Research Title:	Evaluation of Oyster Shell Powder Effectiveness in Prolonging Tomato Senescence and Significant Factors in its Physical Environment in Angsila, Chonburi, Thailand
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Abstract

This environmental research aimed to evaluate the efficacy of Oyster (*Saccostrea cucullata*) Shell Powder in prolonging the senescence of Tomato (*Solanum lycopersicum*). It also intended to evaluate the significant factors commonly present in the physical environment of the said organism. Standard equipment from Extech was used to measure various parameters from the habitat of the shells namely water temperature, TDS, dissolved oxygen, salinity, electrical conductivity, transparency, water pH, air temperature, and relative humidity. Visual comparison was also applied to evaluate the color of the water. Oyster shells were burned at 900°C using the furnace at Kasetsart University, Sri Racha Campus. It was used to prolong the shelf-life of tomatoes. There were 4 treatments used in the said test namely control (distilled water), 1%, 5%, and 10% oyster shell powder solution. The results of the different experiments were observed, gathered, and compared using one-way ANOVA and Tukey HSD Test. Based on the experimentations, results and gathered data, the researchers concluded that there are significant differences in water temperature, DO, TDS, Electrical conductivity, salinity, transparency, relative humidity, and air temperature, except water pH in the physical environment of oyster. Additionally, the shell powder solution of Oyster (*Saccostrea cucullata*) can prolong the senescence of tomatoes.

Keywords: Senescence, Prolong, physical environment

Introduction

In this pandemic season, people in Thailand and around the world have shown a greater interest in the cultivation and consumption of vegetables due to the food value of vegetables in human diets, which can improve immunity against any form of pathogens. Growing and transporting of vegetables are vitally important today in order to suffice the needs of the consumers. Most vegetables are bulky and perish within a short time if not carefully handled. According to Centre for Agricultural Information of Thailand, poor handling conditions account for over 30 percent of losses in vegetables. Unless leafy vegetables and some fruit vegetables are harvested at the right stage of maturity and carefully packed, losses between 10–30 percent can occur at the consumer level. Vegetables, being perishable, are subject to heavy losses after harvesting and during transportation. Tomato (*Solanum lycopersicum*) is among the examples of vegetables that are vulnerable to losses. This very delicate vegetable is widely cultivated and consumed worldwide (Agrios, 2005). It will go through several procedures after the harvest such as packing, processing, transporting, and many more until it can go to the market and meet the customers. Along the way, this product will ripen and when it reaches the marketplace, it will only have few days to remain fresh then, it rots. According to various studies, in most developing countries, microbial infestation of tomatoes is common and have contributed to the rapid rotting process of the plant. (Yeboah, 2011). Microbial spoilage of fruits and vegetable is known as rot, which manifests as loss of texture (soft rot) changes in color (black or grey) and often off odor (Trias et al; 2008). The challenge here in order to prevent tomato from rotting quickly is to find an effective way to prolong its shelf-life.

A well-known location for the abundance of oyster shells is Angsila, Chonburi, Thailand. Due to the presence of this aquatic organism, the said place also became a center for the selling of oyster. This seafood is nutritious and very popular among consumers (Fisheries Statistics of Thailand, 2017). As a result of commercial activities of this valuable seafood, certain part in the coastal area of Angsila has been converted into a dumping site for shell wastes. This type of disposal is detrimental to the environment and can cause major ecological disturbance to the aquatic organisms that are endemic to the said place. These observations have prompted the researchers to evaluate the physical environment of oysters and to use the shells in prolonging the shelf-life of tomatoes. Utilizing the shells in

this research is workable and safe because oyster shells contain 96% of calcium carbonate ($CaCO_3$) (Yoon *et.al.*, 2003). A research revealed that when oyster shell powder was heated to higher temperature, it exhibited a bactericidal activity (*Sawai et. Al., 2001*). When the shells are heated at 700°C or above, its calcium carbonate structure changes to calcium oxide (CaO) (Alidoust *et al*., 2005). According to Miller et al., 2012, if CaO is dissolved or exposed to moisture, it further changes into calcium hydroxide ($Ca(OH)_2$).

The situations above led to the formulation of this environmental research entitled "Evaluation of Oyster (*Saccostrea cucullata*) Shell Powder Effectiveness in Prolonging Tomato (*Solanum lycopersicum*) Senescence and Significant Factors in its Physical Environment in Angsila, Chonburi, Thailand. The main goal of this research is to evaluate the efficacy of Oyster (*S. cucullata*) Shell Powder in prolonging the senescence of Tomato (*S. lycopersicum*). Furthermore, it aimed to evaluate the significant factors commonly present in the environment of the said organism.

Research Questions:

1. Can the Oyster (*Saccostrea cucullata*) shell powder prolong the senescence of Tomato (*Solanum lycopersicum*)?
2. Which concentration of Oyster (*Saccostrea cucullata*) shell powder is the most effective in prolonging tomato senescence?
3. Is there a significant difference in various factors commonly present in the physical environment of Oyster (*Saccostrea cucullata*)?

Objectives:

1. To evaluate the effectiveness of Oyster (*S. cucullata*) shell powder in prolonging the senescence of Tomato (*S. lycopersicum*).
2. To find out if there is a significant difference in various factors found in the physical environment of Oyster (*S. cucullata*).

