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EFFECTS OF DESICCATION ON THE HATCHABILITY RATE OF LABORATORY AND WILD STRAIN AEDES AEGYPTI (L.) AND AEDES ALBOPICTUS SKUSE EGGS

ABSTRACT

Aedes eggs are desiccation resistant and this feature enables them to survive and adapt to prolonged drought. Information on hatchability of desiccated Aedes eggs is crucial for estimation of survivorship and thence disease transmission. This study was conducted to determine the hatchability rate of wild and laboratory strain of Aedes eggs after drying and storing indoor or outdoor. Ae.aegypti and Ae.albopictuseggs were collected onto filter paper 10 fully engorged femalesoviposited for 24h. The egg papers were air-dried and keptinside mosquito cages located indoor and outdoor for 2, 4, 7, 14, 30 and 60 daysof storage interval. After each interval, an egg paper was divided into equal portions and submerged in a medium broth for hatchingand observed under an imaging system to determine the first appearance of the larvae. Eggs of both species stored outdoor showed the highest hatchability rate compared to the eggs stored indoor. The earliest appearance of larvae was at 15th minute after submergence. Eggs were still able to hatch after 60 days of storage, though the hatchability rate was reduced. This study reconfirms that Aedes eggs are desiccation resistant and drying does not exert any negative effects on the eggs.

Keywords Aedes aegypti, Aedes albopictus, eggs, desiccation, hatchability.

1. INTRODUCTION

Aedes aegypti and Aedes albopictusare two main vectors responsible for transmitting diseases such as dengue, yellow fever, West Nile virus, chikungunya virus etc[1,2]. Both species originated from tropical and sub-tropical region [3-7]. Recent study showed that Ae. aegypti and Ae. albopictus were also found in countries in the temperate region such as Europe [8], Nepal and Buenos Aires in Argentina [9]. The dispersal and survival of these tropical mosquito species in temperate zone is a subject of much research. Several studies have reported that Aedes eggs have the ability to survive in the extreme weather and various environment conditions[10, 11]. Ae.aegypti eggs have the capability to withstand the desiccation process and are able to survive from three months to eight month at various temperatures and relative humidity [12]. The ability of the eggs to withstand desiccation for a long period has enabled these vectors to disperse around the world facilitated by the global travel. Christophers[3] reported a total of 34% of a laboratory strain of Ae. aegypti hatched in grass infusion after storage of 2 months at 25°C, while after 4 months only 1 – 2% of eggs were hatched and no hatching was recorded after 5 months.The eggs of a wild strain Ae. aegypti collected from the tree holesanddried for 12 – 14 weeks completely hatched after 48 hours of submergence [3, 18]. In contrast, Weissman - Strum and Kindler [13] reported that80% - 90% eggs of a laboratory strain Ae.aegypti hatched after storage for 1-2 months, while study by Hotchkin[14] showed that 94% of Ae. aegyptieggs hatched within 10-20 min after being stored for ten weeks and took 72 hours for all eggs to fully hatch. Dickerson [12] reported that the percentage of Ae. aegypti and Ae. albopictus eggs hatched was dependent on the temperature and relative humidity of the environment. The eggs once laid by the female will go through the embryogenesis process. Christopher [3] and Farnesiet al[15]reported that the embryonic development of Ae. aegypti eggs took 48 hours to 72 hours to mature and ready to hatch. Sota and Mogi [16] indicated that the embryonated eggs were tolerant to desiccation and they could survive at extreme conditions, a condition known as diapause which allows the Aedes eggs to suspend their development until the condition is suitable for them to continue to the next stage of life cycle or when the environment is suitable for them to hatch. However in the extreme weather and environment, the metabolic activity of the eggs slows down until the weather and condition is suitable to hatch. The study on the egg survivorship at extreme temperature and relative humidity was also conducted by Thomas et al[8] on the European strain Ae. albopictus and tropical strain Ae. albopictus and Ae. aegypti. They found that the eggs were able to survive in the extreme weather conditionsand hatched after a certain period. However, the study by Julianoet al[10] and Dickerson [12] showed that Ae.aegypti eggs could survive better under low relative humidity and high temperature and resistant to warm temperature and desiccation, compared to Ae.albopictus eggs.In this paper, we report the impact of desiccation on the eggs of Ae.aegypti and Ae.albopictus. The results obtained in this study can be used to predict the fertility and mortality of desiccated stored eggs.

