

Efficacy of Homoeopathic Medicines in *Litopenaeus vannamei* Juveniles Experimentally Challenged with White Spot Syndrome Virus: A Pilot Comparative Study

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Abstract—Background: White Spot Syndrome Virus (WSSV) is a severe viral disease of shrimp associated with rapid mortality. **Objectives:** To evaluate and compare the overall survival rate between the Homeopathically treated and placebo groups And Also To understand the effectiveness of Homoeopathic Medicine in One sided Diseases.

Methods: Fifty specific-pathogen-free shrimp were experimentally challenged by intramuscular injection of 0.1 mL WSSV inoculum and randomly allocated to five groups of ten. Arsenicum album, Sulphur, Conium maculatum, and Calcareo carbonica were administered at 200C by aqueous immersion; the fifth group received the vehicle as placebo control. Survival and clinical observations were recorded for 9 days. **Results:** By Day 9, survival was 0% with Arsenicum album, 30% with Sulphur, 90% with Conium maculatum, 30% with Calcareo carbonica, and 0% with placebo. The dissertation-reported chi-square comparison of final alive/dead status showed a statistically significant difference among groups ($\chi^2=25.714$, $df=4$, $p=0.000036$). **Conclusion:** Conium maculatum showed the highest observed survival in this pilot experiment. Because each treatment condition was represented by a single tank, the findings are preliminary and hypothesis-generating and require confirmation in replicated experiments with larger samples and standardized challenge conditions.

Index Terms—White Spot Syndrome Virus; *Litopenaeus vannamei*; homoeopathy; Conium maculatum; shrimp aquaculture; experimental study

I. INTRODUCTION

Global aquaculture of the Pacific white shrimp, *Litopenaeus vannamei*, is important for food security and coastal livelihoods. Intensification of shrimp

farming has, however, been constrained by viral diseases, among which White Spot Syndrome Virus (WSSV) is a major pathogen. WSSV is a large double-stranded DNA virus of the family Nimaviridae associated with rapid disease progression and high mortality. Clinical manifestations described in the dissertation include lethargy, reduced activity, reduced feeding, reddish discoloration and white calcified spots on the carapace.[1–6]

Current management of WSSV is largely preventive, including biosecurity, quarantine, use of specific-pathogen-free stock, water-quality management and control of vectors and carriers. Experimental approaches including herbal products, immunostimulants and molecular strategies have been investigated, but a universally effective curative treatment has not been established.[7–14]

There is limited experimental evidence on the use of Homoeopathic medicines in WSSV-Challenged shrimp. Therefore, this study was conducted to explore their effect under controlled experimental conditions.

II. MATERIALS AND METHODS

Study design and setting

This comparative experimental pilot study was conducted at the Department of Aquatic Animal Health Management, College of Fisheries, Yekkur, Kankanady, Mangaluru, Karnataka, during 20–28 February 2026. The study followed a completely randomized design with a single-blind protocol. Fifty specific-pathogen-free *L. vannamei* juveniles were selected by purposive sampling and acclimatized for 72 hours before viral challenge.

Experimental animals

Shrimp were selected as apparently healthy and active, with normal feeding behaviour. Exclusion criteria included physical trauma or cannibalism injuries, broken antennae, visible structural opacity or cloudy/white tail muscle, lethargy or abnormal clustering, and feed refusal during stabilization. The total sample consisted of 50 juveniles, with 10 shrimp assigned to each of five tanks.

Viral challenge

WSSV inoculum was prepared from naturally infected *L. vannamei* tissue exhibiting classical white spots and lethargy and was verified by the Department of Aquatic Animal Health Management. Infected tissue was homogenized in a 1:10 ratio using TNE/STE buffer. Each shrimp received 0.1 mL of viral homogenate by intramuscular injection at the junction of the fifth and sixth abdominal segments. Inoculation was performed on 20 February 2026 at 14:30 h under departmental supervision.

Treatment allocation and administration

Immediately after viral challenge, shrimp were randomly allocated to five groups of ten. T1 received Arsenicum album 200C, T2 Sulphur 200C, T3 Conium maculatum 200C, T4 Calcarea carbonica 200C, and T5 an unpotentized vehicle as placebo control. Each 200C preparation was dispensed into 100 mL distilled water and introduced into the respective treatment tank by aqueous immersion once weekly at 14:30 h. The control received an equal volume of vehicle on the same schedule. The observer recording daily observations was not informed of tank allocation.