Hypotheses:

Alternative: The Oyster (*Saccostrea cucullata*) shell powder can prolong the senescence of Tomato (*Solanum lycopersicum*) and there is significant difference in all factors measured within the physical environment of *S. cucullata.*

Null: The Oyster (*Saccostrea cucullata*) shell powder cannot prolong the senescence of Tomato (*Solanum lycopersicum*) and there is no significant difference in all factors measured within the physical environment of *S. cucullata.*

Materials:

Materials and equipment used in this science project.

Secchi disc and meterstick	Tomatoes	Microwave Oven
pH, Conductivity, TDS, Temperature Meter	Oyster Shell powder	Beakers
Dissolved Oxygen meter	Digital Balance	Hot plate
Thermo Hygrometer	Petri dishes	Test tubes

Research Methodology

A. Study Site

The study site is located at Angsila, Chonburi Coastal Area with Latitude 13°20'04"N, Longitude 100°55'18"E.

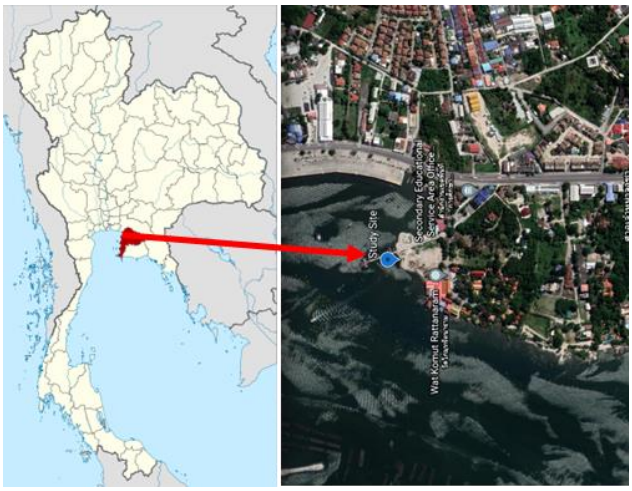


Figure 1. The study site at Angsila, Chonburi, Thailand.

B. Survey and Preparation of Materials

The researchers surveyed and chose Angsila, Chonburi as the study site because it is the main place for oyster production. After the survey and selection of study site, needed laboratory materials and equipment for habitat quality testing were procured from the science laboratory of Chonradsadornumrung School.

Figure 2. The study site at Angsila, Chonburi, Thailand and the researchers.

C. Evaluation of valuable factors in the physical environment of Oyster

Various physical and chemical parameters were considered in assessing the environment of oyster such as Water temperature, Total Dissolved Solids (TDS), electrical conductivity, salinity, hydrogen potential (pH), transparency, air temperature, relative humidity, dissolved oxygen and water color. Secchi disc was used to determine the transparency of the water. Extech standard instruments were used to measure the water temperature, TDS, pH, electrical conductivity, salinity, and dissolved oxygen. Visual comparison was used to evaluate the color of the water. In this test 20ml of the sample and 20ml of distilled water were taken in two separate wide mouthed test tubes. The results were tabulated (as clear, greenish, greyi sh, brownish, and blackish) by comparing the color of the sample with distilled water. Thermo-hygrometer was used to determine the air temperature and relative humidity of the study site. The hydrosphere protocols from www.globe.gov were used in all the tests needed to evaluate the physical environment of the experimental shells.

Figure 3. Assessing various factors in the environment of oyster.

D. Collection and preparation of oyster shell

All shells used in this study were collected from the market after the vendor removed the meat. The shells were then brought to the Science laboratory. 500g of oysters were weighed using the digital balance. All of the samples were washed and cleaned using 2 M HCl then, dried under the sun for 24 hours.

E. Burning of oyster shells

After drying and cleaning process, the shells were brought to Kasetsart University, Sri Racha Campus for burning process. It was heated at 900ºC using the furnace of the said University.

Figure 4. Burning of oyster shell using the furnace.

F. Prolonging the senescence of Tomato

The burned shells from Kasetsart University, Sri Racha Campus were then brought to the Science laboratory of Chonradsadornumrung School for the next procedure.

All equipment needed for prolonging the shelf-life of tomatoes were prepared. Beakers were sterilized in the oven for 2 hours at 180°C. After this, various treatments were then formulated. There were 4 treatments used in this test namely control (Distilled water), 1% oyster shell powder (1g oyster powder + 1000 mL Distilled water), 5% oyster shell powder (50g oyster powder + 1000 mL Distilled water), and 10% oyster shell powder (100g oyster powder + 1000 mL Distilled water). The tomatoes purchased from the market were soaked in each treatment for 15 minutes. After the soaking, the tomatoes were placed in a safe location for observation. The researchers observed each treatment daily until the tomatoes rotted completely.

Figure 5. Soaking tomatoes in oyster shell powder solution.

Results and Discussions

The figures below until the next page show the data encoded on Globe web page from December 2020 to February 2021. Figures 7 to 13 shows the Globe data entry for air temperature, relative humidity, TDS, electrical conductivity, transparency, water pH, DO, and salinity of the physical environment of oyster in Angsila, Chonburi, Thailand.

