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Effect of *Nigella sativa* on growth and survival rate of *Penaeus vannamei*

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Abstract
Nigella sativa oil has been used to determine whether it can improve the immunity of *Penaeus vannamei* Shrimps. In this study, *Nigella sativa* oil was used to coat commercial feed and fed to the *Penaeus vannamei*, where they were observed and data was collected for their growth and survival rate. Twenty shrimps were placed in different tanks and each tank was fed with their assigned type of feed and feeding rate percentage. There were a total of six tanks, two Control tanks, two Treatment 1 tanks and two Treatment 2 tanks. Shrimps in the Control tanks were fed with commercial feed, those in Treatment 1 tanks were fed with 10 mL/kg of *Nigella sativa* coated commercial feed and those in Treatment 2 tanks were fed with 20 mL/kg of *Nigella sativa* coated commercial feed. Before the project, it was hypothesized that the tanks fed with Treatment 2 would have the highest growth and survival rate. At the end of the project, Treatment 2 had the best survival and growth rates compared to Treatment 1 and Control. The results supported the hypothesis that the shrimps fed with more *Nigella sativa* oil had the highest growth rate and survival rate. However, it was also observed that Control had a higher growth rate compared to Treatment 1.

Keywords: *Nigella sativa*, *Penaeus vannamei*, growth, survival rate, effect

1. Introduction
Penaeus vannamei, also known as the white leg shrimp, originates from the Eastern Pacific coast of Central and South America; they are found in marine water where water temperatures are normally above 20 ºC as they are a type of tropical shrimp species. *Penaeus vannamei* mature at about 6-7 months old, when mature, the males will weigh at about 20 g and females 28 g and above. It is a common commercial food shrimp, is easily obtainable, and has reasonable feed requirement prices (FAO) [1]. *Penaeus vannamei* is a crustacean and like all crustaceans, do not possess acquired immunity and is thus unable to produce antibodies. Their only safeguard is their dependence on their innate immunity. Thus, unlike fish, they are incapable of obtaining immunity to diseases through vaccination (Techna, 2018) [2].
Nigella sativa is a medicinal herb and part of the Ranunculaceae family. In the wild, they can be found in Southern Europe, Northern Africa, and Southern-western Asia. The flowers of *Nigella sativa* have white or pale to dark blue petals and are self-branching plants. For human consumption, they are used for allergies, immunity boost, cognitive function, and brain health (Kamal, 2018) [3]. *Nigella sativa* oil particularly consists of thymoquinone (TQ), which is the main component that has shown anti-inflammatory effects. This has been tested on several inflammation-based models which included experimental encephalomyelitis, colitis, peritonitis, oedema and arthritis. This was done by subduing the inflammatory mediators, prostaglandins and leukotrienes (Venkatachallam *et al.*, 2010) [4]. The oil and its active ingredients are said to have anti-microbial, anti-tumor properties towards different microbes and cancers, as well as having antifungal activity, cytoprotective and antioxidant properties (Ijaz H *et al.*, 2017) [5].
The oil used in this study was extracted from the seeds of *Nigella sativa*. So far, there has been no evidence that *Nigella sativa* has any effect on the survival rate, but it is shown to increase macrophage activity in attacking pathogens (Apines *et al.*, 2015) [6] and to have lowered bacterial activity with a higher *Nigella sativa* oil dosage for fish (Awad *et al.*, 2013) [7].
In shrimp aquaculture, there are many diseases that have killed off many shrimps as they are unable to defend themselves from pathogens due to having no acquired immunity. To decrease the chances of introducing pathogens and other harmful bacteria, good husbandry, such as no