Group	Intervention	Potency
T1	Arsenicum album	200C
T2	Sulphur	200C
T3	Conium maculatum	200C
T4	Calcarea carbonica	200C
T5	Placebo vehicle	—

Observation and outcome

The observation window was 9 days. Clinical assessment was performed every 12 hours at 08:00 and 20:00 h, recording swimming behaviour, feeding activity, cuticular colour change and white calcified spots. Mortality was recorded to the nearest observation interval. The primary reported outcome was survival at Day 9.

Statistical analysis

The dissertation reports Kaplan–Meier-style survival curves for the observation period and a chi-square test comparing final alive/dead status across the five groups. The reported result was $\chi^2=25.714$, $df=4$, $p=0.000036$. The statistical software used was not specified in the dissertation.

III. RESULTS

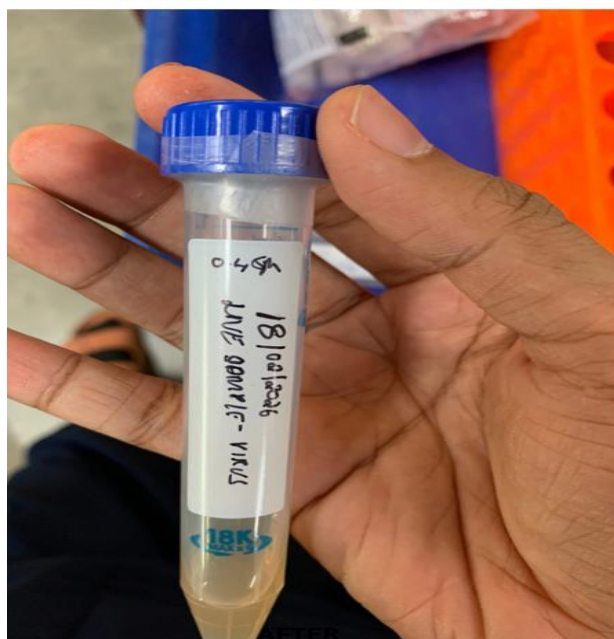
All 50 shrimp survived the immediate post-injection period. Transient lethargy and bottom-settling were observed after injection, with recovery of equilibrium within approximately 45 minutes. Survival declined progressively in the placebo group, which reached zero survival by Day 5. By Day 9, T3 retained nine of ten shrimp, while T2 and T4 retained three of ten each. T1 and T5 had no survivors.

Table 1. Daily number of surviving shrimp in each experimental group over the 9-day observation period.

Day	T1	T2	T3	T4	T5
1	10	10	10	10	10
2	10	10	10	10	10
3	10	10	10	10	5
4	10	7	10	8	4
5	5	7	10	6	0
6	3	6	9	5	0
7	2	5	9	4	0
8	1	4	9	3	0
9	0	3	9	3	0

Table 2. Final Day-9 survival by experimental group.

Group	Alive Day 9	Dead Day 9	Survival (%)
Arsenicum album 200C (T1)	0	10	0
Sulphur 200C (T2)	3	7	30
Conium maculatum 200C (T3)	9	1	90
Calcarea carbonica 200C (T4)	3	7	30
Placebo (T5)	0	10	0





