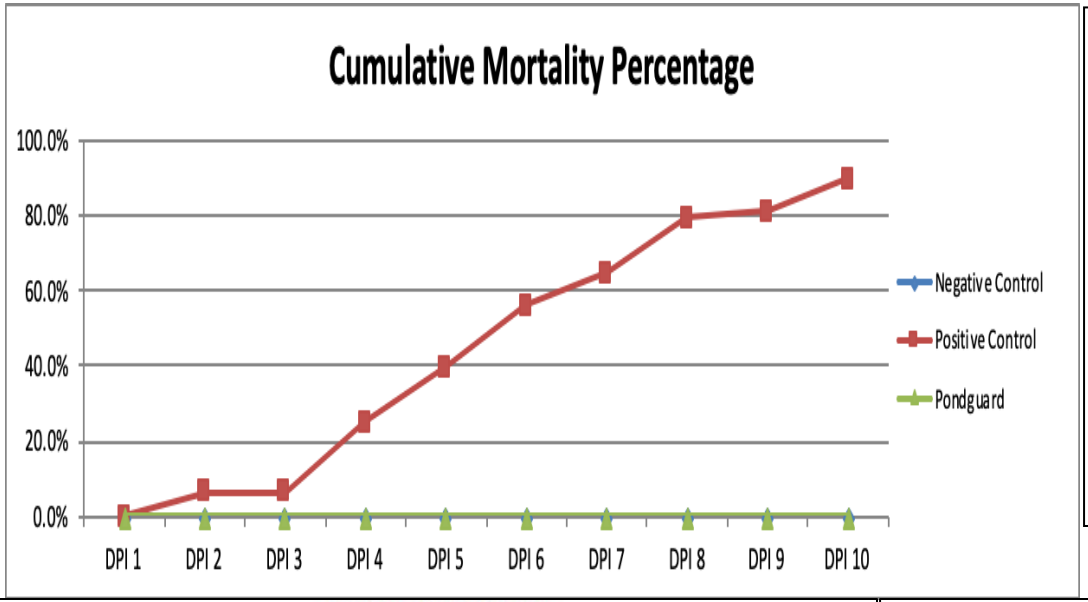
Trial was conducted to determine the effect of Pondguard with treatment feed to the survival rate (SR) in WSSV infected shrimp. The treatment group (Pondguard + feed) showed 100% protection against WSSV.

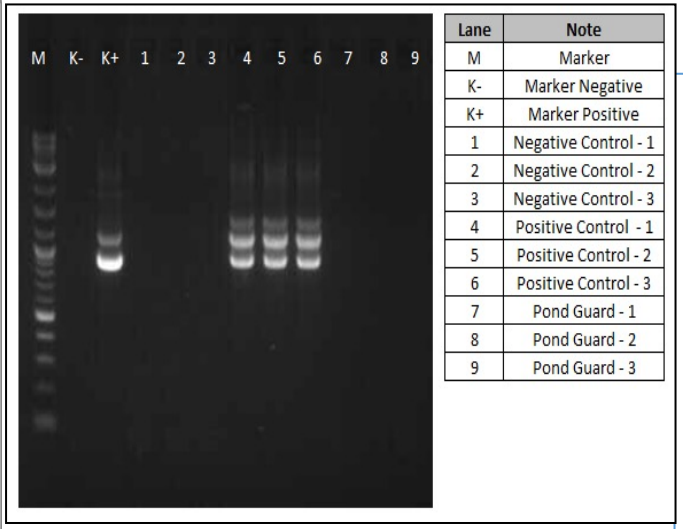
Pondguard application was able to maintain SR 100% against White Spot Syndrome (WSSV).

Note: This study was conducted by DRC Team, Lampung

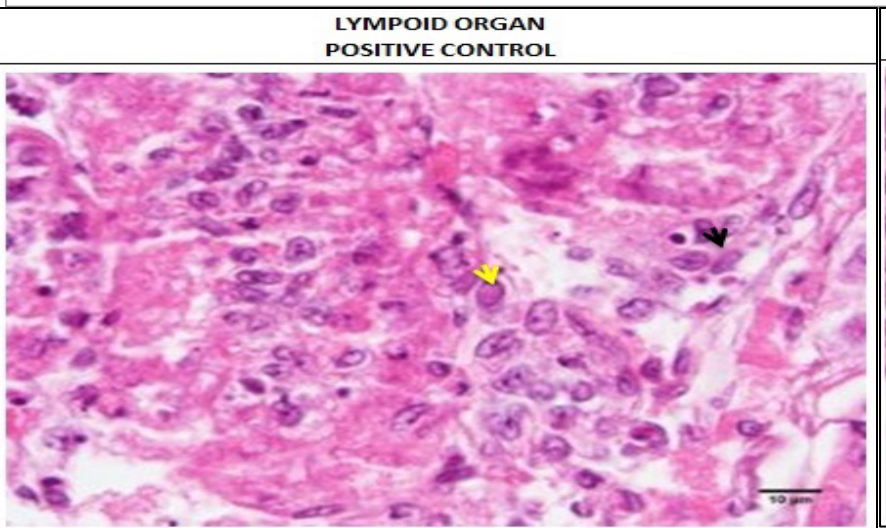
- A small scale bioassay trial was conducted to determine the efficacy of Pondguard against WSS Virus of shrimp.
- The Pondguard was applied for 2 weeks in the tanks prior to challenge.
- The shrimp were challenged by sub-lethal dose of WSS Virus using *per os* method.
- The higher survival rate i.e. 100% was recorded in Pondguard applied tanks after 10 days of observation.
- The trial results prove that Pondguard improved the immune system of shrimp to fight against WSS Virus.

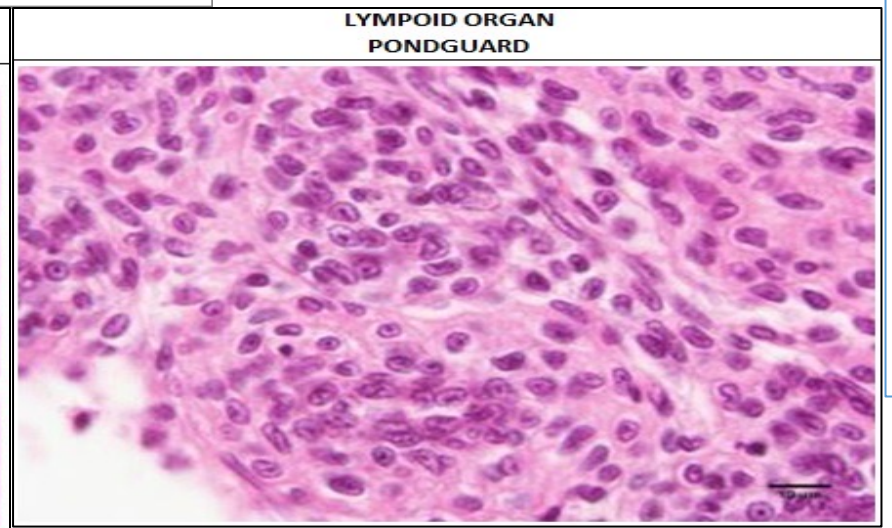
Pondguard against White Spot Syndrome Virus (WSSV)





Lane	Note
M	Marker
K-	Marker Negative
K+	Marker Positive
1	Negative Control - 1
2	Negative Control - 2
3	Negative Control - 3
4	Positive Control - 1
5	Positive Control - 2
6	Positive Control - 3
7	Pond Guard - 1
8	Pond Guard - 2
9	Pond Guard - 3





- **The trial was repeated two times to perform the similar results.**
- **There was 100% protection achieved by applying PG against WSSV.**
- **The PCR test of the remaining shrimp on the day of termination i.e. DPI 10 stated that all the shrimp in PG group were negative to WSSV. The positive control shrimp were positive to WSSV.**
- **The sub-lethal dose of virus was submerged in the PG and incubated for 2-3 hours before feeding to the shrimp.**

DRC, Pasar Kemis
August-October 2019

Trial 5

Efficacy of Pondguard against White Spot Syndrome Virus (WSSV) of Shrimp

Disease Research Challenge lab,

PT. Central Proteina Prima Tbk.

Materials and Methods

- Shrimp : White shrimp (*Litopenaeus vannamei*)
- Product : Pondguard
- Initial MBW : 2.8 g
- Int. Stocking : 12 pcs/tank.
- WSSV shrimp tissue : Copy number log 4
- WSSV challenge : 10% of biomass
- Negative shrimp tissue : negative WSSV & IMNV by PCR
- Water Volume : 70 L
- Total Tanks : 16 tanks.
- Feeding Method : 4 x per day
- Feed Type : Pellet feed no. 03
- Trial Duration : 7 days

Materials and Methods

Per Os Challenge

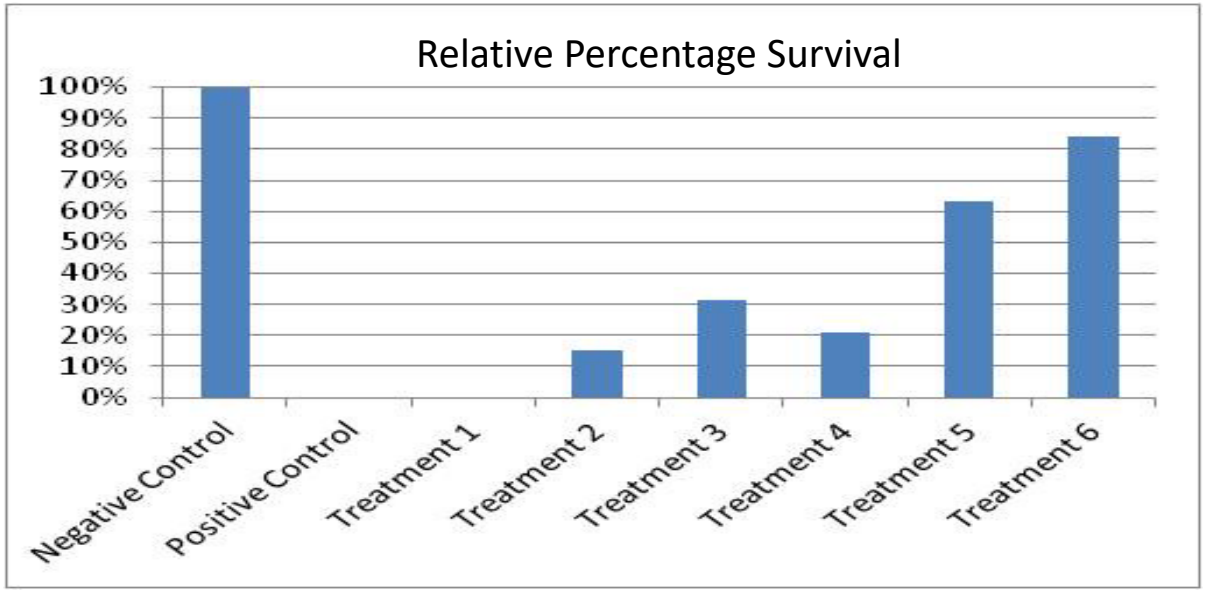
1. WSSV shrimp tissue were sliced into small size
2. 3.36 g WSSV shrimp tissue (10% from biomass) were dipped into 20 ml of Pondguard for 3 hours for treatment group. And for positive control group, WSSV shrimp tissue were dipped into 20 ml of TN Buffer. Negative shrimp tissue dipped into 20 TN Buffer.
3. Washed the shrimp tissue using TN buffer and gave to the shrimps.

