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Influence of Hibernation and Dehydration on the Hatching Percentage of Artemia Cyst Collected from Solar Saltpan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

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The brine shrimp *Artemia franciscana* is an important live feed for fin and shell fish larval culture. Cysts of Artemia are naturally found in a wide variety of harsh environments where they are exposed to different changing environmental conditions. Artemia cysts are also exposed to different hydration/dehydration (H/D) conditions during the post-harvest processing period in the Artemia processing industry. India has enormous quantity of cyst production every year from solar saltpans. But due to the lack of proper post-harvest processing technology, most of the cyst are sold to very low price to larval rearing industries. The aim of the present study was to find out the effect of different rate of dehydration, and different preservation temperatures for enhancing cyst hatchability. The cysts were collected from Thamarikulam saltpan in Kanyakumari District, Tamilnadu, India. The collected cysts were dehydrated to different percentages and preserved at 0º, -10˚ and -20˚C for different durations. The study disclosed that the cyst required 1.3h for full hydration in 30ppt saline water and reached full dehydration at 16h in saturated saline solution. Among the three preservation conditions, the maximum hatching percentage (77.95%) was observed in the cyst that preserved at -20˚C for 400days.Among the different rate of dehydration and hibernation, the 40% dehydrated cyst allowed to hibernate at -20˚C had the maximum hatching percentage. From this study, it is concluded that the optimum of all dehydration percentage, level of chillness and duration of cold storage had equally influenced in the improvement of cyst quality.

Keywords: Artemia; hydration of cyst; dehydration of cyst; hibernation; hatching.

1. INTRODUCTION

In India, *Anemia* has been reported to be present as natural population in the Sambhar and Didwana lakes in Rajasthan and saltpans in the Gulf of Kutch, Gujarat, Vadala in Bombay, Veppalodai near Tuticorin, Karsewar Island off Tuticorin, Kelambak near Madras and Vedaranyam near Muthupet lagoon [1]. Among these places, Sambhar and Didwana lakes are inland and the others are in the coastal belt. The cyst of Artemia can be preserved for a long time makes it convenient to hatch them whenever necessary. This makes the Artemia cyst to find almost usage in larval rearing industries. The natural environment of the Artemia are the salt lakes and saltpans. The cysts are released in to their environments during adverse conditions where the cysts are exposed to various adverse environmental conditions. These reduce the hatching performance of the released cyst. Moreover, due to the lack of proper post harvesting processing technology, tons and tons of cyst naturally released in to the solar saltpan are wasted.

Artemia cysts pass through several cycles of hydration/dehydration (H/D). This may have severe impact on the quality of cysts in terms of hatching percentage, hatching efficiency and performance of the hatched nauplii [2,3]. To a certain extent, some level of hydration/dehydration exposure is an almost unavoidable element in the history of any commercial cyst sample from pre harvesting until marketing. Persoone et al. [4] described about the culture of Artemia and produced cyst. The *Artemia urmiana* in Uremia lake had large production, but did not attain good quality due to the lack of post-harvest technology and proper harvesting technology [5]. Most of the maritime states in India have salt pans for salt production. Nuruddin Mahmood et al. [6] Produced Artemia Cyst in the salt pan. In these areas, Artemia naturally inhabit and release cyst during rainy season due to the unfavorable environment created by raining followed by low salinity. From these salt pans, every year, many tons of cysts are wasted due to improper post harvesting technology and processing [7]. This create a huge economy loss to India and longing for a proper technology development to process the cyst wasted during rainy season.

2. MATERIALS AND METHODS

2.1 Collection of Cyst

Cysts were collected from the saltpan of Thamarikulum. The collected cysts were transported to the laboratory in plastic containers. The cysts were washed with fresh and brine water and dried for dehydration. Then stored at 30 ± 2ºc (Room temperature).

2.2 Determination of 100% Hydration of Cyst

Twenty grams of cleaned cysts were placed in a container having sea water (30ppt and 1lit water for every one gram of cyst), provided with vigorous aeration. One gram of cyst was taken out in every advancing one hour and weighed for the increase of cyst weight by hydration and the same cysts were observed under microscope for it change of shape. The shape of the cyst changes from biconcave to spherical is the indication of complete hydration. The same procedure was repeated for every advancing one hour till the hydrated cyst did not show any increase in weight and become spherical shape. Now it is considered as 100% hydrated cyst.