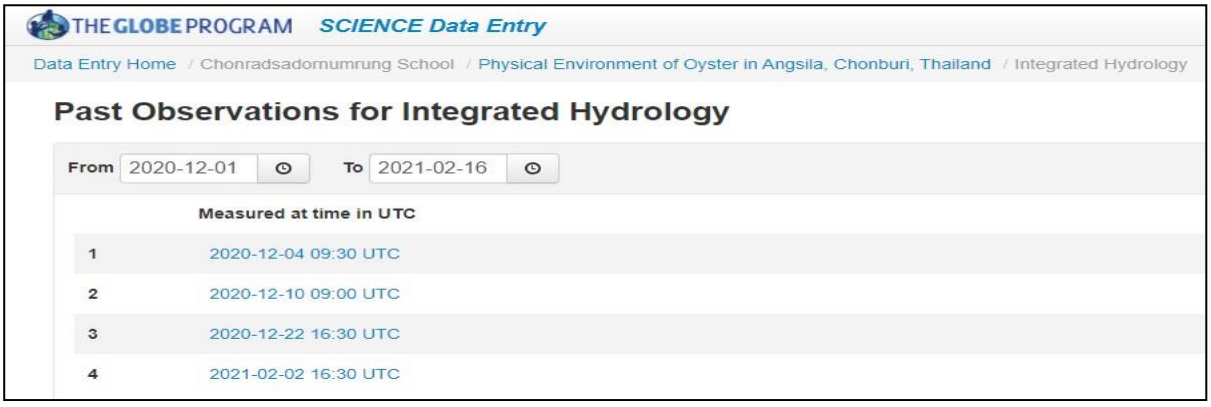


Figure 6. Globe Data Entry that has been entered from December 02, 2020 to February 02, 2021.

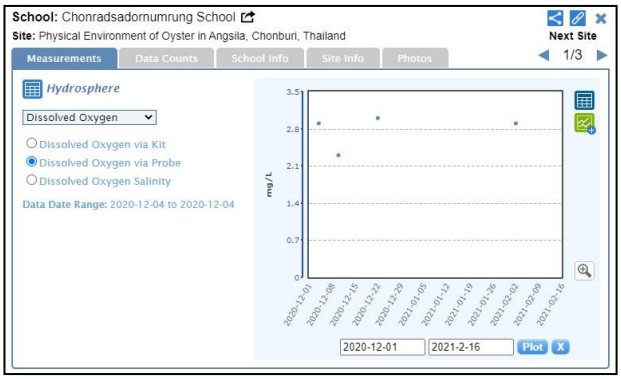


Figure 7. Globe Data Entry for Dissolved oxygen.

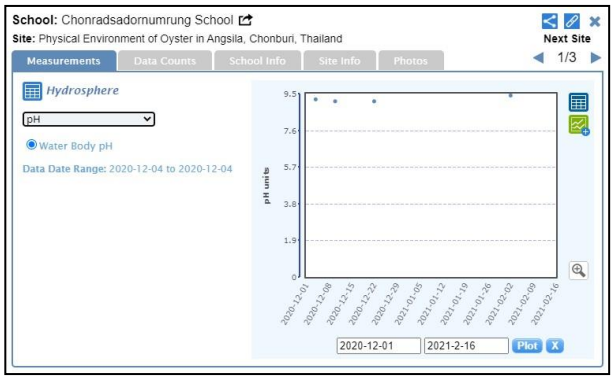


Figure 8. Globe Data Entry for water pH.

Figure 9. Globe Data Entry for relative humidity.

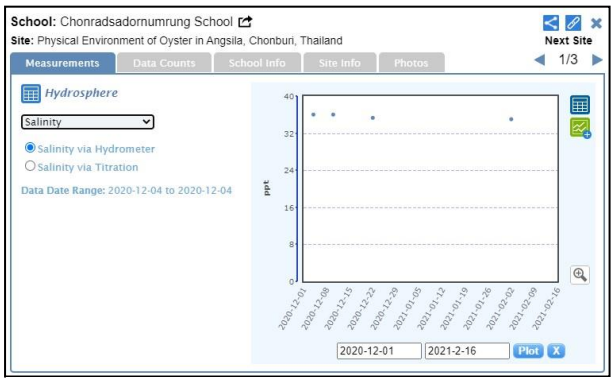


Figure 10. Globe Data Entry for salinity.

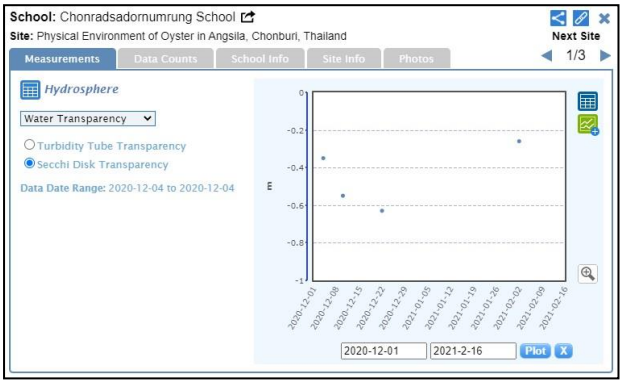


Figure 11. Globe Data Entry for water transparency.

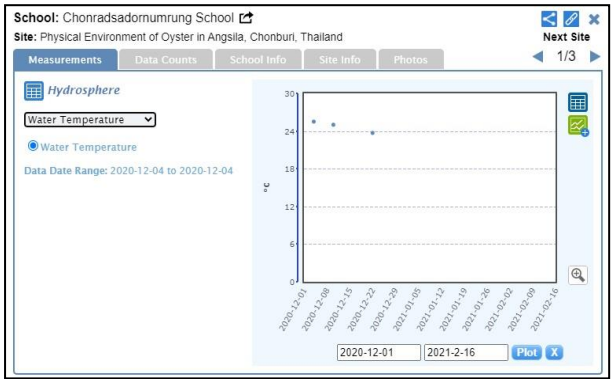


Figure 12. Globe Data Entry for water temperature.