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2.0MATERIALS AND METHODS

2.1Mosquito strains

Mosquito strains used in this study were the Malaysian lab and wild strain Ae.aegypti and Ae.albopictus. The laboratory strain Ae. aegypti (F1034 - F1035) and Ae. albopictus (F49 - F51) have been maintained in the insectarium of the Institute for Medical Research, Kuala Lumpur since 1970s,while the wild strain Ae. aegypti (F1 – F2) was collected from PulauKetam, Selangor(3.0336°N, 101.2493°E) and Ae. albopictus (F1 – F2) was collected from Pulau Carey, Selangor(2.8667°N, 101.3667°E).The wild strain Aedes aegypti and Aedes albopictus were obtained from 200 ovitraps set up in PulauKetam and Pulau Carey area for 5 days.

2.2Mosquito rearing

The eggs were hatched in plastic basins under the following conditions: 26.5± 1.5 °C; 60±10% relative humidity; photoperiod 12:12. The larvae were fed with bovine liver powder for the first two instars and laterinstars fed with small pieces of half cooked bovine liver. Pupae were transferred into a cage (9" x 9" x 9") for eclosion into adults. The adult mosquitoes were allowed to feed on 10 % sugar solution ad libitum. After three days the female mosquitoes were allowed to blood-feed on a restrained mouseuntil fully engorged.

2.3Aedes eggs preparation

The fully engorged female mosquitoes were transferred into another cage (6" x 6" x 6"). A filter paper (Whatman No. 3; ∅ 90mm) was labelled and moistened with distilled water inside a porcelain bowl which was left for 24 hours for female mosquitoes to oviposit. Filter paper with eggswas air dried at room temperature for three hours and storedin a cage placed indoor and outdoor.

2.4Storage condition of dried eggs

The filter papers with eggs were kept at two different locations, indoor and outdoor. The indoor storage was located in the Biting Midges Laboratory (BML), Medical Entomology Unit, IMR, while the outdoor storage was located outside the Main Laboratory of Medical Entomology Unit,IMR. These locations were choosing because theymimicked the environment of a house.The indoor temperature was 26.5°± 1.5 C and relative humidity at 60±10%, while the temperature outdoor was 29.0 ±2.0°C and relative humidity at 55±10% during the study.A total of six different storage intervalsnamely 2, 4, 7, 14, 30 and 60 days were used in this study. For eachstrain, 30 filter papers with eggs were prepared (i.e.1 strain x 1 location x 6 a storage interval x 5 replicates). The filter papers with eggs were kept in the cage to prevent them from predation by other insects such as ants.

2.5Hatchability test.

In this study the reduced pressure technique to hasten hatching was not used because our intention was to study the capability of the eggs to hatch naturally. The filter paper with the test eggs was cut into portions each having 30 – 50 eggsand placed into a glass cavity block (50mm x 50mm)and counted. A medium broth (0.7ml; 0.13% of bovine liver) was pipetted into the cavity block so thatthe eggs were immersed in the broth for hatching toproceed. The broth medium was used because Aedes egg is a facultative organism which needs the lower oxygen concentration to hatch (Farnesi et al, 2009; Barbosa and Peters, 1969). The eggs must be submerged into the medium becauseAedes eggs would not hatch if they are in a floating position (Christophers, 1960). The hatching rate at15,30, 45, 60min and 24 hours was recorded. The laboratory temperaturewas controlled at 28.5± 2°C, while relative humidity was at60±10% during the test.The hatching process was observed closely using an imaging system equipped with microscope (RaxVision, US) and software (DIMAS ver 5.0).The number and time of the eggs hatched were recorded for data analysis.

2.6Statistical analysis

Numbers of eggs hatched were counted and recorded. The data including species, strain, location of the storage, storage interval and time of hatchingwere analysed using a statistical software (SPSS Inc, version 17.0, US).The data were tested for normality (Kolmogorov-Smirnov test) andparametric tests were used. The effect of species, strain, locations and storage interval on the hatchability rates were

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analysed using one way ANOVA.For post-hoc analysis, Bonferonni analysis was used to evaluate the effect of location and storage on the hatching rate of eggs.