Trial Design

Group	Rep	Pondguard			Shrimps	
		%	N oil/L aquadest	Application	MBW	N
Negative Control	2	-	-	Negative Tissue (10% of biomass) was dipped into 20 mL of TN Buffer	2.8 gram	12/tank
Positive Control	2	-	-	WSSV Tissue (10% of biomass) was dipped into 20 mL of TN Buffer		
Treatment 1	2	0.2%	2 ml	WSSV Tissue (10% of biomass) was dipped into 20 mL of each treatment		
Treatment 2	2	0.4%	4 ml			
Treatment 3	2	0.6%	6 ml			
Treatment 4	2	0.8%	8 ml			
Treatment 5	2	1.0%	10 ml			
Treatment 6	2	1.2%	12 ml			

Results

Group	Rep	No. of shrimp	Cumulative Mortality							RPS
			DPI 1	DPI 2	DPI 3	DPI 4	DPI 5	DPI 6	DPI 7	
Negative Control	2	12	0%	0%	0%	0%	0%	0%	0%	100%
Positive Control	2	12	0%	8%	17%	33%	54%	67%	79%	0%
Treatment 1	2	12	0%	8%	29%	33%	71%	88%	96%	0%
Treatment 2	2	12	0%	4%	4%	13%	21%	25%	67%	15%
Treatment 3	2	12	0%	4%	4%	17%	21%	33%	54%	32%
Treatment 4	2	12	0%	4%	4%	13%	25%	50%	63%	21%
Treatment 5	2	12	0%	0%	0%	4%	8%	25%	29%	63%
Treatment 6	2	12	0%	0%	0%	0%	4%	4%	13%	84%



Trial 6

Efficacy of Pondguard against Green Pathogenic Vibrio

Disease Challenge lab,

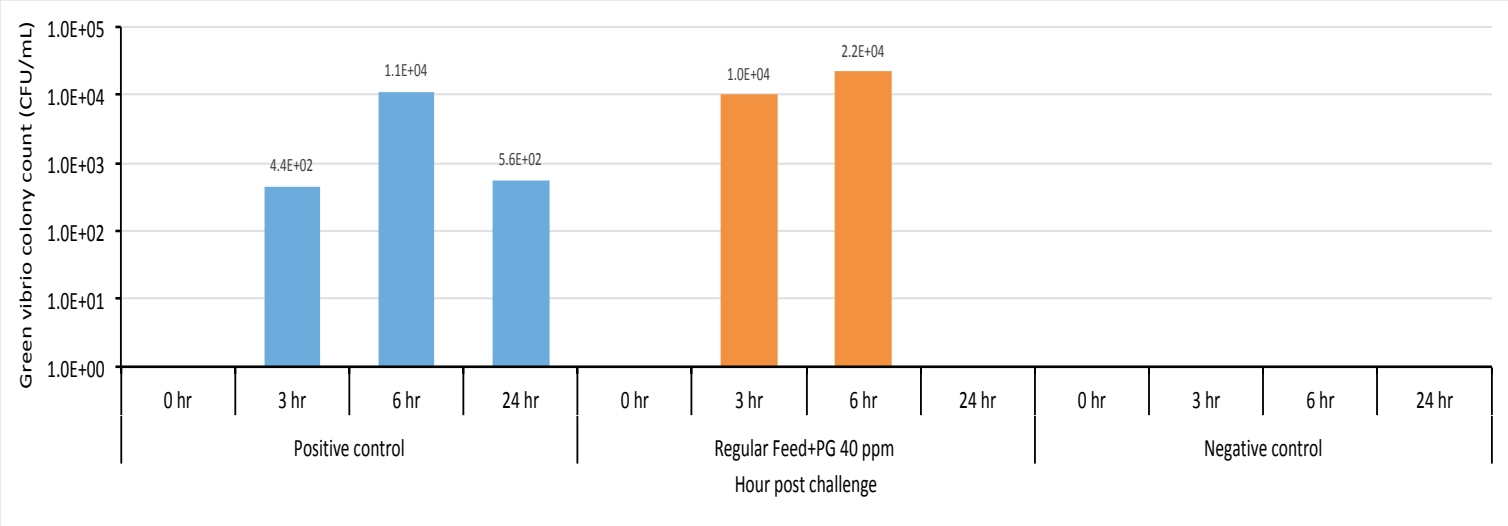
Can Tho University, Can Tho, Vietnam

Efficacy of Pondguard against Green Pathogenic Vibrio

- Trial was conducted to determine the effect of Pondguard on the reduction of Green pathogenic Vibrio.
- The Pondguard showed significant reduction in Green Vibrio colonies.

Pondguard application was able to reduce 100% green Vibrio in 24 hours

Figure 9. Green colony of bacteria in water sample of part 4 (NO BAV Feed+BAV Liquid)



Note: This study was conducted by Cantho University, Vietnam

- A small scale bioassay trial was conducted to determine the efficacy of Pondguard against Green Vibrio.
- The Pondguard was applied for 2 weeks in the tanks prior to challenge.
- The shrimp were challenged by sub-lethal dose of VP-AHPND i.e. log 6.
- The obtained result shows that Pondguard was able to reduce the green Vibrio load up to zero % in 24 hours of challenge period.
- The trial results prove that Pondguard is able to reduce the Vibrio load in controlled environment.

Trial 7

Efficacy of Pondguard against Luminiscent Vibrio

CP PRIMA

Disease Research Challenge lab,

PT. Central Proteina Prima Tbk.

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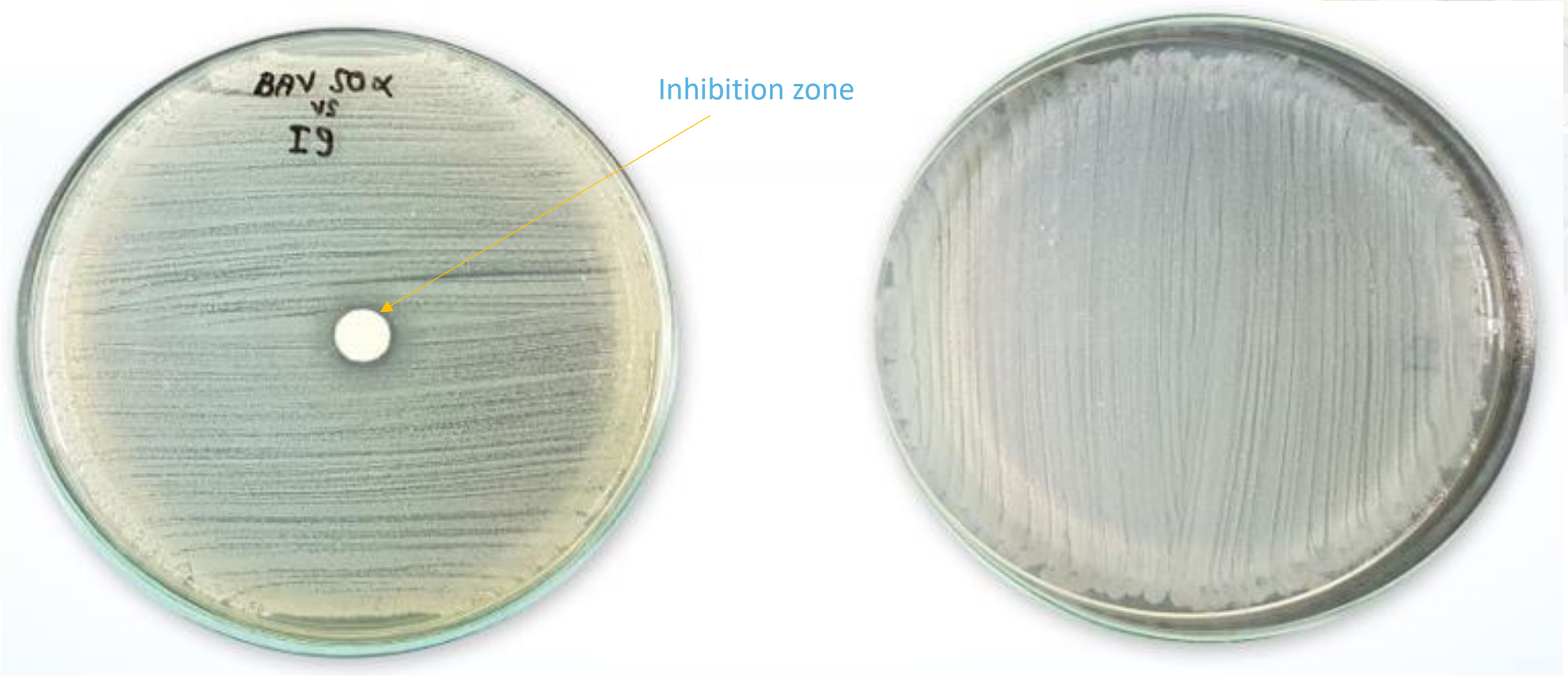
Lum Bac 1.9