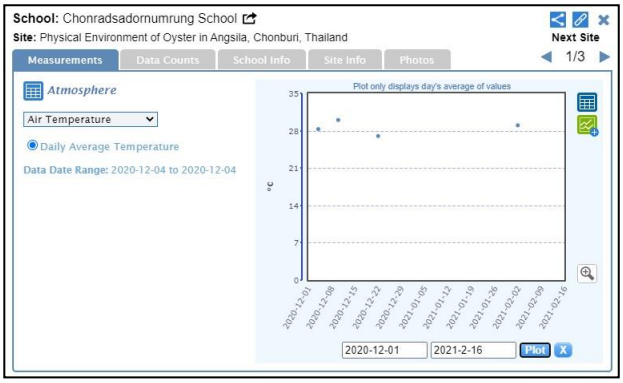


Figure 13. Globe Data Entry for Air Temperature.

Table 1. Average results of all factors measured in the physical environment of oyster.

Parameters	04 Dec. 2020 (4:00 PM)	10 Dec. 2020 (9:00 AM)	22 Dec. 2020 (4:30 PM)	02 Feb. 2021 (4:30 PM)
Water Temperature (°C)	25.5	24.97	23.67	28.77
Dissolved Oxygen (mg/L)	2.91	2.34	2.98	2.95
TDS (ppm)	3124	3061	3117	3516
Electrical Conductivity (µS/cm)	6112	5993	6190	6849
Transparency (m)	0.35	0.55	0.63	0.26
Water pH	9.19	9.36	9.08	9.36
Salinity (%)	36	36	35.33	35
Water color	Clear	Clear	Clear	Clear
Relative Humidity (%)	49	43	51	64
Air Temperature (°C)	28.3	30.9	27	31.27

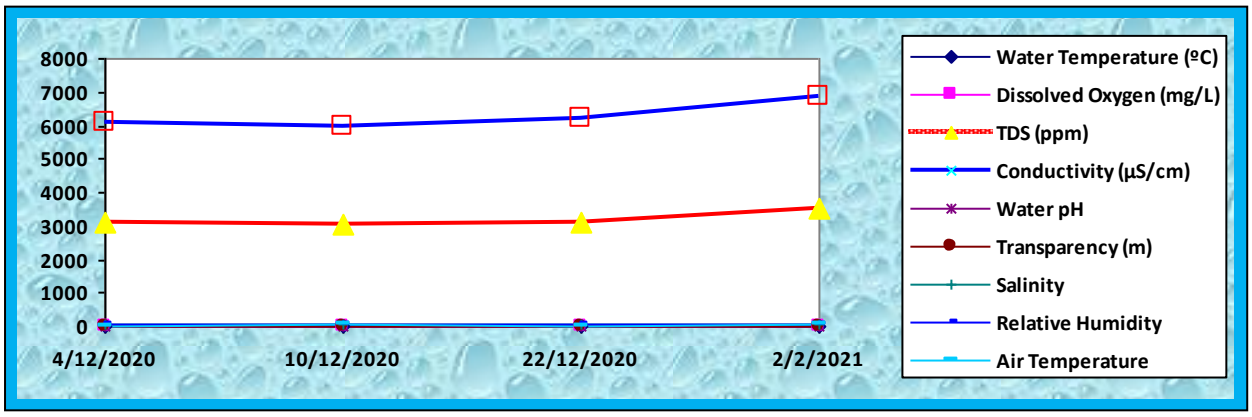
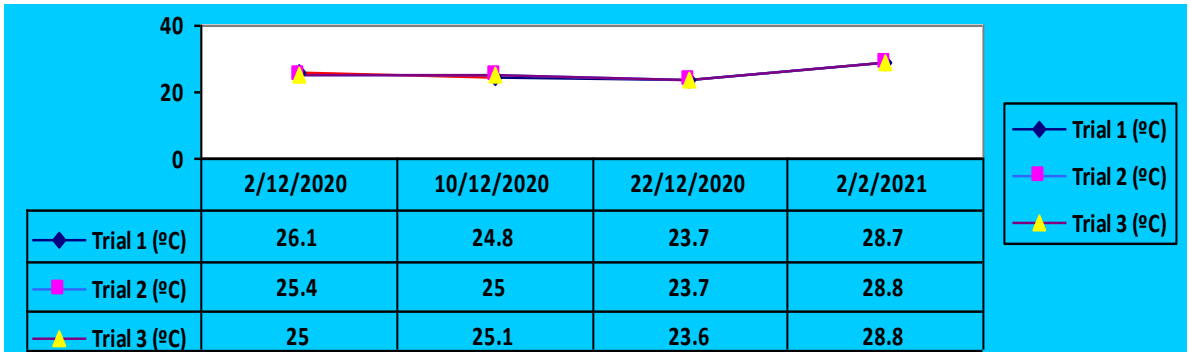


Figure 14. Average results of all parameters measured at oyster physical environment.