2.3 Dehydration and Preservation of Cyst

Twenty grams of cysts were taken in a container and added 500ml of saturated salt water of 270 ppt. The containers were provided with vigorous aeration. Every hour, 1gm of dehydrating cysts were taken out and filtered by 100µm mesh silk cloth. The dehydrated cysts were dipped in distilled water for a while to remove the external salt spread on them by keeping on plotting paper. Then the cysts were weighed for knowing the percentage of dehydration. In this way the cyst was dehydrated in to different percentages.

2.4 Hibernation or Cold Preservation of Dehydrated Cyst

After determining the different percentage of dehydration of cyst (10,20,30,40,50 and 60%), 100g (5gx20 bags) of cyst from each percentage of dehydration was taken separately and kept in 0°C, -10°C, and -20°C deep freezer for 400 days. Every 50days interval, 5g cysts were taken out and checked for hatching percentage. Three replicates were maintained for all temperatures and all dehydrations. The hibernation experiment was conducted for 400 days.

2.5 Hatching of Dehydrated Cyst

2.5.1 Decapsulation of cysts

1g of the cysts were suspended in one liter, sea water by gentle aeration. After 30 minutes 1gm of bleaching powder was added in to the sea water containing the suspended cysts. The color of the cysts was observed. When the color of the cysts changed from dark brown to orange, the cysts were filtered out from the decapsulation solution and rinsed with cool tap water until no chlorine smell persist. Now the cyst is ready to allow in hatching cylinder for hatching.

2.5.2 Determination hatching percentage of hibernating Cyst

From the preserved cyst, 1g of decapsulated cyst, was taken in cylindroconical flask containing one litre of 28ppt brine solution. The cysts were kept in suspension by gentle aeration. After 24 hours of incubation, the hatched out nauplii were counted by using 1ml pipette. Percentage of hatching was calculated using this formula.

Hatching percentage (%) = No. of nauplii hatched / Total no. of Cysts * 100

3. RESULTS

3.1 Rate of Hydration of Cyst in Different Hours

One gram of collected cyst were allowed for hydration in different hour intervals (1–4 hours). In this experiment, the collected cysts fully hydrated in 4 hours (1.31±0.006) (Table. 1). After that the cyst did not show any change in weight even allowed them further for dehydration.

3.2 Rate of Dehydration of Cyst in Saturated Salt Solution in Every One-hour Intervals

In this experiment, the collected cysts dehydrated fully in saturated salt solution in 16 hours (0.58±0.01) (Table. 2). After that the cyst did not show any change in weight loss when the time was increased.

3.3 Influence of Different Dehydration on the Hatching Percentage of Cyst when Stored at Ambient Room Temperature (30±4ºC)

The cysts dehydrated to different percentages were when preserved at ambient room temperature, the hatching percentage increased till 200 days of preservation. The hatching percentage of cyst also increased in 10 to 40% dehydration, but the 50% dehydration had a positive effect only up to 100 days of storage and after that the hatching percentage started declining (Table 3).

3.4 Effect of 10% Dehydration and 0ºC, -10ºC and -20ºC Hibernation on Hatchability of Cyst

The cyst collected from the condenser region of solar saltpan were cleaned, 10% dehydrated and preserved in the cold storage at 0°C, -10°C and -20 °C for 400 days. The preserved cysts were taken out from the cold storage in different time duration and tested for its hatchability. The hatchability of 20% dehydrated cyst increased in dried relation with the days. The maximum hatching percentage in 0° C, -10° C and -20° C were 40, 55 and 58 % respectively. Among the 0° C,10° C and -20° C preserved cyst, the maximum hatching percentage was observed in the cyst that was preserved at -20° C for 400 days, but there was no considerable difference found between the 350 and 400 days preserved cyst (Table 4).

3.5 Effect of 20% Dehydration and 0ºC, -10ºC, and -20ºC Hibernation on Hatchability of Cyst

The cyst dehydrated to 20% and stored at 0° C, -10° C and -20° C increased the hatching percentage when taken out and tested for the hatchability. The hatching percentage not only increased based on the advancement in the time duration of storage, but also the preservation percentage. Among the three-preservation temperatures, the highest hatching was noticed in the -20° C preserved cyst in 400 days. There was no considerable variation in the hatchability of cyst when preserved after 350 days and up to 400 days (Table 5).