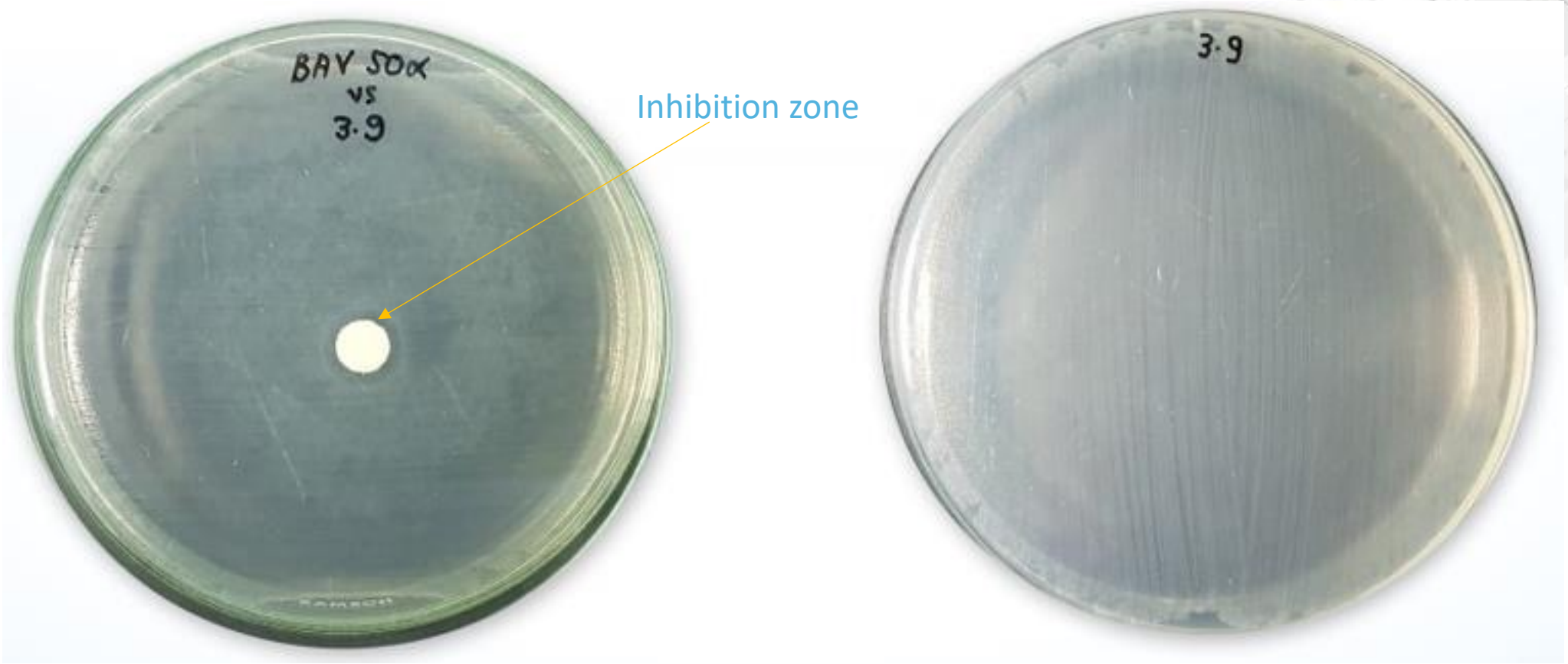


BAV 50 Vs 1.9 Vibrio from Situbundo

CONTROL1.9 Vibrio from Situbundo

Note: This study was conducted in DRC, Pasar Kemis

Lum Bac 3.9 *Photobacterium*



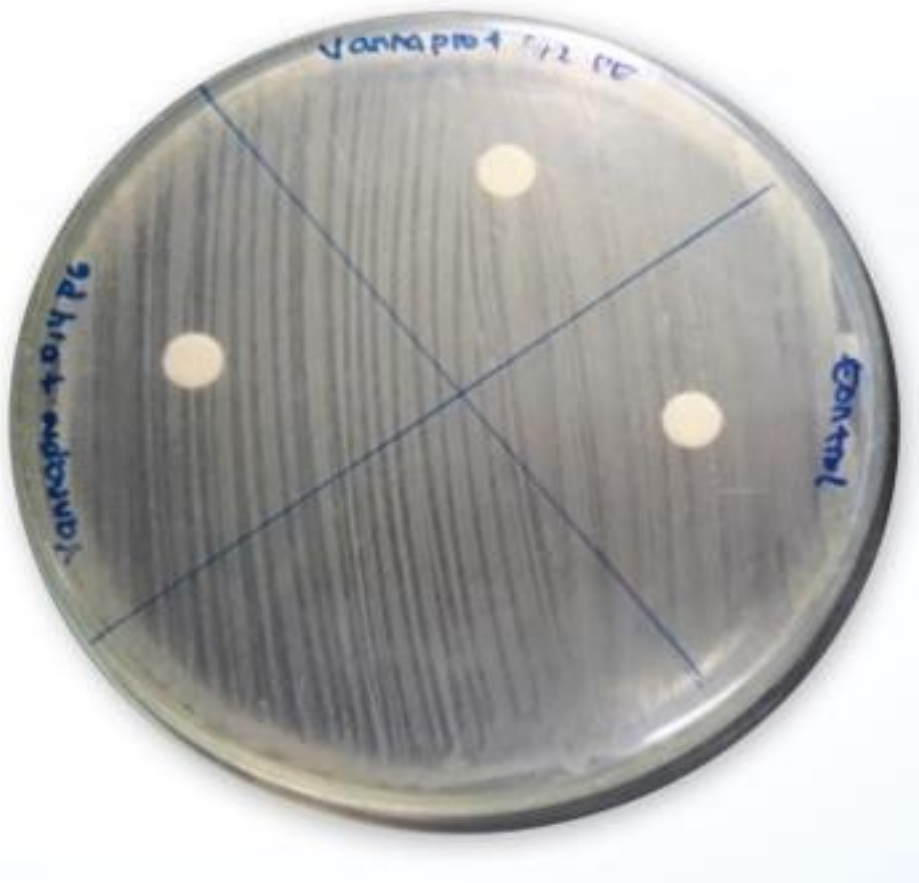
BAV 50 Vs 3.9 Photobacterium from Situbundo

CONTROL 3.9 Photobacterium from Situbundo

Note: This study was conducted in DRC, Pasar Kemis

Impact of Pondguard on Common Probiotics

Biosol VS Pondguard



Vannapro VS Pondguard

Note: This study was conducted in DRC, Pasar Kemis

Indoor trial to Study the Effect of Pondguard on overall performance of shrimp and its impact on critical water quality parameters

Objective

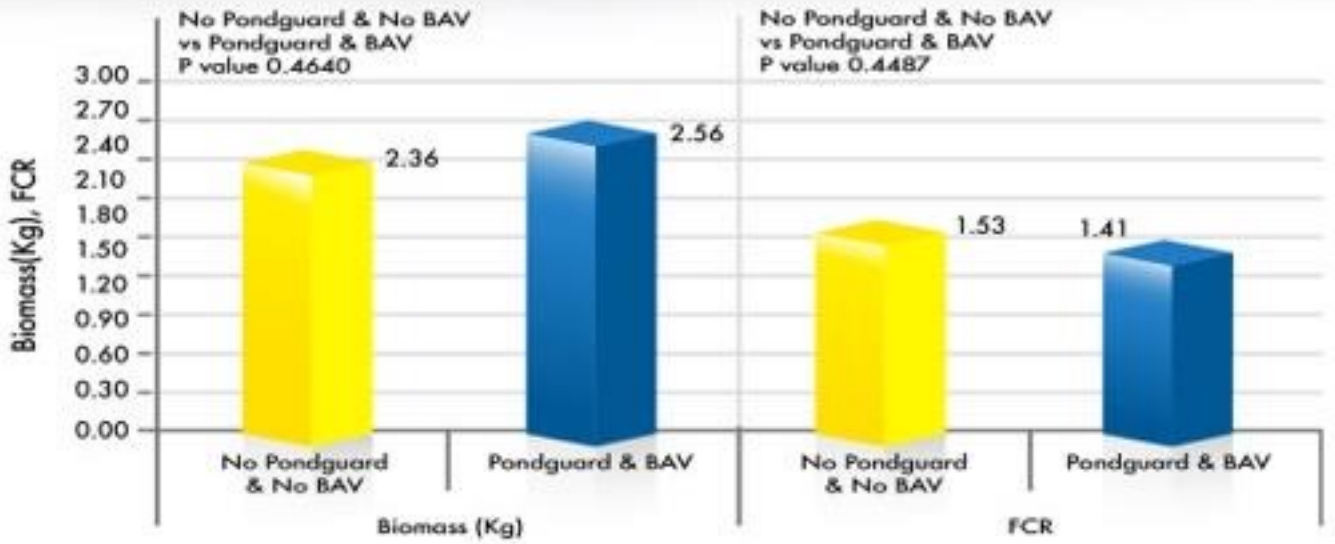
To study the effect of Pondguard on growth performance and water quality parameters of white shrimp (*Litopenaeus vannamei*)

- Shrimp: White shrimp (*Litopenaeus vannamei*)
- Stocking Density: 70 shrimp/m3
- Initial MBW (g): 0.15 ± 0.006*
- DOC: 90 days (March 23, 2015 – June 20, 2015)
- Tank Volume: Fiber Tank 2 m3

There is no negative effect on shrimp harvest performance

Treatment	Description	Number of Tank	St. Density (pcs/m3)	DOC	Harvest MBW (g)	Acc. ADG (g/day)	Biomass (kg/tank)	Productivity (kg/m3)	SR (%)	FCR (%)
To	Non Pondguard & Non BAV	4	70	90	21.01±0.355a	0.232 ±0.004a	2.363±0.062a	1.18±0.31a	80.36±1.071a	1.53 ±0.040a
T1	Pondguard & BAV	4	70	90	21.04 ±0.339a	0.233 ±0.004a	2.562±0.073a	1.28±0.37a	86.96±3.600a	1.41 ±0.041a
P value					0.4384	0.4384	0.4640	0.4640	0.1290	0.4487

Values are means ± SEM
Means in the same row with different superscripts are significantly different at p<0.05



Note: This study was conducted by Feed Tech Team at MRC

Trial 9

Study on the Effect Pondguard on natural food Plankton

Objective

To evaluate the effect of Pondguard on growth population and viability of *Thallasiosira weisflogii*, *Chaetoceros muelleri*, *Tetraselmis* sp.)