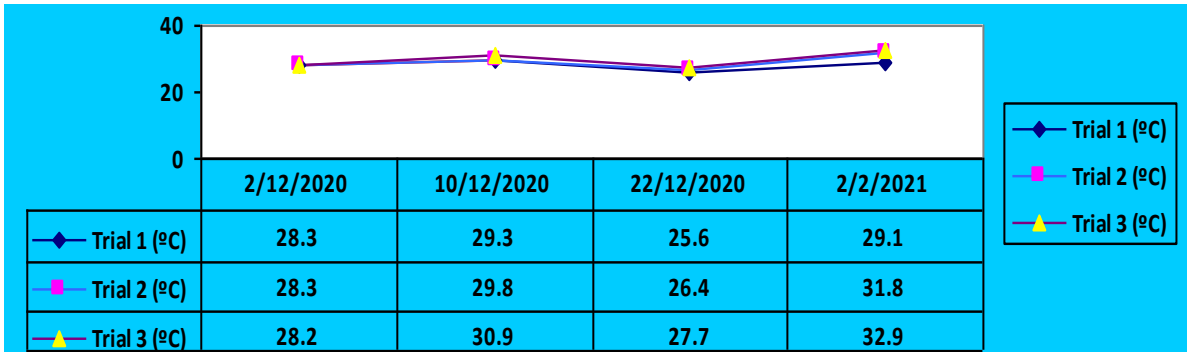
Table 1 and Figure 6 shows the average results of all parameters measured in the physical environment of oyster found in Angsila, Chonburi, Thailand. These results were summarized after 4 series of experiments that started from 04 December 2020 to 02 February 2021. The average water temperature ranges from 23.67-28.77ºC, DO ranges from 2.34-2.98 mg/L, TDS ranges from 3061-3516 ppm, Electrical conductivity ranges from 5993-6849 µS/cm, Transparency from 0.26-0.63 m, water pH from 9.08-9.36, salinity from 35-36%, relative humidity from 43-64%, air temperature from 27-31.27ºC. The water color was consistently clear throughout the experiment.

One-way ANOVA and Tukey HSD test were used to determine if there is significant difference in all parameters measured in the physical environment of oyster in Angsila. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05 for water temperature, DO, TDS, Electrical conductivity, transparency, salinity, relative humidity, and air temperature suggesting that the one or more treatments is/are significantly different. For water pH, the p-value corresponding to the F-statistic of one-way ANOVA is higher than 0.05, suggesting that the treatments are not significantly different for that level of significance. The statistical tools further revealed that there were slight changes in most of the factors measured for 4 times in Angsila.



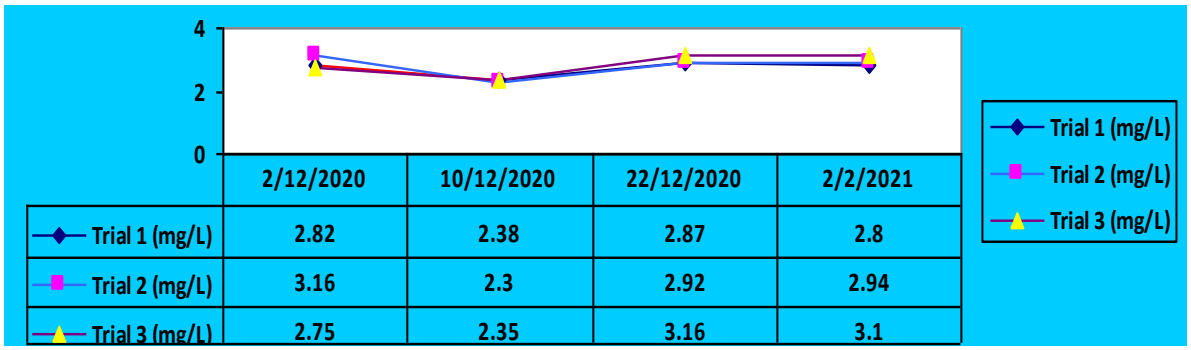
	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (ºC)	26.1	24.8	23.7	28.7
Trial 2 (ºC)	25.4	25	23.7	28.8
Trial 3 (ºC)	25	25.1	23.6	28.8

FIGURE 15. Water temperature (ºC).



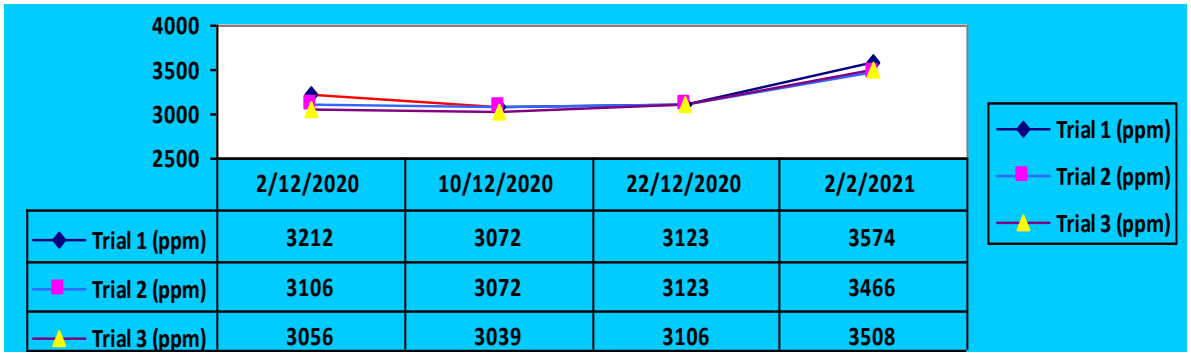
	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (ºC)	28.3	29.3	25.6	29.1
Trial 2 (ºC)	28.3	29.8	26.4	31.8
Trial 3 (ºC)	28.2	30.9	27.7	32.9

FIGURE 16. Air temperature (ºC).