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overfeeding, regular water changes and water quality tests should be practiced. Diseases had caused a high mortality rate for shrimp farmers and caused economic loss (Seibert *et al.*, 2012) [8]. Immunostimulants are used to help fish and shellfish be more resistant to microbial infections by activating their immune system (Raa, 1996) [9]. They help to boost the speed of recognition and removal of a wide variety of infectious pathogens and foreign substances, increase the strength of the immune systems and resistance to pathogens when exposed to stress. Medicinal herbs have been hypothesized to be capable of improving the growth and survival rate, this has caused belief that using phytochemicals could possibly replace the usage of chemotherapeutic chemicals in the aquaculture industry (Hai, 2015) [10]. It has been studied that many plant natural products (medicinal and dietary plants) have exhibited potent anti-inflammatory *in vivo* by distinct molecular mechanisms (Gertsch et al., 2011) [11]. The possible usage of using medicinal herbs to replace chemical immunostimulants has appealed to many countries due to being cheap, simple to produce and have lesser side effects when treating diseases (Hai, 2015) [10].

In this study, *Nigella sativa* oil was used to coat commercial feed and fed to the shrimp of *Penaeus vannamei*. This study was to observe whether the usage of *Nigella sativa* will positively affect the growth and survival rates of *Penaeus vannamei*. The shrimp's growth and survival performance were observed, recorded and analysed.

2. Materials and Methods
The 3 kg of fresh shrimp juveniles (*Penaeus vannamei*, around 15 g each) were purchased from Ng Yong Hock Investments Pte Ltd, a subsidiary of Apollo Aquaculture Group Pte Ltd in Singapore. These juveniles were delivered to the experimental site in oxygenated plastic bags. A 60-day experiment was conducted during July to September 2018 at School of Applied Science, Temasek Polytechnic.

2.1 Water quality
2.1.1 Preparation
A total of fourteen falcon tubes, two cuvettes and twelve water samples from each shrimp tank were prepared for water quality testing.

2.1.2 Nitrite test
Nitrite test was conducted every week during the experiment. Each falcon tube was filled up with 10 mL of each tank's water sample respectively, and 10 mL of deionized water was used to fill up a falcon tube for the blank. One packet of nitrite reagent (Nitri Ver, 3 Nitrite reagent for 10 mL sample) will be added into each falcon tube. The samples were left to rest for 20 minutes for the reaction to occur.

2.1.3 Ammonia test
Like the nitrite test, the ammonia test was conducted every week during the experiment. Each falcon tube was filled with 25 mL containing each tank's water sample respectively and 25 mL of deionized water was used to fill up a falcon tube for the blank. 1 mL of mineral stabilizer was added into each sample and 3 drops of mineral stabilizer were added to the blank. Mineral stabilizer was used to break down any sediments in the water since it is saltwater. Each sample was capped and inverted 10 times to mix the solution, 3 drops of polyvinyl alcohol were added into the samples and blank, to allow the colour to form in the solution after the addition of

Nessler's reagent which was followed by inversion of the tubes. Using a pipette, 1 mL of Nessler's reagent was added into each sample and blank and will be mixed again. The solutions were left to rest for 1 minute for the reaction to take place.

2.1.4 Spectrophotometer test
The spectrophotometer was turned on to test for nitrite and ammonia. The cuvette was first filled to 10 mL of the blank sample and was placed into the spectrophotometer and tallied to zero. The cuvette was rinsed with deionised water before being filled up to 10 mL with each sample. The external surface of the cuvette was cleaned with paper towels before it was placed in the spectrophotometer, allowing the readings to be more accurate. The results for the ammonia and nitrite test as well as the dilution were recorded down. The process was repeated with the next sample until all of the samples were done.

2.1.5 Dilution
During the experiment, there were some water samples where the nitrite and ammonia levels were above the range the spectrophotometer can read. To resolve this issue, dilution of the samples with distilled water was conducted. During the experiment dilution of 5X, 10X, 20X and 50X were used.