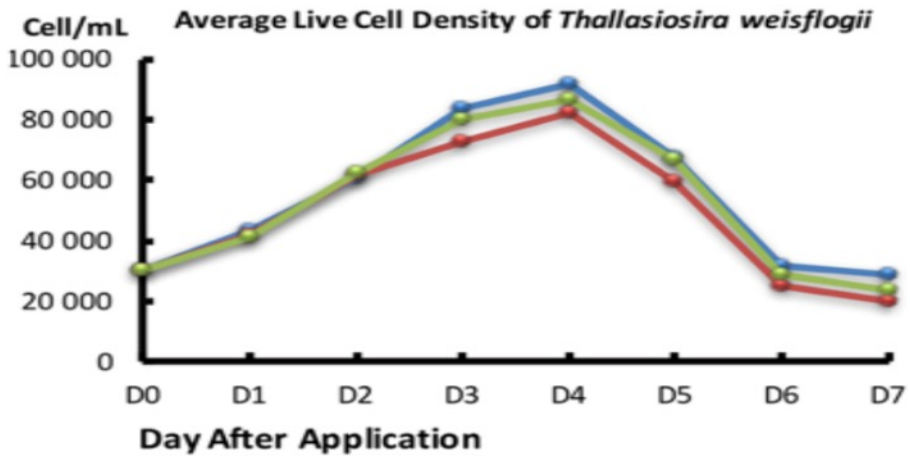
Location and time

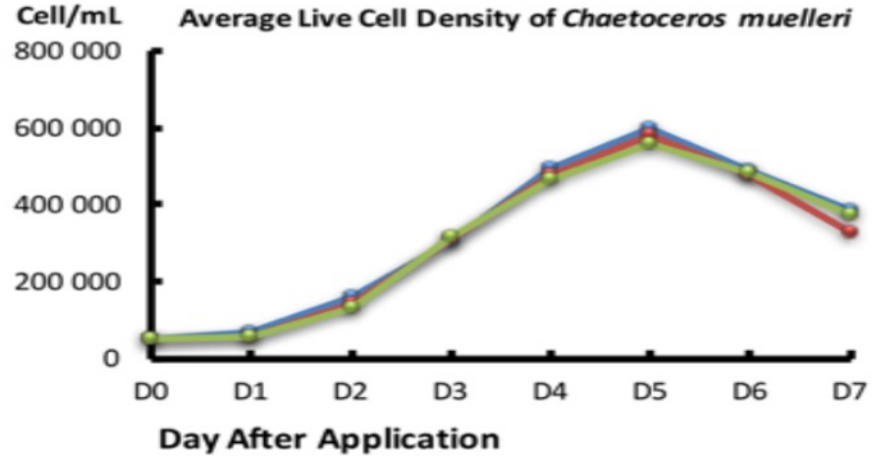
Started studies on August 2016 – until finish, seven days.
Indoor studies at Marine Research Center (MRC), Bandar Lampung.

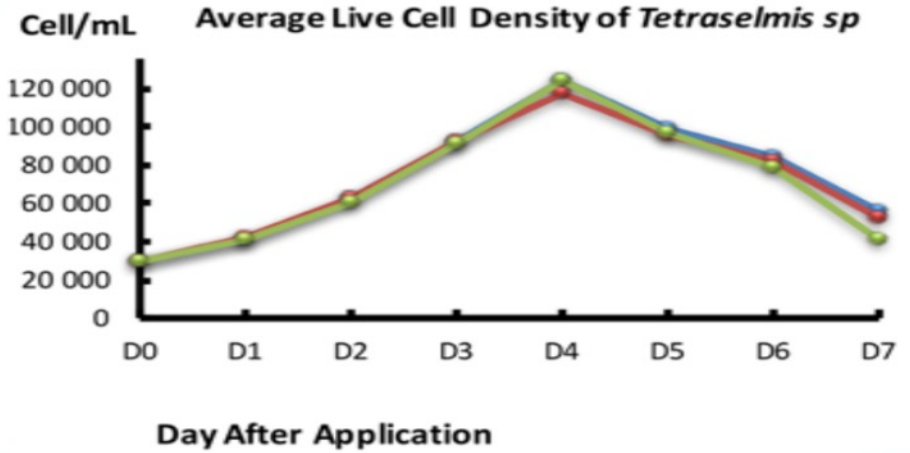
Materials and method

The study had 3 treatments, control and 0.60 ppm as Pondguard 1 and 0.80 ppm as Pondguard 2. Each treatments had 4 replications. Twelve 500 L tanks were used for this trial.

There is no negative effect on algae live cell density







Field Trial

Trial1

Pond trial to Study the Effect of Pondguard on overall performance of shrimp and its impact on critical water quality parameters

CP PRIMA

SFS, SHiS and TRD

PT. Central Proteina Prima Tbk.

POND GUARD

Objectives

- To observe and evaluate the impact of Pondguard on growth and Water quality parameters.
- To evaluate the efficacy of Pondguard against Total Vibrio and Green Vibrio in culture Pond water.
- To evaluate the efficacy of Pondguard against WSSV and IMNV in culture Pond.

Trial Design

PL Source	Treatment	Replication
CP Rembang	Control	2
CP Rembang	Pondguard	2

Pondguard application

Dose at DOC -7 and -3 : 0.4 ppm

Dose during culture: 0.2 ppm, once a week

Feed: IRAWAN

Other parameters were same as Free market SOP

PONDGUARD

Shrimp Production performance

Protocol	PONDGUARD				CONTROL			
Unit 2 Pond Address	Pond 1		Pond 2		Pond 3		Pond 4	
Hatchery	REMBANG		REMBANG		REMBANG		REMBANG	
PL Code	C13.1.5		C13.1.5		C13.1.5		C13.1.5	
Pond Type	Full PE		Full PE		Full PE		Full PE	
Area (m2)	1000		1000		1000		700	
PWA (HP)	5		4		5		4	
Stocking Date	9-May-19		9-May-19		9-May-19		9-May-19	
SD (pcs/m2)	106		106		106		116	
Init Biomass (kg)	0.53		0.53		0.53		0.41	
Mortality Incident_DOC (pcs)			62 (34)				94 (4487)	
Partial Harvesting Date	16-Jul-19	31-Jul-19	16-Jul-19	31-Jul-19	16-Jul-19		16-Jul-19	31-Jul-19
DOC (days)	69	84	69	84	69		69	84
Shrimp size	71	53	76	55	56		80	61
Biomass (kg)	161.0	176.6	151.6	174.3	105.9		114.3	150.9
Populasi (pcs)	11448	9381	11519	9598	5922		9200	9265
Tot. Biomass (kg)	337.55		325.94		105.9		265.19	
Tot. Pop (pcs)	20829		21117		5922		18465	
MBW (g)	16.21		15.43		17.88		14.36	
Final Harvesting Date	16-Aug-19		13-Aug-19		13-Aug-19		16-Aug-19	
DOC (days)	100		97		97		100	
Shrimp size	42.6	53	43.6	59	38.7	55	49	64
Biomass (kg)	992.33	8.81	970.3	33.5	796.7	31.1	763.79	16.65
Populasi (pcs)	42273	467	42305	1977	30832	1711	37426	1066
Tot. Biomass (kg)	1001.14		1003.80		827.80		780.44	
Tot. Pop (pcs)	42740		44282		32543		38491	
MBW (g)	23.42		22.67		25.44		20.28	
Shrimp Production								
Tot. Biomass (kg)	1338.69		1329.74		933.7		1045.63	
Tot. Pop (pcs)	63569		65399		38465		56956	
SR (%)	59.92		61.64		36.25		70.34	
MBW (g)	21.06		20.33		24.27		18.36	
FCR	1.31		1.28		1.24		1.44	
Acc.feed (kg)	1757.4		1704.2		1153.70		1506	
Productivity (ton/Ha)	13.386		13.297		9.34		14.937	
Productivity (kg/HP)	267.74		332.44		186.74		261.41	
Salmofan Score (min-max)	21	23	20	23	20	21	20	22

Note: The dead shrimp about 84 kg at DOC 94 also included in the harvest performance of Pond 4 (control)

Summary and Conclusion

- The overall productivity was higher in PG ponds (13.3 ton/Ha) than Control ponds (12.1 ton /Ha).
- There was no negative impact on critical water quality parameters, like plankton, pH, DO and Alkalinity etc. recorded in PG applied ponds as compared to control ponds.
- There is no significant difference in Plankton fluctuations between PG and Control ponds.
- Total Vibrio and Green Vibrio in culture Pond water of both PG ponds and Control ponds are within the maximum limit. There is no significant difference between PG and control ponds.
- All the culture ponds and reservoir were negative to WSSV. The ponds were also detected negative to EMS, IMNV and EHP during the trial period.
- In addition, the cooked shrimp on Salmon fan scale from PG applied ponds performed one score higher than the Control ponds.

POND GUARD

Trial 2

Pond trial to Study the Effect of Pondguard on overall performance of shrimp and its impact on critical water quality parameters

CP PRIMA

SFS, SHiS and TRD

PT. Central Proteina Prima Tbk.

POND GUARD

Objectives

- To observe and evaluate the impact of Pondguard on growth and Water quality parameters.
- To evaluate the efficacy of Pondguard against Total Vibrio and Green Vibrio in culture Pond water.
- To evaluate the efficacy of Pondguard against WSSV and IMNV in culture Pond.