3.6 Effect of 30% Dehydration and 0ºC, -10ºC, and -20ºC Hibernation on Hatchability of Cyst

The Artemia cysts when dehydrated to 30% and preserved at 0° C, -10° C and -20°C, the maximum hatching percentage of 79.12% were observed at -20°C cold preservation for 350 up to 400 days. But difference in hatching percentage were found among the different preservation temperature (Table 6).

3.7 Effect of 40% Dehydration and 0ºC, -10ºC, and -20ºC hibernation on hatchability of cyst

The Artemia cyst when dehydrated to 40% and stored at different preservation temperature of (0°C, -10°C and -20°C) and durations, the highest hatching percentage (87.50%) was noticed at -20 °C preservation temperature for from 350 days (Table 7).

Table 1. Rate of hydration of collected cyst in different hours

Sl. No	Hours	Cyst weight (g) after Hydration (1g cyst)
1	1	1.10±0.03
2	2	1.24±0.06
3	3	1.30±0.01
4	4	1.31±0.01

Table 2. Rate of dehydration of cyst (1gram) in saturated salt solution during different hours

Hours	Weight loss(g)	Dehydration Percentage (%)
1	0.90±0.05	90
2	0.84±0.04	84
3	0.79±0.02	79
4	0.74±0.05	74
5	0.69±0.04	69
6	0.65±0.01	65
7	0.61±0.05	61
8	0.58±0.01	58
9	0.55±0.03	55
10	0.53±0.07	53
11	0.50±0.04	50
12	0.49±0.03	50

Table 3. Influence of different Dehydration on the Hatching percentage of cyst when stored at ambient room temperature (30±2ºC)

Hibernation Period (Days)	Percentage of Hatching Percentage on Different Dehydration				
	10%	20%	30%	40%	50%
0	20.26±1.75	21.45±2.35	20.76±1.80	21.44±2.03	21.67±2.68
50	24.67±2.48	26.80±1.78	27.92±1.87	29.45±2.68	32.80±2.55
100	27.48±1.55	30.52±2.17	33.10±2.75	35.40±1.70	35.89±3.95
150	30.98±2.75	33.28±3.65	37.16±2.04	39.50±1.98	34.64±1.80
200	29.67±3.14	34.18±2.75	36.40±2.60	39.49±1.46	33.10±2.44
250	27.16±2.68	32.40±1.70	33.85±2.78	37.46±3.60	31.42±3.48
300	24.08±3.70	27.55±2.75	29.16±2.85	34.60±2.78	25.45±3.60
350	18.77±1.94	21.20±2.50	22.28±3.55	26.72±1.92	19.47±2.68
400	15.08±2.75	17.40±1.98	17.96±2.63	20.68±2.80	15.18±2.70

Table 4. Effect of 10% dehydration and 0ºC, -10ºC and -20ºC hibernation on hatchability of cyst

Serial No	Hibernation Period	Hatching percentage		
		0ºC	-10ºC	-20ºC
1	0 days	20.14±3.52	20.34±2.54	20.07±2.02
2	50 days	25.75±2.31	26.66±1.69	32.42±1.74
3	100 days	27.64±3.46	30.75±2.31	35.40±2.05
4	150 days	29.50±2.54	37.64±3.46	39.56±2.78
5	200 days	31.42±1.69	40.50±2.54	44.48±3.14
6	250 days	34.57±2.15	43.42±1.69	47.80±3.45
7	300 days	36.48±1.78	49.57±2.15	52.62±2.80
8	350 days	39.29±1.40	54.48±1.78	57.80±3.65
9	400 days	40.92 ±1.80	55.29±1.40	58.64±3.08

Table 5. Effect of 20% Dehydration and 0ºC, -10ºC, and -20ºC hibernation on hatchability of cyst

Serial No	Hibernation Period	Hatching percentage		
		0ºC	-10ºC	-20º
1	0 days	20.33±0.47	20.66±1.24	20.33±1.69
2	50 days	36.44±2.89	38.52±3.54	40.34±2.54
3	100 days	39.05±3.15	41.08±2.40	43.58±1.90
4	150 days	43.64±2.10	44.65±2.95	46.80±2.55
5	200 days	46.56±3.10	49.50±1.94	51.45±3.17
6	250 days	49.42±2.68	53.45±1.67	56.56±1.87
7	300 days	55.60±3.16	58.05±2.60	62.95±2.64
8	350 days	60.14±3.52	64.54±2.07	68.07±2.02
9	400 days	60.14±3.52	64.07±3.84	67.18±1.80