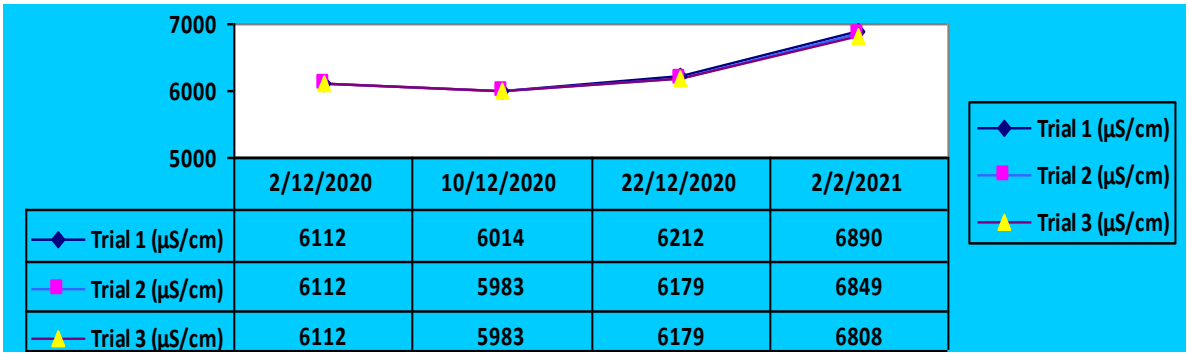
	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (mg/L)	2.82	2.38	2.87	2.8
Trial 2 (mg/L)	3.16	2.3	2.92	2.94
Trial 3 (mg/L)	2.75	2.35	3.16	3.1

FIGURE 17. Dissolved oxygen (mg/L).



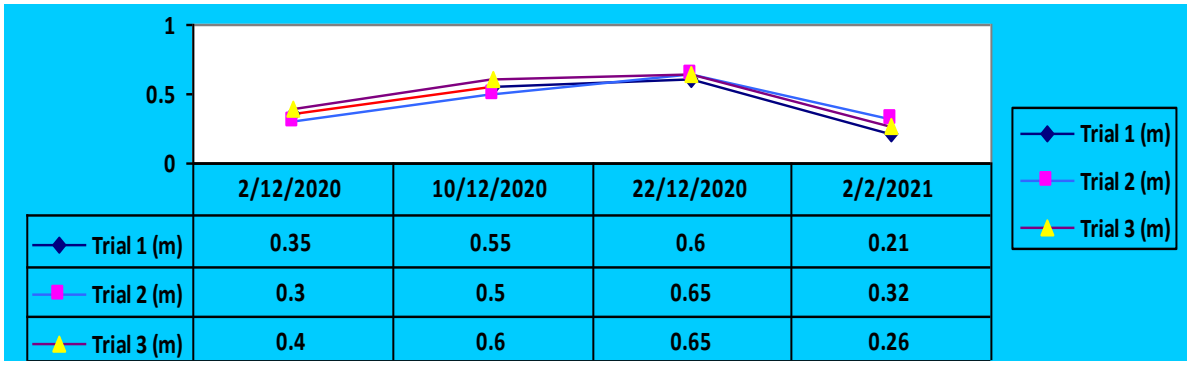
	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (ppm)	3212	3072	3123	3574
Trial 2 (ppm)	3106	3072	3123	3466
Trial 3 (ppm)	3056	3039	3106	3508

FIGURE 18. Total Dissolved Solids (ppm).



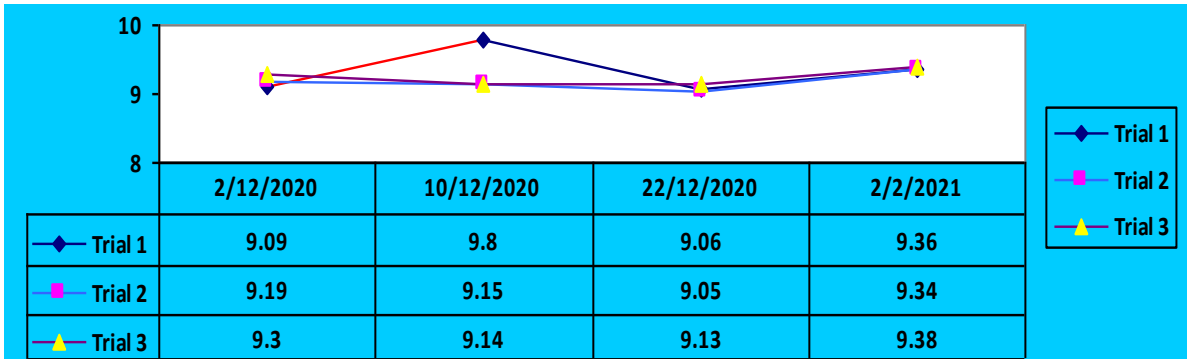
	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (µS/cm)	6112	6014	6212	6890
Trial 2 (µS/cm)	6112	5983	6179	6849
Trial 3 (µS/cm)	6112	5983	6179	6808

FIGURE 19. Electrical Conductivity (µS/cm).