Trial Design

PL Source	Treatment	Replication
CP Rembang	Control	3
CP Rembang	Pondguard	3

Pondguard application

Dose at DOC -7 and -3 : 0.4 ppm

Dose during culture: 0.2 ppm, once a week

Feed: IRAWAN (BAV 150 mixed in premix)

Other parameters were same as Free market SOP

PONDGUARD

Shrimp Production Performance

Shrimp Production	PG			Control		
	AVG	±	SD	AVG	±	SD
No of Pond	3			3		
Stocking Density	107	±	2	105	±	0
No of Partial harvest	3 times (DOC 64, 74, 88)			3 times (DOC 64, 74, 88)		
Partial Harvest pop (%)	47.6			51.6		
- MBW (g)	14.88	±	0.48	14.26	±	0.22
- Biomass (kg)	582.54	±	26.10	592.99	±	13.79
- Final Harvest (DOC)	100	±	3	97	±	3
Final Harvest Pop (%)	52.4			48.41		
- MBW (g)	22.20	±	1.18	21.17	±	1.11
- Biomass (kg)	959.57	±	94.26	831.00	±	181.76
Overall Performance						
Tot. Biomass (kg)	1,542.1	±	104.8	1,424.0	±	189.4
SR (%)	76.7	±	4.8	76.5	±	7.4
SR Diff. (%)	0.32					
MBW (g)	18.7	±	0.8	17.6	±	0.7
FCR	1.3	±	0.1	1.4	±	0.1
FCR Diff. (%)	-4.21					
Productivity (kg/ha)	15,421.1	±	1048.5	14,239.9	±	1893.6
Productivity Diff. (%)	7.66					

Shrimp Production performance

- Total 6 ponds (3 ponds with PG application and 3 ponds as Control) have been harvested.
- The Mean Body Weight of PG ponds (18.7g) is higher than Control ponds (17.6 g).
- The production of PG ponds are better (15.4 ton/ha) than Control ponds (14.2 ton/ha).
- The Survival rates of PG ponds are better (76.70%) is slightly better than control ponds (76.46%).
- The FCR of PG ponds (1.31) are better than the control (1.37) ponds

POND GUARD

Profit & Loss Report

Description	PG Treatment			AVG	Control			AVG
	P-1	P-2	P-3		P-4	P-5	P-6	
Stock. Density - (Pcs / m^2)	101	101	99	101	99	99	99	99
DOC - (Days)	101	100	95	99	94	100	95	96
MBW - (Gr)	19.6	18.5	18.1	18.7	16.9	18.2	17.8	17.6
ADG - (Gr / Day)	0.19	0.18	0.19	0.19	0.18	0.18	0.19	0.18
FCR	1.33	1.25	1.35	1.31	1.51	1.22	1.37	1.37
SR (%)	73.11	82.11	74.89	76.7	69.17	83.91	76.29	76.46
Biomass - (Kg)	1,554	1,642	1,434	1,544	1,232	1,610	1,429	1,424
Area - (m^2)	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Productivity/Ha	15,543	16,419	14,343	15,435	12,318	16,104	14,289	14,237
Gross Profit (Rp. / Kg)	21,964	23,678	18,667	21,436	9,396	23,268	17,437	16,700
Revenue (Rp. Mn)	109,181,319	113,536,131	97,637,811	106,785,087	80,615,337	110,806,286	96,211,743	95,877,789
COGS (Rp. Mn)	74,361,514	73,854,153	70,350,501	72,855,389	68,713,619	72,472,548	70,691,840	70,626,002
Fry	*4,566,330*	*4,566,330*	*4,442,985*	*4,525,215*	*4,442,985*	*4,442,985*	*4,442,985*	*4,442,985*
Feed	*29,702,024*	*29,353,625*	*27,729,657*	*28,928,435*	*26,784,575*	*28,243,044*	*28,179,956*	*27,735,859*
Chemical & Others	*3,159,938*	*3,155,829*	*3,135,282*	*3,150,350*	*3,131,173*	*3,155,829*	*3,135,282*	*3,140,761*
Man Power Expenses	*12,669,169*	*12,543,732*	*11,916,545*	*12,376,482*	*11,791,108*	*12,543,732*	*11,916,545*	*12,083,795*
Electricity + Fuel	*16,149,565*	*15,989,669*	*15,190,185*	*15,776,473*	*15,030,289*	*15,989,669*	*15,190,185*	*15,403,381*
PG usage	*100,800*	*100,800*	*100,800*	*100,800*	*0*	*0*	*0*	*0*
Others	*8,013,687*	*8,144,169*	*7,835,047*	*7,997,634*	*7,533,490*	*8,097,289*	*7,826,886*	*7,819,222*
Gross Profit - Cash (Rp. Mn)	34,819,805	39,681,977	27,287,310	33,929,698	11,901,719	38,333,738	25,519,903	25,251,786
Depreciation	*22,683,190*	*22,683,190*	*22,683,190*	*22,683,190*	*22,683,190*	*22,683,190*	*22,683,190*	*22,683,190*
Gross Profit Margin (%) - Cash	31.9%	35.0%	27.9%	31.8%	14.8%	34.6%	26.5%	26.3%
Gross Profit (Rp. Mn)	12,136,615	16,998,787	4,604,120	11,246,507	(10,781,472)	15,650,547	2,836,713	2,568,596
Gross Profit Margin (%)	11.12%	14.97%	4.72%	10.53%	-13.37%	14.12%	2.95%	2.68%
Cost per Kg	47,842	44,980	49,047	47,201	55,781	45,002	49,474	49,607
Gross Profit_Cash Diff. (%)	25.6							

Profit & Loss

- Cost per kg of shrimp production of PG ponds (Rp. 47,201) is lower than Control ponds (Rp. 49,607).
- Gross profit margin of PG ponds are 25.6% higher than Control ponds.

Summary and Conclusion

Harvesting performance

- The productivity of PG ponds are about 7.79 % better than Control ponds.
- The production of PG ponds are better (15.4 ton/ha) than Control ponds (14.2 ton/ha).
- The Survival rates of PG ponds are better (76.70%) is slightly better than control ponds (76.46%).
- The FCR of PG ponds (1.31) are better than the control (1.37) ponds.
- Cost per kg of shrimp production of PG ponds (Rp. 47,201) is lower than Control ponds (Rp. 49,607).
- Gross profit margin of PG ponds are 25.6% higher than Control ponds.

Summary and Conclusion

Pathogen Screening of Shrimp

- Both PG and Control ponds were negative to WSSV, IMNV, EHP except one pond in Control group was positive to EHP by PCR.

Vibrio Screening of Pond water

- Av. Total Vibrio Count during culture period: Total Vibrio of PG ponds is 17.9 % less than Control ponds (Control ponds = 1,836 cfu/ml and PG Ponds = 1,556 cfu/ml).
- Av. Total Green Vibrio Count during culture period : Green Colony of PG is 95.3 % less than Control ponds (Control Ponds= 508 cfu/ml and PG Ponds = 260 cfu/ml).
- Av. Total Bacteria Count during culture period : TBC of PG ponds is 36.11 % lower than Control ponds (Control = 154,861 cfu/ml; Treatment = 113,773 cfu/ml).

POND GUARD

Summary and Conclusion

Water Quality Parameters

- Average Nitrite level : Nitrite level of PG ponds are 1.23% higher than Control ponds (Control ponds= 4.82 ppm and PG ponds = 4.88 ppm).
- Average TAN level : TAN level of PG is 1.75% lower than Control ponds (Control ponds= 0.58 ppm and PG ponds = 0.57 ppm).
- Average DO level : The DO level of PG ponds are 0.47 % higher than Control ponds (Control ponds = 6.35 ppm and PG ponds = 6.38 ppm).
- Average pH level : The pH level of PG ponds are 0.24% higher than Control ponds. (Control ponds= 8.14; PG ponds = 8.16).

Certificate

SURAT NOMOR PENDAFTARAN OBAT IKAN

Nomor: KKP RI NO. D 16060285 - HBC

Nama Produsen Obat Ikan	: PT. CENTRAL BALI BAHARI
Alamat lengkap Produsen Obat Ikan	: Jl. Yos Sudarso No. 257, Kelurahan Garuntang, Kecamatan Bumi Waras, Kota Bandar Lampung, Provinsi Lampung
Alamat tempat Produksi obat ikan	: Jl. Yos Sudarso No. 257, Kelurahan Garuntang, Kecamatan Bumi Waras, Kota Bandar Lampung, Provinsi Lampung
Nama Produsen obat ikan diluar negeri	: -
Nama Pemberi Licensi	: -
Nama dagang/merek obat ikan	: PONDGUARD
Klasifikasi Obat Ikan	: Obat bebas
Bentuk Obat Ikan	: Cair
Jenis Sediaan Obat Ikan	: Obat Alami
Komposisi Obat Ikan	: Minyak Lavender, Minyak Eucalyptus, Minyak Pinus, Methyl Paraben, Kupri Sulfat, Emulgator (Tween 80), Brilliant Blue, Camnolaine, Air Suling
Ukuran Kemasan	: 300 ml; 500 ml;800 ml; 1 Lt; 1,2 Lt; 3 Lt; 5 Lt; 10 Lt; 20 Lt

dinyatakan

DAPAT

Diedarkan, diedarkan, dan digunakan obat ikannya di seluruh wilayah Negara Republik Indonesia. Surat Nomor Pendaftaran Obat Ikan ini berlaku untuk jangka waktu 5 (lima) tahun terhitung sejak tanggal diterbitkan dan tidak dapat dipindahkan kepada pihak lain.

Jakarta, 10 Juli 2018
Direktur Jenderal Perikanan Budidaya

Dr. Ir. Slamet Soejatto, M.Si

SURAT NOMOR PENDAFTARAN OBAT IKAN

Nomor: KKP RI NO. D 1804350 HBS

Berdasarkan hasil evaluasi teknis oleh Direktorat Jenderal Perikanan Budidaya, maka obat ikan dari:

Nama Produsen Obat Ikan	: PT. CENTRAL BALI BAHARI
Alamat lengkap Produsen Obat Ikan	: Gedung Puri Matari II Lt. 2 Jl. HR Rasuna Said Kav. H 1-2 Kelurahan Karet, Kecamatan Setiabudi Jakarta Selatan
Alamat tempat Produksi obat ikan	: Jl. Yos Sudarso No. 257 Kelurahan Garuntang, Kecamatan Bumi Waras, Kota Bandar Lampung
Nama Produsen obat ikan diluar negeri	: --
Nama Pemberi Licensi	: --
Nama dagang/merek obat ikan	: BAV SALT
Klasifikasi Obat Ikan	: Obat Bebas
Bentuk Obat Ikan	: Serbuk
Jenis Sediaan Obat Ikan	: Herbal (Alami)
Komposisi Obat Ikan	: Zat aktif: Garam (NaCl), Minyak Herbal Alami: minyak pinus (Pinus sp), minyak lavender (Lavandula sp), minyak eucalyptus (Eucalyptus sp). Zat tambahan: Emulsifier (Polysorbate 80)
Indikasi	: Meningkatkan kesehatan hewan aquatik
Ukuran Kemasan	: 250 gr; 500 gr; 1 kg; 2 kg; 5 kg; 10 kg; 20 kg; dan 50 kg

dinyatakan

DAPAT

Diperedarkan, diedarkan, dan digunakan obat ikannya di seluruh wilayah Negara Republik Indonesia. Surat Nomor Pendaftaran Obat Ikan ini berlaku untuk jangka waktu 5 (lima) tahun terhitung sejak tanggal diterbitkan dan tidak dapat dipindahkan kepada pihak lain.