3.8 Effect of 50% Dehydration and 0ºC, -10ºC, and -20ºC Hibernation on Hatchability of Cyst

The 50% dehydrated cysts, when stored at 0°C, -10°C and -20°C preservation temperatures under different time durations, the hatching percentage increased in accordance with the increasing durations (day) and decrease of temperature. The highest hatching percentage (77.33) was notice on 350 days of cold preservation at -20°C (Table 8).

3.9 Effect of Different Dehydration and Hibernation on Hatching Percentage of Artemia

The hydration and hibernation equally effect the hatching percentage of Artemia cyst. The 40% of hydration at -20ºC had the maximum hatching

percentage (87.50%). But the 50% dehydration at -20ºC gave less hatching percentage than 40% dehydration. Irrespective of all dehydration percentages, an increasing trend of hatching percentage were observed in all the three hibernation temperatures (Fig. 1).

4. DISCUSSION

Production of Artemia cyst from the solar salt pan of India is estimated as >2000 tons / year (Sivagnanam etal 2014). But due to the lack of post-harvest technology, the hatching percentage of the cyst reduced consistently (<10%) Bam Deo Pandey [8]. This study has been carried out with the aim of enhancing the hatching percentage of cyst collected from solar salt pan. The frequency of hydration and dehydration in the salt pan makes the naturally collected cyst in to inferior quality [9]. Preliminary study conducted by our research team proved that the constant level of water content retained the hatching percentage of cyst.

Table 6. Effect of 30% Dehydration and 0ºC, -10ºC, and -20ºC hibernation on hatchability of cyst

Serial No	Hibernation Period	Hatching percentage		
		0ºC	-10ºC	-20ºC
1	0 days	20.42±2.57	20.75±2.31	20.15±2.04
2	50 days	42.60±2.55	43.74±2.56	39±2.44
3	100 days	46.64±1.17	47.89±2.24	45.92±2.74
4	150 days	48.15±2.04	50.40±3.48	48.36±2.25
5	200 days	50.25±2.08	52.46±2.80	55.75±3.90
6	250 days	54.50±2.48	55.54±2.48	63.64±2.80
7	300 days	59.45±2.83	65.33±2.94	72.32±3.42
8	350 days	64.75±1.62	74.68±3.44	78.76±3.18
9	400 days	68.43±2.44	74.40±4.68	79.12±2.60

Table 7. Effect of 40% Dehydration and 0ºC, -10ºC, and -20ºC hibernation on hatchability of cyst

Serial No	Hibernation Period	Hatching percentage		
		0ºC	-10ºC	-20ºC
1	0 days	20.29±2.15	20.50±2.54	20.53±2.49
2	50 days	42.89±2.17	44.92±1.80	47.62±2.87
3	100 days	48.80±2.25	49.55±2.64	52.54±4.28
4	150 days	50.22±4.62	52.26±2.95	54.28±3.54
5	200 days	55.35±2.66	58.60±1.55	63.34±2.90
6	250 days	59.33±1.24	61.42±2.16	70.40±2.68
7	300 days	62.44±3.80	66.54±3.94	75.33±2.49
8	350 days	67.50±2.52	76.80±5.42	86.26±3.90
9	400 days	70.92±3.46	75.59±2.20	87.50±5.62

Table 8. Effect of 50% Dehydration and 0ºC, -10ºC, and -20ºC hibernation on hatchability of cyst

Serial No	Hibernation Period	Hatching percentage		
		0ºC	-10ºC	-20ºC
1	0 days	21.66±2.05	21.33±1.24	21.33±1.69
2	50 days	41.28±2.16	44.33±2.49	50.33±2.49
3	100 days	42.68±2.94	49.66±2.49	54.66±2.05
4	150 days	45.38±1.63	53.33±2.49	60.66±1.69
5	200 days	47.33±2.05	58.66±2.86	63.33±2.86
6	250 days	54.33±2.05	62.66±2.05	68.24±2.16
7	300 days	57.66±2.62	69.66±2.49	70.34±1.63
8	350 days	64.66±2.05	71.33±2.05	77.66±1.69
9	400 days	65.33±1.24	71.66±2.49	77.33±2.05