3RESULTS

3.1Storage location

The storage locations affected the hatchability rates of the Aedes eggs (Table 1). There were significant differences in mean percentage of hatching for the Aedes eggs stored at different locations ($p< 0.05$).The mean percentage of hatching for Aedeseggs stored outdoor was higher compared to the eggs stored indoor. More than 50% of the Ae.aegypti eggs stored indoor and outdoor hatched during this study, while the mean percentage of hatching forAe. albopictuswas <50% for the eggs stored indoor compared to the eggs stored outdoor. These resultssuggested that the Ae. aegypti eggs were more competitivein the wild environment compared to Ae. albopictus. T-test analysis of hatching time for Ae.aegypti and Ae. albopictus both showed no significant difference between eggs stored indoor and outdoor ($df = 46$, $t = 0.18$, $p > 0.05$)

3.2Effect of storage time onhatchability of Aedes eggs stored indoor and outdoor

The eggs of Ae.aegypti and Ae.albopictus were storedfor 2 to 60 days to observe the hatchability (Figure 1). There was significant difference between the hatchability rates of Aedes eggs and the storage interval ($p < 0.05$). The highest mean percentage hatching was in eggs stored for 4 days and the lowest was 2 days of storage interval for laboratory strain Ae. aegypti,while the wild strain Ae. aegyptiexhibited the highest mean of hatching rate at 7 days post-storage. The laboratory strain Ae. albopictus eggs hatchedin large number 7 days after storage indoor, while the eggs of wild strain Ae. albopictus hatched 4 days after indoor storage. The highest number of eggs hatched for both species occurred 7 to 14 dayspost- indoor storage.Aedes eggs stored outdoor were capable of hatching in large number compared to eggs stored indoor (Figure 2). Eggs of the laboratory strain Ae. aegypti stored outdoor showed the lowest mean hatching percentage compared to the eggs stored indoor at 7 days and 14 days post-storage. Only wild strain Ae.albopictus failed to hatch after 2 days ofstorage. The highest mean percentage of hatching for outdoor eggs was recorded for the wild strain Ae. aegyptikept for 7 days, while laboratory strain Ae. aegypti hatched in large number after 14 days of storage. There was a decrease in hatching rates of wild strain Ae. aegypti30 days post-storage,butthe mean percentage of hatching increased 60 days after storage. Laboratory strain Ae. albopictus eggs showed high mean percentage in hatching rates after 7 days of storage, while wild strain Ae. albopictuseggs exhibited the highest hatchability rates 4 days after storage. Hence Aedes eggs kept outdoor was more fecund compared to eggs kept indoor

3.3Hatching time

The univariate test for within subject effect showed there was significantdifference for all main parameters measured: species, location and storage time interval ($df = 60$, $F = 6.284$, $p < 0.05$). Mean percentage of hatching for laboratory strain Ae. aegypti eggs stored indoor showed that the eggs started to hatch one hour after submergence in medium(Fig 3a). No sign of hatching was observed prior to one hour of submergence. The highest mean number of eggs hatched was those storedfor 4 days, while the lowest were eggs stored for 2 days. All laboratory strain Ae. aegypti stored indoor hatched after 1 hour. Meanwhile, laboratory strain Ae. aegypti eggs stored outdoor hatched 30 minutes after submergence (Fig 3b). The eggs stored for7 days, 14 days and 30 days showed the highest hatching, while eggs stored at 2 days, 60 days and 4 days showed the lowest. Wild strain Ae.aegypti eggs stored after 7 days and located indoor hatched after 15 minutes in the medium (Fig 3c), followed by eggs stored for 4 days which hatched after 30 minutes in the medium. Eggs stored for 14 days hatched after 1 hour and for the eggs stores for 2 days, 30 days and 60 days hatched after 24 hours. The wild strain Ae.aegypti eggs stored outdoor showed different patterns of hatching time (Figure 3d). The earliest hatching (30 min) was observed in eggs stored for 4 days , followed by 7 days storage at 45 minutes and 14 days storage after 1 hour. The eggs stored for 2 days, 30 days and 60 days hatched after 24 hours. Eggs of laboratory strain Ae. albopictus stored indoordid not hatch after 2 to 4 days of storage , however the eggs stored for 7 to 60 days hatched after one hour of submergence but the hatching rate was low (Fig 4a). In comparison, the laboratory strain Ae. albopictus eggs stored outdoor for 30 days hatched after 30 minutes, while eggs stored after 60 days hatched after 45 minutes (Fig 4b). On the other hand, eggs stored for 2-14 days hatched after 24 hours submergence in the medium. The mean number of eggs hatched washigher after 7 days of storage in both indoor and outdoor. The wild Ae.albopictus eggs stored indoor for2 days, 30 days and 60 days did not hatch at all (Fig 4c),while eggs stored for 4 days hatched after 1 hour. After 7 days of storage wild strain Ae.albopictuseggs stored indoor hatched after 15 minutes and after 14 days eggs hatched 24 hours after submergencein medium. The eggs stored for 7 days