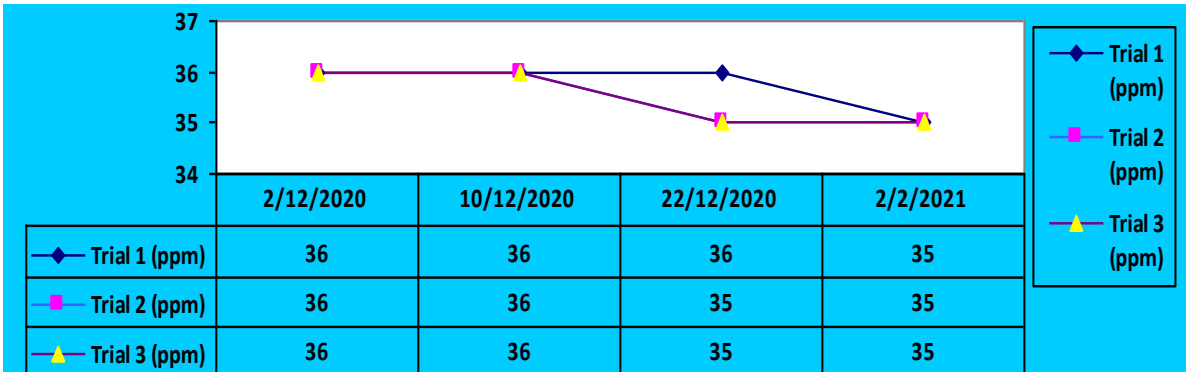
	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (m)	0.35	0.55	0.6	0.21
Trial 2 (m)	0.3	0.5	0.65	0.32
Trial 3 (m)	0.4	0.6	0.65	0.26

FIGURE 20. Transparency (m).



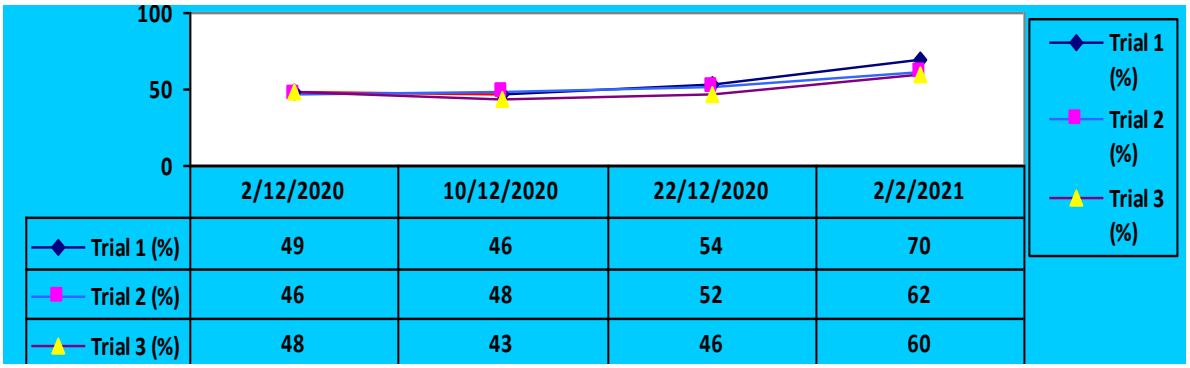
	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1	9.09	9.8	9.06	9.36
Trial 2	9.19	9.15	9.05	9.34
Trial 3	9.3	9.14	9.13	9.38

FIGURE 21. Water pH.



	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (ppm)	36	36	36	35
Trial 2 (ppm)	36	36	35	35
Trial 3 (ppm)	36	36	35	35

FIGURE 22. Salinity (ppm).



	2/12/2020	10/12/2020	22/12/2020	2/2/2021
Trial 1 (%)	49	46	54	70
Trial 2 (%)	46	48	52	62
Trial 3 (%)	48	43	46	60

FIGURE 23. Relative humidity (%).

FIGURE 15 to 23 shows all the parameters (water temperature, dissolved oxygen, TDS, electrical conductivity, transparency, water pH, salinity, air temperature, and relative humidity) measured in Angsila, Chonburi, Thailand from December 04, 2020 to February 02, 2021. Each parameter was measured 3 times to get the valid result. All of the graphs above revealed that there are changes in all factors measured for 4 times. Analysis of variance and Tukey HSD test were the statistical method used to compare these results and to find out if there are significant differences among the collected data.

Table 2. Results of Prolonging the Shelf-life of Tomato after 12 days observation.

Treatments	Qualitative Observations
Control (Distilled Water)	The tomatoes soaked in distilled water are still fresh from day 1 to 11. It started to show wrinkles on its pericarp at day 12.
1% Oyster Shell Powder Solution	The tomatoes soaked in 1% oyster shell powder solution showed a comparable result with the control set-up. The product was fresh from day 1 to 11. However, at day 12 one of the samples showed a part that is about to rot but the rest especially the smaller tomatoes are still in

	good condition without any manifestation of wrinkles or rotting part.
5% Oyster Shell Powder Solution	The samples soaked in 5% oyster shell powder solution started to show wrinkles on the pericarp at day 6. Bigger tomatoes rotted faster compared to smaller tomatoes.
10% Oyster Shell Powder Solution	Bigger tomatoes soaked in 10% oyster shell powder solution have the same result with 5% solution. It started to show wrinkles on the pericarp at day 6.