2.2 Feed preparation
2.2.1 Feed preparation of control, treatment 1 and treatment 2
For Control, the normal commercial feed was given to them after measuring the correct amount. Treatment 1 feed was prepared by measuring 350 g of feed. 3.5 mL of *Nigella sativa* oil was measured and was applied to the measured feed until the oil evenly coat the feed pellets. For Treatment 2, the exact same steps were done except 7.0 mL of *Nigella sativa* oil was evenly coat the 350 g of feed.

2.2.2 Feed weight measurement
After the weight of the shrimps was taken, the amount of feed given for each time of the day (Morning-9am, afternoon-1pm and evening-5pm) were calculated by multiplying the total weight of the shrimp in each tank with the desired feeding rates (1%, 2% and 3%). The feeding rates were changed depending on the weight of the shrimp, their feeding habits, the feed conversion rate (FCR) and their health.

2.3 Weighing of the shrimps
The weighing of the shrimps was done once every week. The total weight of the shrimps was used to calculate the amount of feed to be given to the shrimps daily and to help calculate the feed conversion rate (FCR) of these shrimps.

2.4 Calculation
2.4.1 Survival rate

$$\text{Survival rate (\%)} = \frac{\text{Number of shrimp at the end of the experiment}}{\text{Number of shrimp at the beginning of the experiment}} \times 100$$

2.4.2 Specific growth rate

$$\text{Growth rate (\%)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Number of days}} \times 100$$

2.4.3 Mean weight gain
Mean weight gain = Final mean weight − Initial mean weight

2.4.4 Feed conversion rate (FCR)

$$FCR = \frac{Total\ amount\ of\ feed\ given}{Total\ weight\ gain\ of\ shrimps}$$

2.5 Statistical analysis
Data obtained from the experiments for growth parameters and survival were analysed by one-way ANOVA. Student-Newman-Keuls test was used to determine the significant difference between the treatments.

3. Results
3.1 Water quality
During the experiment, water parameter tests were done once a week. Table 1 featured the ammonia level range, table 2 featured the nitrite level range and table 3 featured the pH level range when doing water quality test. From the data collected, it was shown that Control and Treatment 1 were beyond the normal ammonia level range of 0.1 mg/L to 2.4 mg/L during week 1. Control, Treatment 1 and Treatment 2 also had abnormal nitrite levels and were beyond the normal range, which are usually below 4.5 mg/L. They were also below the pH normal range of 7.4 – 7.9 during week 2, the pH for the tanks in Control, Treatment 1 and Treatment 2 were slightly below the normal range and had higher nitrite levels than normal. The ammonia levels, nitrite and pH levels have remained optimum and within normal range for the rest of the experiment.

Table 1: Range of ammonia levels during the experiment

Ammonia					
Week	Week 1	Week 2	Week 3	Week 4	Week 5
Control	1.25 - 4.30	0.31 - 1.96	0 - 0.25	0	0.16 - 0.29
Treatment 1	1.55 - 6.60	0.48 - 1.68	0 - 0.25	0	0.29
Treatment 2	1.07 - 1.56	0.80 - 2.75	0	0	0.30

Table 2: Range of nitrite levels during the experiment

Nitrite					
Week	Week 1	Week 2	Week 3	Week 4	Week 5
Control	11.7 - 12.2	6.56 - 6.85	0.25 - 0.50	0.25 - 0.50	0.17 - 1.88
Treatment 1	9.08 - 12.55	3.90 - 8.20	0.50	0.50	0.261 - 0.63
Treatment 2	10.96 - 15.25	3.32 - 8.60	0.50	0.25 - 0.50	0.218 - 0.42