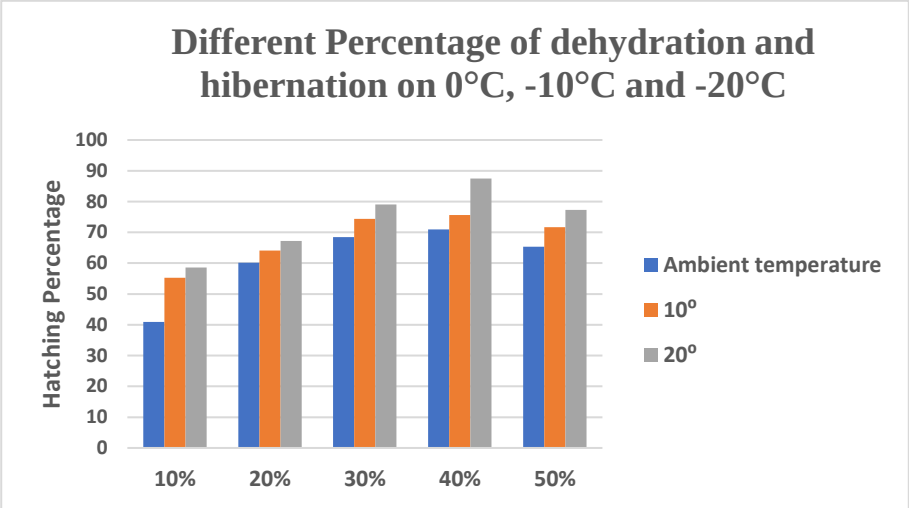

Fig. 1. Effect of different dehydration and hibernation on hatching percentage of Artemia

In this study, the cyst collected from the solar saltpans were stored in different percentage of dehydration (10-50%). In the hyper saline treatment method of this experiment, 50% of dehydration was only obtained even the cyst were retained for more than 11 hrs. in supersaturated saline solution. From these experiments, it is proven that the percentage of dehydration had influence on hatching percentage. But the percentage of dehydration had its limitation. The increase of hatching percentage due to dehydration was observed only up to 40% dehydration of cyst and after that the hatching percentage started declining. Aliakbar Hedayati and Tahere Bagheri [10] also described the effect of dehydration on the hatching percentage of cyst.

From this result, it may be inferred that an optimum percentage of water is needed to be retained in the cyst during its hibernation period. This finding is very important in the preservation of Artemia cyst in cold storage for hibernation to increase the hatching percentage of cyst collected from the slat pans. Royan et al. [11] also confirmed our findings. In his study, the effect of dehydration due to hyper saline storage after collection from salt pan was found to be enhancing the cyst quality and hatchability. But in their study the optimum dehydration required for the maximum hatching percentage was not found out.

The storage temperature or the hibernation temperature also had influences in the increasing hatching percentage. Among the three storage temperatures studied (0ºC,-10 ºC and - 20 ºC), the cyst hibernated at -20ºC gave the maximum hatching percentage. Apart from low temperature which is responsible for increasing the hatching percentage, the dehydration percentage is also equally influencing the hike in hatching percentage. The maximum cyst hatching percentage was observed in 40% dehydration than the 50% dehydration. From this study, the dehydration percentage and hibernation temperature are equally influential to evoke the positive result in cyst hatching. A study conducted by Gary E. Belovsky et al. [12] also confirmed our findings. In their experiment, an increase of hatching percentage was found in the brine shrimp cysts obtained from Great Salt Lake (Utah, U.S.A.) up to 65.5% when stored at -20 ºC.

The result obtained from this study showed that the period of hibernation is also very important criteria to enhance the cyst hatching. The hatching result obtained in every advancing 50 days interval was favorable till 350 days. After this time period, there was no considerable increase in the hatchability of Artemia cyst. So, there is an optimum time period for the maximum hatching percentage of cyst.

5. CONCLUSION

The main finding in this study is that the hibernation period, preservations temperatures and percentage of dehydration all act together for the enhancement of cyst hatching percentage. The result observed from this study will help to reduce the wastage of cyst collected from the natural saltpans. Moreover, this finding will also

help the industries that are involving in purchasing the raw cyst from the salt farmers to enhance the quality of cyst.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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