Jakarta, 30 April 2018
Direktur Jenderal Perikanan Budidaya

Dr. Ir. Slamet Soejatto, M.Si

Congresses & Publications

Multidisciplinary Advances in Veterinary Science

Scientia Ricerca

Volume 1 • Issue 2 • 2017

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Research Article

Multidisciplinary Advances in Veterinary Science

Effectiveness of Natural Herbal Oil Formulation against Infectious Myonecrosis Virus in Whiteleg Shrimp *Penaeus vannamei*

Haig Yousef Babikian MSc[2], Rajeev Kumar Jha PhD[1]*, Yousep Haig Babikian PhD[1], Daniel Wisoyo BSc[1], Yuli Asih BSc, Sarayut Srisombat MSc[1] and Benjamin Jiaravanon MSc[1]

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[2]*Panacea Natural Sciences, Indonesia*

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Received: February 17, 2017; **Published:** May 10, 2017

Abstract

Aim: Infectious Myonecrosis Virus (IMNV) of *Penaeus vannamei* shrimp is considered as a chronic virus. An effort was made to develop an anti-Infectious Myonecrosis Virus formulation by using the combination of oil blends.

Material and Methods: Altogether 10 plant oils extract *Lavandulalatifolia, Pinussylvestris, Jasminumofficinale, Citrus limon, Prunumsavium, Viola odorata, Gardenia jasminoides, Cocos nucifera, Rosa damascene and Eucalyptus globulus*, were selected as anti-IMNV candidates. The oil blends were mixed with the shrimp feed in required quantity. The experimental shrimp were fed on the blend oil enriched feed for 14 days prior to challenge. The per os challenge method was applied to infect the shrimp. Simultaneously, to support the feed, a liquid supplement named as " Pondguard" (Reg no. KKP RI No. D16060285-HBC) was applied directly in the tank water.

Results: The cumulative mortality recorded in positive control was 58.3% whereas 8.3% in the exerimental group. The rate of mortality in experimental group were significantly lesser than positive control group. The typical gross sign appearance i.e. whitish muscle in 2nd and 3rd segment, in the control group were higher than the treatment.

Conclusion: The trial results showed that the developed blended oil extract formulation has significant effect against IMNV in controlled conditions. The developed product is user friendly as it is mixed and incorporated into the shrimp feed in specific amount to have anti-viral properties.

Volume 1 Issue 2 May 2017

Introduction

Infectious myonecrosis is known to cause significant outbreak and mortalities in penaeid shrimp [1]. In Indonesia the first outbreak was in Situbondo in 2006, and it spread to all over the country: East Java, Bali, Lampung, Central Java, West Kalimantan and West Nusa Tenggara [3]. In Brazil IMNVcaused up to 70% mortality [2]. IMNV has been demonstrated to be horizontally transmitted by ingestion of infected moribund shrimp [4], and live feed [8].

Citation: Rajeev Kumar., *et al.* "Effectiveness of Natural Herbal Oil Formulation against Infectious Myonecrosis Virus in Whiteleg Shrimp *Penaeus vannamei*". *Multidisciplinary Advances in Veterinary Science* 1.2 (2017): 50-56.

Study on the Efficacy of Pondguard in Improving Clinical Performance of White Leg Shrimp

American Journal of Biomedical Science & Research

www.biomedgrid.com

ISSN: 2642-1747

Research Article

Study on the Efficacy of Pondguard in Improving Clinical Performance of White Leg Shrimp (*Penaeus Vannamei*) in an AHPND Bacterial Challenge Model

Haig Yousef Babikian[1]*, Rajeev Kumar Jha[1], Dang Thi Hoang Oanh[2] and Truong Quoc Phu[2]

[1]*PT Central Proteina Prima, Indonesia*

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***Corresponding author:** Haig Yousef Babikian, PT Central Proteina Prima, Indonesia.

To Cite This Article: *Haig Yousef Babikian. Study on the Efficacy of Pondguard in Improving Clinical Performance of White Leg Shrimp (Penaeus Vannamei) in an AHPND Bacterial Challenge Model. Am J Biomed Sci & Res. 2019 - 5(3). AJBSR.MS.ID.000914. DOI: 10.34297/AJBSR.2019.05.000914.*

Received: September 19, 2019; **Published:** September 25, 2019

Abstract

A bioassay trial was conducted to determine the efficacy of developed product, Pondguard as an anti-Acute Hepatopancreatic Necrosis Disease (AHPND) candidate. The Pondguard (Registration no. D 16060285-HBC) is consisting of natural oils, like, lavender oil, Eucalyptus oil and Pine oil. It maintains the immunity level of shrimp, which helps to protect shrimp from infectious diseases. Two doses of Pondguard i.e. 40 ppm and 80 ppm were selected for the trial. The treatment groups tanks were applied Pondguard whereas no application in control tanks throughout the experiment. The shrimp of both treatment and control were challenged by immersion method. The cumulative mortality reached up to 56.7% in positive control whereas 23.3 % in 80 ppm pondguard group and 13.3% in 40 ppm pondguard group at dpi 8 whereas no mortality recorded in negative control. The Relative Percent Survival of 80 ppm group was 64.7% and of 40 ppm group was 76.5%. The trial results show that the developed Pondguard has significant effect against AHPND-Vibrio *parahaemolyticus* in a controlled condition.

Keywords: AHPND; *Vibrio parahemolyticus;* Essential Oil Blend; Pondguard

Journal of Pharmacognosy & Natural Products

Journal of
Pharmacognosy & Natural Products

Jha et al., J Pharmacogn Nat Prod 2016, 2:4
DOI: 10.4172/2472-0992.1000123

Research Article OMICS International

Effectiveness of Natural Herbal Oil Formulation against White Spot Syndrome Virus in *Penaeus vannamei*

Rajeev Kumar Jha*, Yousef Haig Babikian, Haig Yousef Babikian, Soy Daniel Wisoyo, Yuli Asih, Sarayut Srisombat and Benjamin Jiaravanon

PT Central Proteina Prima, Indonesia

Abstract

The Natural Herbal Oil Formulation (NHOF) have been designed to work against White Spot Syndrome Virus (WSSV) in Shrimp. The essential oil blend extracted from the following plants, *Lavandula latifolia, Pinus sylvestris, Jasminum officinale*, *Citrus limon*, *Prunus avium, Viola odorata, Gardenia jasminoides, Cocos nucifera, Rosa damascene* and *Eucalyptus globulus,* mixed together to develop as anti-WSSV product. The product were added in the feed, as feed additive. Another product developed which was applied in tank water as water supplement named as "Pondguard" (Reg no. KKP RI no. D16060285-HBC). The Pondguard is composed of three essential oils i.e., *Eucalyptus globulus*, *Pinus sylvestris* and *Lavandula latifolia*. A bioassay trial was conducted to determine the efficacy of developed formulation as an anti-WSSV candidate. The experimental shrimp was fed on NHOF-mixed feed and "Pondguard" applied in the tank water whereas control shrimp were fed on the regular feed. The shrimp of both experimental and control were challenged by WSSV on 15th day of feeding. Two methods of WSSV challenge were applied, per-os challenge and immersion challenge. The cumulative mortality in positive control reached up to 100% on DPI 10 whereas no mortality appeared in the experimental group and in negative control. The trial results show that the developed natural herbal formulation has significant effect against WSSV in a controlled condition.

3rd International Conference on Aquaculture & Fisheries

Efficacy of Anti-AHPND formulated feed in vannamei shrimp in Vietnam

3rd International Conference on Aquaculture & Fisheries
September 29-October 01, 2016 London, UK

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Posters & Accepted Abstracts: J Aquac Res Development

DOI: 10.4172/2155-9546.C1.009

Abstract

A bioassay trial was conducted to determine the efficacy of developed feed as an anti-AHPND candidate. The treatment was fed on anti-AHPND feed whereas control fed on the regular feed throughout the experiment. The shrimp of both treatment and control were challenged by per os method on day 8. The cumulative AHPND-gross sign appearance in positive control reached up to 95% at dpi 8 whereas no gross sign appeared in treatment and in negative control. The cumulative mortality reached up to 90% at dpi 8 in positive control whereas no mortality recorded in treatment and negative control. The Vibrio parahaemolyticus isolated from the hepatopancreas of infected shrimp were matched 100% with the existing AHPND strain. The trial results show that the developed anti-AHPND feed has significant effect against AHPND pathogen in a controlled condition.

5th Annual World Congress of Aquaculture and Fisheries 2016

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2016中国（青岛）第五届国际藻业大会
BIT's 5th Annual International Congress of Algae–2016

时间：2016年11月4–6日　　地点：中国青岛金沙滩希尔顿酒店
Time: November 4–6, 2016　　Venue: Hilton Qingdao Golden Beach, China

Title: Development of Natural Herbal Oil Formulation as an Anti-White Spot Syndrome Virus Agent in *Penaeusvannamei*