Table 3: Range of pH levels during the experiment

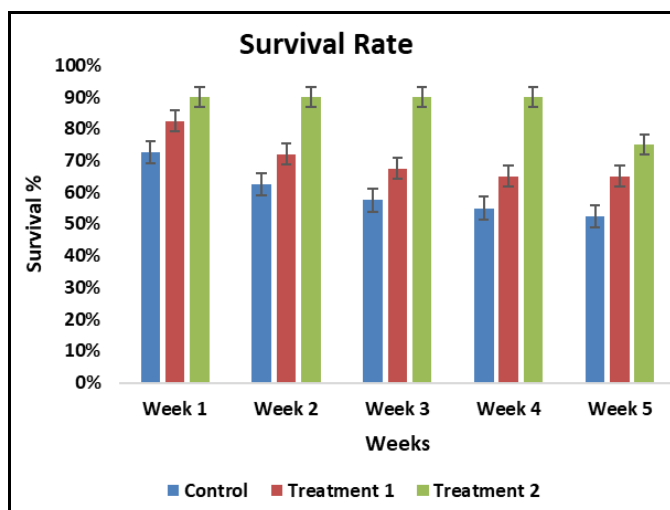| pH					
Week	Week 1	Week 2	Week 3	Week 4	Week 5
Control	6.1 - 6.3	7.2 - 7.6	7.4 – 8.0	7.4	7.4 - 7.5
Treatment 1	6.3 - 6.7	7.2 - 7.3	7.4	7.4 - 7.8	7.4
Treatment 2	6.3 - 6.5	7.3 - 7.4	7.4	7.8	7.4 - 7.6

3.2 Survival rate

Fig 1: Bar graph that shows the survival rate throughout the experiment

Based on Fig 1, the survival rate decreased at a slow rate for Control and Treatment 1 while Treatment 2 had a sudden slight drop on the last week. From the Control graph, we can see that there was a sudden drop at week 1 and 2 before maintaining a steady decrease in the survival rate from week 3 to week 5. From Treatment 1, there was a sudden drop at week 1 as well as a slight drop in survival rate from week 2 to week 3 too, before maintaining an even decrease and became even from week 4 onwards. For Treatment 2, there was a sudden drop in survival rate, though not as bad as Control and Treatment 1 at week 1. There was no decrease in survival rate until week 5 where there was a sudden drop by 15%.

Based on the results, Treatment 2 had the best survival rate compared to Control and Treatment 1 throughout the experiment.
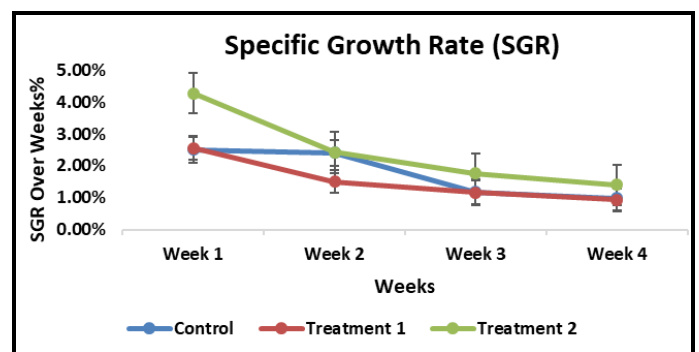
3.3 Specific growth rate

Fig 2: Line graph that shows the specific growth rate throughout the experiment

In Control, the SGR decreased very slightly from week 1 to week 2, but there was a deep drop between week 2 and week 3. Between week 3 and week 4, the drop in SGR is more gradual as compared to week 2 and week 3.

In Treatment 1, the SGR decreased drastically between week 1 and week 2 before it maintained a slow descend between week 2 and week 4. In Treatment 2, the SGR decreased drastically between week 1 and week 2 before it maintained a

slow descend between week 2 and week 4. On the whole, Treatment 2 has the best specific growth rate among all the treatments and Control during the experiment. At week 2, it was observed that the specific growth rate was around the same for Control and Treatment 2 and the same thing was observed in week 3, except that it was between Control and Treatment 1 (Fig 2).
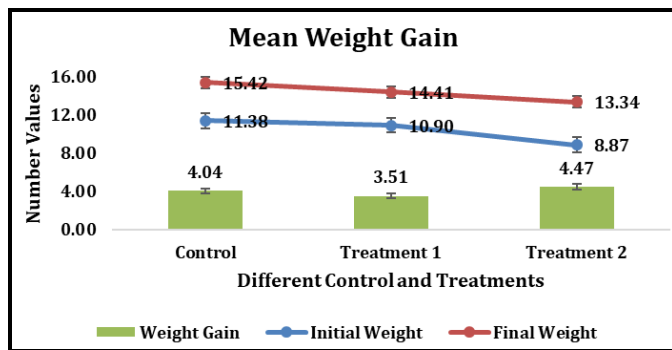
3.4 Mean weight gain