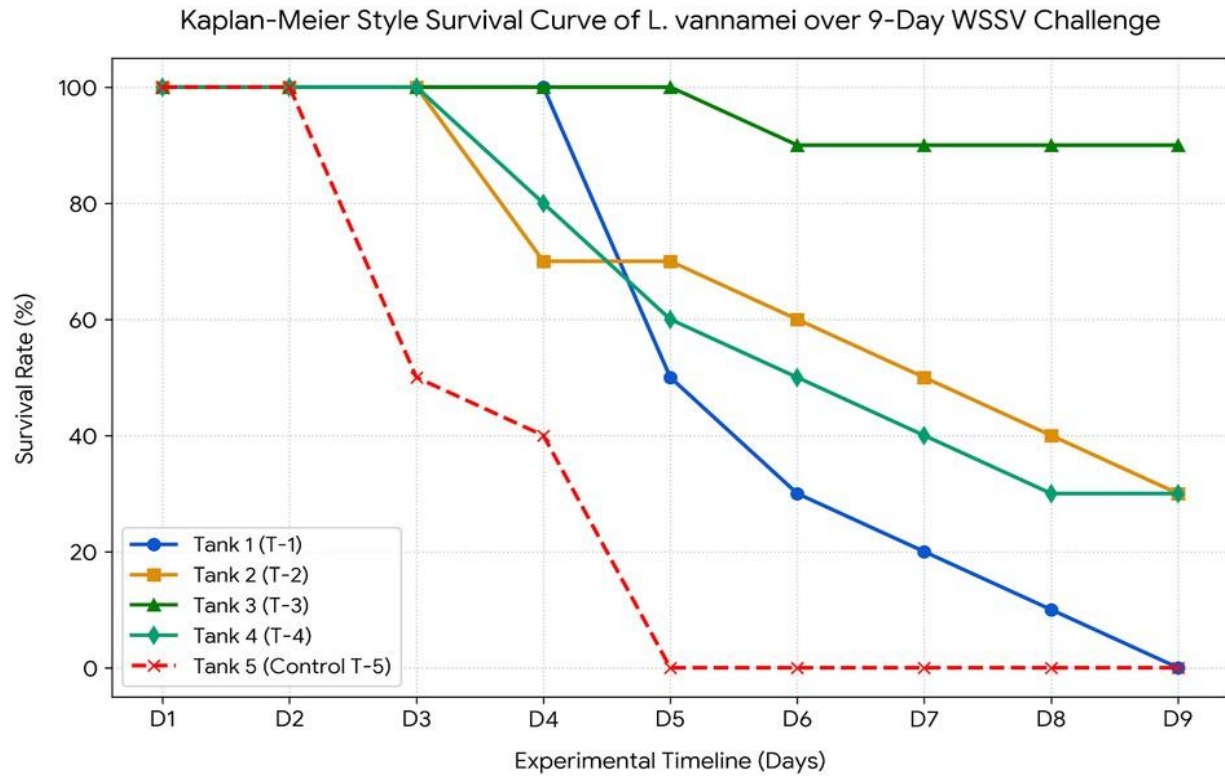


Figure 1. Comparative Kaplan–Meier-style survival curves of *L. vannamei* juveniles ($n=10$ per cohort) over the 9-day post-inoculation observation period. Reproduced from the dissertation.