Dr. Rajeev K. Jha*, Benjamin Jiaravanon, Yousef H. Babikian, Haig Y. Babikian, Sarayut Srisombat, Soy D. Wisoyo, and Yuli Asih
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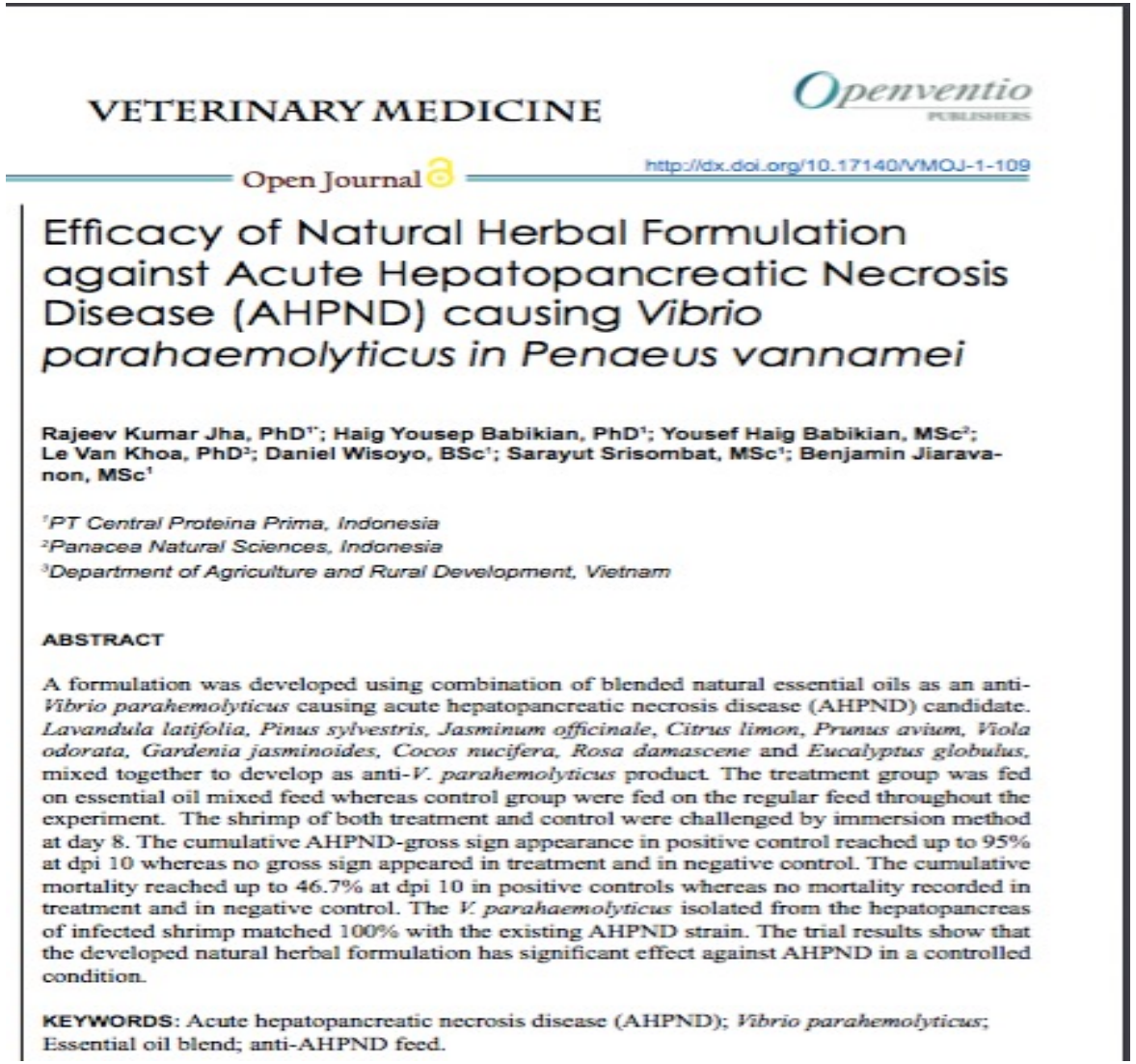
Journal of Veterinary Medicine



VETERINARY MEDICINE

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Open Journal

http://dx.doi.org/10.17140/VMOJ-1-109

Efficacy of Natural Herbal Formulation against Acute Hepatopancreatic Necrosis Disease (AHPND) causing *Vibrio parahaemolyticus in Penaeus vannamei*

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ABSTRACT

A formulation was developed using combination of blended natural essential oils as an anti-*Vibrio parahemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) candidate. *Lavandula latifolia, Pinus sylvestris, Jasminum officinale, Citrus limon, Prunus avium, Viola odorata, Gardenia jasminoides, Cocos nucifera, Rosa damascene* and *Eucalyptus globulus,* mixed together to develop as anti-*V. parahemolyticus* product. The treatment group was fed on essential oil mixed feed whereas control group were fed on the regular feed throughout the experiment. The shrimp of both treatment and control were challenged by immersion method at day 8. The cumulative AHPND-gross sign appearance in positive control reached up to 95% at dpi 10 whereas no gross sign appeared in treatment and in negative control. The cumulative mortality reached up to 46.7% at dpi 10 in positive controls whereas no mortality recorded in treatment and in negative control. The *V. parahaemolyticus* isolated from the hepatopancreas of infected shrimp matched 100% with the existing AHPND strain. The trial results show that the developed natural herbal formulation has significant effect against AHPND in a controlled condition.

KEYWORDS: Acute hepatopancreatic necrosis disease (AHPND); *Vibrio parahemolyticus*; Essential oil blend; anti-AHPND feed.

Ministry of Fisheries, Vietnam

Hiệu quả của Pondguard trong việc phòng trị bệnh hoại tử gan tụy cấp (AHPND) trên tôm nuôi

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Đặt vấn đề

AHPND (Bệnh hoại tử gan tụy cấp) hay EMS (Hội chứng chết sớm) trên tôm nuôi lần đầu tiên được phát hiện tại Trung Quốc năm 2009 (nhưng tại thời điểm đó chưa được quan tâm). Đầu năm 2011, bệnh bùng phát và trở nên nghiêm trọng, 80% sản lượng tôm bị chết tại các tỉnh Hải Nam, Quảng Đông, Phúc Kiến và Quảng Tây, Trung Quốc (*Leaño & Mohan, 2012*). Dịch bệnh xuất hiện và bùng phát trên TTCT được nuôi ở Việt Nam năm 2010, ở Malaysia năm 2011, ở Thái Lan năm 2012 và 2013 và ở Philippines năm 2013 và 2014. Ước tính thiệt hại trực tiếp và gián tiếp do AHPND gây ra cho ngành nuôi tôm của khu vực châu Á lên tới 1 tỷ USD (*FAO, 2013*).

Đặc điểm của bệnh AHPND là lây lan rất nhanh, tỷ lệ chết cao (có thể lên tới 100%), thường trong vòng 30 - 35 ngày thả ao nuôi với tôm post hoặc tôm nhỏ. Bệnh do thể thực khuẩn của vi khuẩn *Vibrio parahaemolyticus* gây ra, chúng đi qua đường miệng và xâm nhập vào đường tiêu hóa của tôm, sau đó tạo ra độc tố phá hủy cấu trúc và chức năng cơ quan tiêu hóa của tôm là gan tụy. Do đó, việc đánh giá thử nghiệm này rất cần thiết nhằm xác định khả năng chống lại vi khuẩn *Vibrio parahaemolyticus*.

Pondguard, chế phẩm do nhóm nghiên cứu thuộc PT Asclepius Pharmaceutical Sciences Indonesia, Indonesia nghiên cứu sản xuất và cung cấp cho thử nghiệm này. Đây là một hỗn hợp các tinh dầu chiết xuất từ thiên nhiên như tinh dầu oải hương, tinh dầu khuynh diệp, tinh dầu thông giúp duy trì khả năng miễn dịch của tôm, giúp bảo vệ tôm khỏi các bệnh truyền nhiễm. Sản phẩm Pondguard đã được cấp phép bởi Bộ Thủy sản Indonesia, số đăng ký D 16060285 - HBC. Sản phẩm ở dạng lỏng, hòa tan tốt trong nước, không màu và có pH 6,8 - 7,4 (*Jha và ctv, 2016*).

Phương pháp thí nghiệm

TTCT dùng cho thí nghiệm này có khối lượng trung bình 0,6 - 0,8 g/con (đây là kích cỡ tôm dễ bị tấn công bởi vi khuẩn *Vibrio parahaemolyticus*). Tôm thí nghiệm đã được sàng lọc bằng Real time PCR các tác nhân gây bệnh đốm trắng, AHPND và vi bào tử trùng gây bệnh tại Chi cục Thú y vùng II vào tháng 12/2019.

Tác nhân gây bệnh là *Vibrio parahaemolyticus* (VP) - chủng gây bệnh AHPND được sử dụng trong thí nghiệm là VP36, được phân lập từ tôm bị nhiễm AHPND (chủng đã được phân lập từ tôm bệnh lấy ở Sóc Trăng vào tháng 9/2016). Chủng VP36 được bảo quản ở - 80°C trong môi trường. Vi khuẩn được xác nhận là VP khi cho khuẩn lạc màu xanh trên môi trường TCBS agar (Thiosulfate Citrate Bile Salts Sucrose Agar) và khuẩn lạc màu tím trên môi trường CAV (Chrom Agar Vibrio), dương tính khi kiểm tra bằng phương pháp PCR với mồi AP3 (*Sirikharin và ctv, 2014*).

Tôm thí nghiệm được cho ăn 3 lần một ngày vào 8h, 12h30 và 17h hàng ngày. Bể nuôi, hệ thống sục khí và sản phẩm Pondguard được sử dụng theo liều lượng hướng dẫn của nhà sản xuất.

Thiết kế thí nghiệm

Chuẩn bị bể thí nghiệm bằng thủy tinh. Mỗi bể chứa 30 lít nước biển (nồng độ 15‰), có hệ thống sục khí liên tục và duy trì nhiệt độ nước từ 28 - 30°C. Nhóm thí nghiệm và nhóm đối chứng được bố trí trong hai phòng riêng biệt, khoảng cách giữa hai phòng khoảng 15 m. Tôm được thả với mật độ 15 - 20 con/bể.

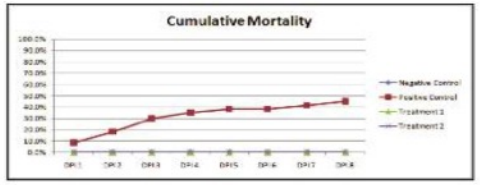
Hoạt hóa vi khuẩn VP36 trên môi trường CAV, vi khuẩn được nuôi cấy trong môi trường TSB+ trong 18 giờ, ở nhiệt độ 28°C.

Sản phẩm Pondguard được cho vào trong bước nuôi cấy vi khuẩn. Có hai nhóm thí nghiệm: Thí nghiệm 1, sản phẩm được cho trực tiếp sau khi cấy vi khuẩn và nuôi lắc trong 18 tiếng. Nhóm 2, sau 18 giờ nuôi lắc vi khuẩn, sản phẩm được cho vào môi trường và giữ tiếp trong 3 giờ trước khi tiến hành cho lây nhiễm. Dùng hộp nhựa chứa khoảng 300 ml dung dịch gồm nước biển và 30 ml môi trường vi khuẩn TSB+, có sục khí liên tục, ngâm 15 - 20 con tôm trong 15 phút. Sau đó, chuyển tất cả vào bể nuôi có chứa nước biển, giảm mật độ vi khuẩn xuống 10^6 tế bào/ml.