Fig 3: Line and bar graph that shows the initial and final mean weight with difference between the two

In the Mean weight gain (Fig 3), the results were collected over a range of 31 days (1 month) to calculate the mean weight gain. As seen in the Fig 3, Treatment 2 had the highest weight gain compared to Control and Treatment 1.

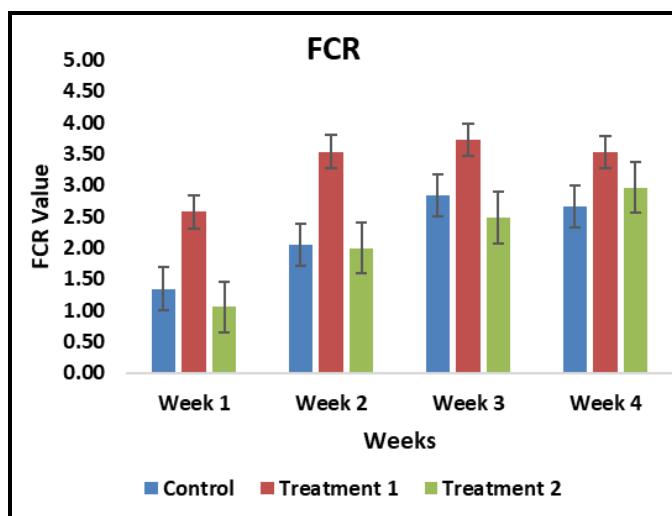### 3.5 Feed conversion rate (FCR)

Fig 4: Bar graph that shows the FCR throughout the experiment

As seen in Fig 4, during week 1 Control was within the normal range before it increased gradually between week 1 and week 3. Between week 3 and week 4, the data show that the FCR for Control decreased slightly.

For Treatment 1, throughout the experiment, the FCR was constantly above the normal range. Between week 1 and week 2, there was a spike in the FCR before it increased slightly between week 2 and week 3. Between week 3 and week 4, there was a slight decrease in the FCR.

For Treatment 2, during week 1 and week 2, the FCR was within normal range despite the spike between week 1 and week 2. In week 3 and week 4 however, it can be shown that the FCR has increased to over the normal range. From week 1

to week 4, it can be observed that the FCR was steadily increasing.

Overall, Treatment 2 had the best FCR among all the groups, followed by Control and Treatment 1. In week 4, it be seen that the FCR for the Control became the lowest among all 3 groups. In conclusion, Treatment 2 overall has a better FCR than Control and Treatment 1.