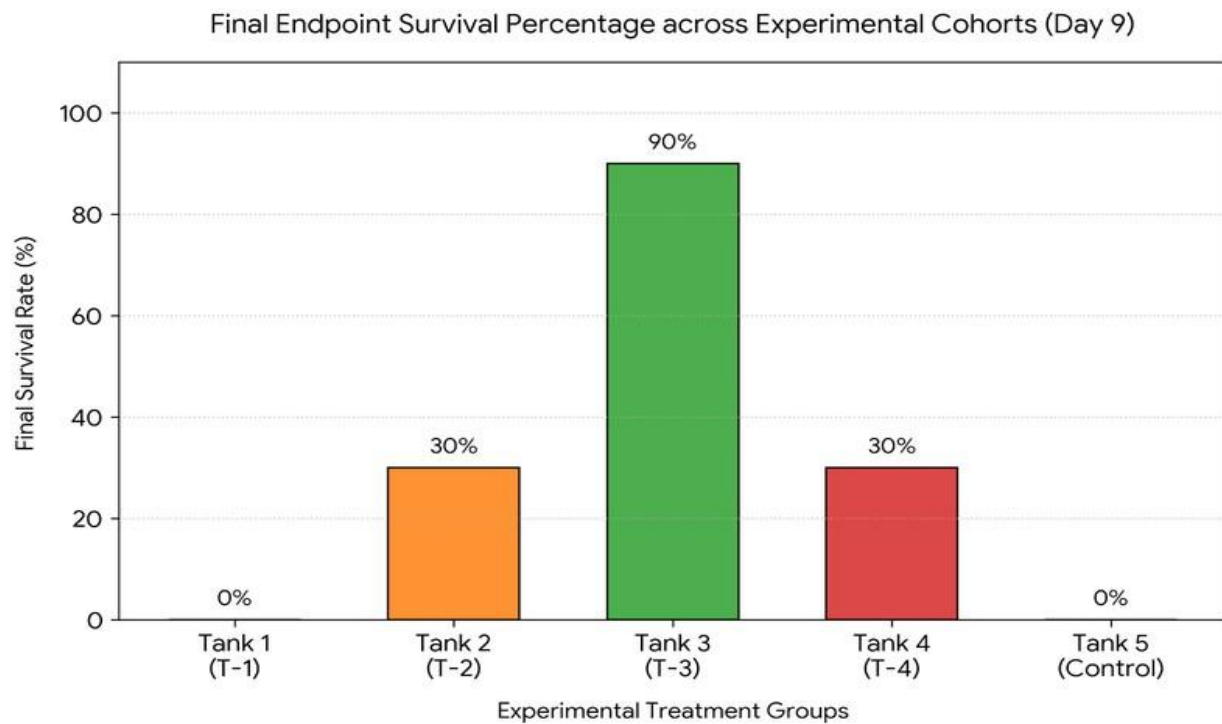


Figure 2. Final endpoint survival percentages of *L. vannamei* juveniles across the five experimental groups on Day 9. Reproduced from the dissertation.

The dissertation-reported chi-square analysis of final survival showed a statistically significant difference in alive/dead outcomes across the five groups ($\chi^2=25.714$, $df=4$, $p=0.000036$). Since only one tank was used for each treatment, the result should be interpreted with caution and cannot be taken as definite evidence of treatment effects.

IV. DISCUSSION

The present pilot study evaluated four homoeopathic medicines in WSSV-challenged *L. vannamei*. Complete mortality in the placebo group by Day 5 was consistent with the rapid and severe course of WSSV described in the dissertation and its cited literature.[1,3,5,6]

Conium maculatum 200C showed the most favourable observed outcome, with 90% survival at Day 9. Sulphur 200C and Calcarea carbonica 200C each showed 30% survival, whereas Arsenicum album 200C showed no survival at the endpoint. The variation indicates that the observed response was not uniform among the tested protocols.

However, these findings should be interpreted cautiously because this was a pilot study. The most important limitation is the use of a single tank for each treatment condition. Shrimp kept in the same tank share the same water, Feed and environmental conditions. Therefore, they cannot be considered completely independent replicates. Differences between tanks may also have influenced the results. The chi-Square test may therefore give an overly confident estimate if individual shrimp are considered independent observations.

Other limitations were the small sample size and the use of only one trial. Individual molecular confirmation of infection or viral-load assessment, and biomarkers such as haemocyte count, phenoloxidase activity and antioxidant status were also not assessed.

Therefore, the present study cannot establish the mechanism behind the higher survival observed in T3. Future studies should use multiple independent replicate tanks per treatment, larger samples and standardized viral challenge conditions. Survival should be accompanied by molecular confirmation, viral-load assessment, histopathology and relevant immune or biochemical endpoints.

V. CONCLUSION

In this pilot experimental study, CONIUM MACULATUM 200C WAS ASSOCIATED WITH THE HIGHEST OBSERVED SURVIVAL among the four homoeopathic treatment groups, with 9 of 10 shrimp surviving to Day 9, whereas the placebo group showed complete mortality by Day 5. These findings are preliminary and need to be confirmed by further studies. Further studies with more animals and independent replicate tanks are needed before the efficacy of these medicines can be evaluated reliably.

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Certificate of Acknowledgment

This is to certify that Ms. Lamiya P., BHMS intern of Alvas Homeopathic Medical College, Mijar, Moodbidri has successfully conducted her research experiment titled: "Efficacy of Homeopathic Medicine on Shrimp Affected from WSSV: An Experimental Study" in our laboratory from 20th February to 28th February 2026.

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Ethics and institutional permission

This study involved *Litopenaeus vannamei*, a crustacean invertebrate, rather than a fish. The

dissertation documents institutional permission and technical supervision at the College of Fisheries, Yekkur, Kankanady, Mangaluru. It does not report a formal animal ethics committee approval or exemption number. Accordingly, no ethics approval number has been invented or stated in this manuscript.

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