Tỷ lệ thức ăn là 7% trọng lượng tôm, cho ăn 3 lần/ngày trong suốt thời gian tiến hành thử nghiệm. Trong vòng 48 giờ sau khi cho lây nhiễm sẽ không thay nước, sau đó sẽ thay 20% nước trong bể mỗi ngày. Quan sát lượng tôm chết vào cùng thời gian cho tôm ăn. VP tổng số trong nước được đếm bằng cách đếm khuẩn lạc trên môi trường TCBS. Mẫu nước được lấy hàng ngày sau khi thay nước. Mẫu nước được lấy ở 3 bể trong mỗi nhóm. VP tổng số trên tôm được đếm bằng phương pháp RT-PCR. Mẫu tôm (tôm sống) được lấy vào lúc 0 giờ (trước khi tiến hành lây nhiễm) và lấy 2 ngày/lần.

Kết quả

Trong nhóm thí nghiệm 1, Pondguard được cho vào môi trường nuôi TSB+ với tỷ lệ 0,2% canh trường cùng lúc với cấy vi khuẩn, nuôi trong vòng 18 giờ. Trong thí nghiệm 2, Pondguard được cho với tỷ lệ 0,3% canh trường, cho vào sau khi vi khuẩn đã được nuôi 18 giờ.



Hình1: Tỷ lệ tôm chết cộng dồn sau 8 ngày gây nhiễm

Mật độ của vi khuẩn trong nhóm đối chứng dương sau 18 giờ nuôi cấy là $9{,}4x10^8$ cfu/ml, lượng vi khuẩn phát triển đã được phát hiện trong hai nhóm.

Không có trường hợp tôm chết nào được ghi nhận trong nhóm thí nghiệm dùng sản phẩm Pondguard. Trong nhóm đối chứng dương, tỷ lệ chết là 45% vào ngày thứ 8 sau khi gây nhiễm. Không có tôm chết trong nhóm đối chứng âm.

Trong nhóm đối chứng dương, tôm bắt đầu chết sau 12 giờ gây nhiễm. Nhóm đối chứng âm và nhóm thí nghiệm không có tôm chết.

Các dấu hiệu biểu hiện trên tôm bị nhiễm bệnh AHPND rất điển hình, như: dạ dày và ruột rỗng, gan tụy nhợt nhạt. Không quan sát được các dấu hiệu điển hình đó trong nhóm thí nghiệm 1. Trong nhóm thí nghiệm 2, các dấu hiệu bệnh AHPND xuất hiện trong ngày đầu tiên cho lây nhiễm với tỷ lệ 6,7%. Trong nhóm thí nghiệm 2, tôm dần phục hồi theo từng ngày và hoàn toàn bình thường vào ngày thứ 8 sau khi cho lây nhiễm; các dấu hiệu nhiễm bệnh đạt tỷ lệ tối đa 100% trong ngày đầu tiên gây nhiễm. Không có dấu hiệu nhiễm bệnh AHPND ở nhóm đối chứng âm.

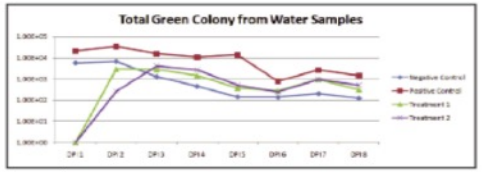
Hình 2: Từ trái qua phải: đối chứng âm, đối chứng dương, thí nghiệm 1 và thí nghiệm 2. Các dấu hiệu đặc trưng của AHPND gồm ruột rỗng, gan tụy nhợt nhạt được thấy rõ trong nhóm đối chứng dương.

Khả năng tiêu thụ thức ăn cũng được sử dụng là chỉ số để đánh giá về tình trạng sức khỏe cũng như mức độ stress của tôm. Trong nhóm thí nghiệm 1, tôm duy trì tiêu thụ thức ăn ở mức tối đa. Trong nhóm thí nghiệm 2 có sự giảm nhẹ về việc tiêu thụ thức ăn xảy ra đồng thời với việc xuất hiện các dấu hiệu bệnh AHPND. Việc tiêu thụ thức ăn trong nhóm đối chứng dương giảm mạnh. Điều này cho thấy trong nhóm thí nghiệm có dùng sản phẩm Pondguard, tôm không xuất hiện bệnh AHPND.

Phân tích RT - PCR: Phản ứng RT - PCR được thực hiện để phân tích mẫu gộp (dạ dày, gan tụy và ruột được cắt nhỏ lẫn với nhau). Nhóm đối chứng dương có giá trị Ct (Threshold cycle) thấp có nghĩa là số lượng mầm bệnh có mặt cao. Sự có mặt của mầm bệnh thấp đồng nghĩa với giá trị Ct cao, trong ngày đầu tiên và ngày thứ 3 của nhóm thí nghiệm 1. Tôm trong nhóm thí nghiệm được phục hồi sau 3 ngày và tất cả các mẫu sau đó đều cho kết quả âm tính với AHPND.

Tổng số khuẩn lạc Vibrio màu xanh từ mẫu tôm: Gan tụy, dạ dày và ruột được lấy và nghiền lần từ mẫu tôm sống. Mẫu lấy từ nhóm đối chứng dương có số khuẩn lạc xanh cao nhất. Tôm trong nhóm thí nghiệm có số khuẩn lạc thấp hơn 10^1 - 10^2 lần, đây là con số được coi là ở mức độ không nhiễm bệnh.

Mẫu nước được lấy hàng ngày trong suốt thời gian thí nghiệm để kiểm tra lượng Vibrio tổng số có trong môi trường nuôi tôm, cấy trên môi trường TCBS và đếm khuẩn lạc sau 20 giờ.



Hình 3: Tổng số khuẩn lạc Vibrio parahaemolyticus từ mẫu nước

Kết quả cho thấy tổng số khuẩn lạc VP trong mẫu nước của nhóm đối chứng dương cao hơn so với các nhóm khác.

Kết luận

Chủng VP gây chết trên tôm với các biểu hiện đặc trưng của AHPND. Pondguard có khả năng kìm hãm sự phát triển của VP trên tôm, vì vậy mà không có tôm bị chết và cũng không có các đặc điểm của AHPND xuất hiện trên tôm. Trong nhóm thí nghiệm sử dụng sản phẩm Pondguard, kết quả RT-PCR âm tính cho thấy sự phát triển của VP đã bị ức chế hoàn toàn. Pondguard có khả năng tiêu diệt vi khuẩn VP trong nước nuôi tôm.

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2nd INTERNATIONAL CONFERENCE ON
Aquaculture and Fisheries
August 28-29, 2020

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Abstract

Early Mortality Syndrome (EMS) is highly infectious in the early culture stage of *Penaeus vannamei*. Successful efforts have been made to develop a "Natural Herbal Blend Formulation (NHBF) " with anti-EMS properties. A trial was set-up in a biosecure bioassay lab of CeRAF, Hanoi, Vietnam using 0.6 to 0.8 g SPF Penaeus vannamei shrimp as a challenge model. The lethal dose of *Vibrio parahemolyticus*- EMS (VP-EMS) strain procured from Prof. Don Lightner, University of Arizona was used in the experiment. The lethal dose of VP-EMS (log 7) incubated into the treatment dose (2 ml/liter) of NHBF before immersion challenge to treatment group. The VP-EMS (log 7) was incubated with TS buffer before immersion challenge to positive control shrimp. The 100% survival recorded in NHBF treated group and negative control group whereas 48.6% cumulative mortality recorded in the positive control group. The VP-EMS presence was confirmed by using RT-PCR analysis and chrome agar platting methods. ponds. The findings suggest that NHBF has anti-EMS properties.

Biography

Haig Yousef Babikian in a Ph. D. and working as research director at Asclepius Pharmaceutical Sciences. He has vast knowledge and research experience on Essential Oil Blend formulations. He has been working on the formulations of blend essential oils to develop anti-viral, anti-bacterial products to be applicable on human, animals as well as on aquatic animals. He has more than 10 research articles published in peer reviewed journals and holding several patents.

Efficacy of natural herbal blend formulation against White Spot Syndrome Virus (WSSV) in Vannamei shrimp and its application optimization in shrimp culture ponds

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Abstract

The White Spot Syndrome Virus (WSSV) is lethal in all the penaeid shrimp including Penaeus vannamei. Successful efforts have been made to develop a "Natural Herbal Blend Formulation (NHBF)" with immunomodulatory properties in its immune dose and anti-viral properties in its treatment dose. A bioassay trial was set-up using 1 g SPF *Penaeus vannamei* shrimp as a challenge model. The lethal dose of White Spot Syndrome virus-infected tissue was incubated into the treatment dose (2 ml/liter) of NHBF before per os challenge to shrimp. The 100% survival recorded in NHBF treated group and negative control group whereas 89.6% cumulative mortality recorded in the positive control group. The Relative Percent Survival was 100%. The WSSV presence was confirmed by histopathology and nested PCR analysis. The second part of the study was to application optimization of NHBF in the culture ponds and measures its benefits. Total six pounds (3 each for Pondguard and control) of 1000 m2 area selected for the studies. There was no significant difference recorded in critical water quality parameters, like, plankton population and fluctuation, Dissolved Oxygen, Ammonia, pH, Total Alkalinity, etc. between treatment and control ponds studied. The pathogenic *Vibrio* spp. of NHBF applied ponds were having a lower count than the control ponds. All the trial ponds were negative to WSSV, IMNV, EHP, and EMS. There was an increase in immunity level and, Survival rate (0.32%), Feed conversion ratio (-4.21%) higher productivity (7.66% higher) in NHBF applied ponds. The findings suggest that the NHBF has anti-pathogenic properties with no side effects on the pond environment. It enhances the productivity and profitability of shrimp farms.

Biography

Haig Yousef Babikian in a Ph. D. and working as research director at Asclepius Pharmaceutical Sciences. He has vast knowledge and research experience on Essential Oil Blend formulations. He has been working on the formulations of blend essential oils to develop anti-viral, anti-bacterial products to be applicable on human, animals as well as on aquatic animals. He has more than 10 research articles published in peer reviewed journals and holding several patents.

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