Figure 24. Physical appearance of tomatoes after 12 days observation.

Table 2 and Figure 24 shows the results of the second test on prolonging the shelf-life of tomatoes using the oyster shell powder solution. After 12 days observation, it was observed that 1% oyster shell powder solution can prolong the senescence of the experimental tomato. The other treatments such as 5% and 10% are not effective in prolonging the shelf-life of tomatoes because the samples exhibited wrinkles and rotting part at day 6. It is due to the fact that 5% and 10% solutions are hypertonic to tomatoes. Hence, the alternative hypothesis has to be accepted because one of the experimental set-ups can prolong the senescence of tomatoes.

Discussions

The results of field measurement, ANOVA, and post-hoc Tukey HSD test showed that there was a significant difference in parameters measured in the physical environment of oyster except for water pH. Despite of this, the physical environment of the said organism is

still stable because the data did not change to a higher level which means that there was a minimal change occurred after 4 times of field measurement. These results also revealed that dumping of oyster shells in the coastal are of Angsila did not cause major problems to the aquatic organisms especially to the abundance of oysters. Currently, there is an ample supply of oysters in Angsila, Chonburi (Fisheries Statistics of Thailand, 2017).

Inorganic substances are more in demand nowadays because they have good stability compared to organic substances (Hewitt et. Al., 2001). With this, oyster shells that possesses an inorganic compound known as $CaCO_3$ (Yoon *et.al*., 2003) can be used in many researches. Utilizing the shells in this research is workable and safe because oyster shells contain 96% of calcium carbonate ($CaCO_3$) (Yoon *et.al.*, 2003). Some research said that shell powder heated to higher temperature exhibited a bactericidal activity (*Sawai et. al., 2001*). Pathogenic microbes are normally present in tomatoes which can be bacteria and fungi that may contribute to the rapid rotting process of the plant. (Yeboah, 2011). In this report, it was discovered that oyster shell powder can prolong the senescence of tomatoes. This observation is relevant to the research done by Sawai et. Al., 2001 because the shells have antibacterial property, therefore it has the capacity to clean the pericarp of tomatoes from pathogens allowing the product to have a longer life span.

Conclusion

Based on the experimentations, results and gathered data, the researchers concluded that there are significant differences in water temperature, DO, TDS, Electrical conductivity, salinity, transparency, relative humidity, and air temperature, except water pH in the physical environment of oyster. Additionally, the shell powder solution of Oyster (*Saccostrea cucullata*) can prolong the senescence of tomatoes.

Recommendations

For the improvement of the study, more research should be done to test the effectiveness of oyster shell powder in inhibiting various bacterial cultures including fungi. Moreover, oyster shells will be burned at various temperatures such as 500ºC, 600ºC, 700ºC, 800ºC, and 900ºC then, all of them will be used as an alternative agent in prolonging the shelf-life of many fruits and vegetables.

GLOBE Badges

Collaboration

This environmental research was finished completely because of the collaborative efforts of various individuals. During the conduct of the study, the researchers were guided and given knowledge by their consultant from Kasetsart University- Dr. Suree Thongwanitniyom and their teachers namely Ms. Warunee Surangsee, Mr. Marvin Servallos and Ms. Denjel Casona. Furthermore, Dr. Suree facilitated the burning of shells using the furnace of Kasetsart University, Sri Racha Campus. Thorough guidance and invaluable ideas from the above names were very significant to completely understand all the scopes of this research. Finally, the researchers of this science project have cooperated to finish the work entirely from the planning stage, experiments, analyzing of data, and packaging of the final research paper.

Make an Impact

The success of this experiment would greatly benefit the public and government officials because the results of physico-chemical factors measurement in the physical environment of oyster would give them valuable information about the current condition of Angsila seawater where many oyster shells are dumped along the coastal area of the place. Moreover, the result of prolonging the senescence of tomatoes using the oyster shell powder solution is very valuable to local and international communities especially the farmers, sellers, and consumers because there is an alternative way to prolong the life of the product to avoid heavy losses. With this experiment, shells will not be thrown away to become a threat to the environment, rather it will be turned into a usable substance that will benefit everyone.

Data Scientist

The researchers have studied systematically the current condition of the seawater at Angsila Chonburi, Thailand where oysters are found. In addition, botany experiment was integrated to this environmental research. All of the data gathered from the field measurement were analyzed using some statistical models like ANOVA (Analysis of Variance) with post-hoc Tukey HSD (Honestly Significant Difference). The results of the analysis were

discussed and presented properly. Moreover, the results of the experiment were linked to the research done by other researchers.

Acknowledgment

The researchers of the study would like to acknowledge the following for making this science project possible. First, they would like to convey their genuine thanks to the Head of CRU English Program Ms. Warunee Surangsee for her utmost support, suggestions, and encouragement as well as for providing all the Laboratory equipment and chemicals that they need in their study. Second, the researchers would like to thank Dr. Suree Thongwanitniyom from Kasetsart Univeristy, Sri Racha for her invaluable ideas to improve and finish this research. Third, heartfelt thanks are also conveyed by the researchers to their Science teachers- Mr. Marvin Servallos and Ms. Denjel Casona, for their guidance towards the completion of the study. Finally, the researchers would like to give their special thanks to the committee of IPST and Globe Student Research Competition for conducting this prestigious event that enabled young scientists to share their scientific discoveries.

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Books

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