4. Discussion

For week 1, the ammonia levels and pH were all above the normal range or below the normal range for Control and Treatment 1. This was the same week where there was one of the most mortality in a week during the project. From this, we can see that *Nigella sativa* has helped to improve the shrimp's immunity as there was the lesser mortality for Treatment 1 than Control despite having abnormal ammonia levels and Treatment 2 having the least mortality despite having lower than normal pH levels. During week 2, we can see that the ammonia level and nitrite levels for all the tanks were near to the highest optimum range or above the normal range. However, there was no mortality during that period for Treatment 2 and there were lesser mortality for Treatment 1 than Control. This could be due to the thymoquinone (TQ) and its properties of antioxidant, antibacterial and other properties that helped with the survival of the shrimp as well as the water quality (Ijaz *et al*., 2017) [5]. For the specific growth rate, Treatment 1 had a lower than Control near the end of the project. This was an unusual result as the expected result was being higher than Control and lower than Treatment 2. It was also shown in previous researches done that certain plant products used in feed have similar or better results (Amaya *et al*., 2007) [12]. It can be hypothesized that the shrimps had a hard time digesting the feed or did not adjust to the feed fast enough. This was evidenced when there was quite a bit of uneaten feed in the Treatment 1 and 2 tanks and none in the control tanks. A possible reason Treatment 2 had a better result despite not consuming all the feed is due to there being more *Nigella sativa*, thus thymoquinone and other components to have a more positive result (Towers, 2016) [13]. Further research can be done in the future to observe if that was the case. For the rest of the experiment there were no large number mortalities. Treatment 2 has the best specific growth rate, mean growth rate and survival rate overall, which shows the effectiveness of the *Nigella sativa* where it was shown improve the health, growth and general performance of the *Penaeus vannamei*. Throughout most of the project, Treatment 2 had the lowest FCR compared to Treatment 1 and Control, thus the shrimps in Treatment 2 had the optimal growth among the groups. It could also be noted that a certain amount of *Nigella sativa* might be required to be effective.

5. Conclusion

According to the results and discussion, it can be concluded that *Nigella sativa* helps to increase the survival rate and growth rate of *Penaeus vannamei* when it is added to their diet. The reason is perhaps that *Nigella sativa* is able to enhance the immunity of *Penaeus vannamei* acting a form of an immunostimulant (Flores-Miranda *et al*., 2011) [14]. This part of research work is still in progress.

7. References

1. Cultured Aquatic Species Information Programme *Penaeus vannamei*. Food and Agriculture Organization of the United Nations (FAO).
2. Techna. Particularities of the shrimp immune system: what are the impacts on overall stress resistance in shrimp farming? Aquaneo-techna.com, 2018.
3. Kamal Patel. Summary of *Nigella sativa*. Examine.com 2018.
4. Venkatachallam SKT, Pattekhan H, Divakar S, Kadimi US. Chemical composition of *Nigella sativa* L. seed extracts obtained by supercritical carbon dioxide. Journal of Food Science and Technology. 2010; 47:598-605.
5. Ijaz H, Tulain UR, Qureshi J, Danish Z, Musayab S. *Nigella sativa* (prophetic medicine): A review. Pakistan Journal of Pharmaceutical Sciences. 2017; 30:229-234.
6. Apines-Amar MJS, Amar EC. Use of immunostimulants in shrimp culture: An update. Biotechnological Advances in Shrimp Health Management in the Philippines. 2015, 45-71.
7. Awad E, Austin D, Lyndon AR. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture. 2013; 388-391:193-197.
8. Seibert CH, Pinto AR. Challenges in shrimp aquaculture due to viral diseases: distribution and biology of the five major penaeid viruses and interventions to avoid viral incidence and dispersion. Brazilian Journal of Microbiology 2012, 857-864.
9. Raa J. The use of immunostimulatory substances in fish and shellfish farming. Reviews in Fisheries Science 1996; 4:229-288.
10. Hai NV. The use of medicinal plants as immunostimulants in aquaculture: A review. Aquaculture 2015; 446:88-96.
11. Gertsch J, Viveros-Paredes JM, Taylor P. Plant immunostimulants-Scientific paradigm or myth? Journal of Ethnopharmacology. 2011; 136:385-391.
12. Amaya E, Davis DA, Rouse DB. Alternative diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 2007; 262:419-425.
13. Towers L. Management of intensive vannamei shrimp ponds. Thefishsite.com, 2016.
14. Flores-Miranda MC, Luan-Gonzalez A, Campa-Cordova AI, Gonzalez-Ocampo HA, Fierro-Coronado JA. Microbial immunostimulants reduce mortality in whiteleg shrimp (*Litopenaeus vannamei*) challenged with *Vibrio sinaloensis* strains. Aquaculture. 2011; 320:51-55.