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had the highest mean percentage of hatchability compared to others stored at different intervals indoor. In contrast, the wild strain Ae. albopictus eggs stored outdoor hatched for all storage intervals except for the eggs stored for 2 days (Fig 3d). The eggs stored for 4 days hatched 30 min after being submerged, while eggs stored for 7 days hatched after 1 hour of submergence. The eggs stored at 14 days, 30 days and 60 days hatched after 24 hours. Based on the hatching rates of both species, eggs stored indoor and outdoor were hatchable regardless of the location of the eggs.

4.DISCUSSIONS

The origin of the Aedes eggs, either wild strain or laboratory strain did not exert a significant effect onhatchability. Both strains were able to hatch after submergence. Vitek and Livdahl[17] compared the hatching rates of laboratory and wild strain of Ae. albopictus and reported no significant differencein hatching rate of eggs of both strains. Wild strain Ae.aegypti and Ae. albopictuseggs located indoor experienced the highest percentage of eggs hatching compared to the laboratory strain, indicating that wild strain Aedes eggs are more viable compared to the laboratory strain Aedes eggs.However, the location of the eggs has significant effect on the hatching rate. The eggs stored outdoor hatched in highest number compared to the eggs stored indoor. Data from this study also suggested that the temperature outdoor induced newly laid eggs to complete the embryogenesis process faster compared to the eggs stored indoor.The mean indoor temperature in Malaysia is 26.5 ±1.5 °C and the relative humidity is between 70±10%, while the outdoor temperature is 29.0±2.0°C and the relative humidity is 60±10%. Such range of temperature and relative humidity is conducive for embryogenesis. Raminani and Cupp[18] reported that the embryogenesis in the Ae.aegypti eggs was completed after 3 - 4 days at 25°C. In this study, the temperature outdoor was 29±2°C and such condition allowed the eggs to complete embryogenesis in less than 4 days. This wassimilarly reported by Farnesiet al[15]who showed that at 26.5 ± 1.5°C Ae. aegyptieggs completed embryogenesis after 2.5 days – 3 days, while at 29±2°C embryogenesiswas completed after 2 days – 2.5 days with percentage of hatching more than 80%.The Aedes eggs stored indoor had the highest mean percentage of hatching after 7 days and 14 days of storage,while after 2 days of storage, only eggs from laboratory strain and wild Ae.aegypti hatchedsuccessfully. In comparison, laboratory strain Ae. albopictuseggs stored for 4days failed to hatch. These results showed that Aedes eggs probably did not complete the embryogenesis development and failed to hatch within 4 days. In contrast toAedes eggs stored indoor, laboratory strain, wild strain Ae. aegyptiand laboratory strain Ae. albopictuseggs storedoutdoor successfully hatched after 2-60 days ofstorage,while the wild strain Ae. albopictuseggs hatched 4 days after storage. Dickerson [12] reported that at 26 ±1 °C, both the laboratory strain and wild strain eggs of Ae. aegypti and Ae. albopictus hatched.The percentage ofhatching of wild strain Ae.aegyptiwas higher than laboratory strain eggs. Aedes eggs after completing the embryogenesis process are in the dormant state which enables them to be inactive until the environment condition is conducive for egg eclosion. During storage the eggs are already inthe dormant stage. In this study Ae. aegypti eggs of both laboratory strain and wild strain showed that the storage timedid not affect their viability. Both Ae.aegypti and Ae.albopictus eggs were able to hatch after 60 days of storage, even though the mean percentage of hatching decreased over time. Dickerson [12] also reported that more than 70% of the wild strain eggs of Ae. aegypti and Ae. albopictus stored for 14 days hatched after submergence. In generalthe hatching rate of eggs of both species either stored indoor or outdoor was similar,even though the hatching was increased. Both laboratory strain Ae. aegypti and Ae. albopictus eggs stored indoor hatched after 1 hour of submergence. In comparison, eggs stored outdoor for 7-14 days hatched after 30 minutes, whileeggs stored 1-7 days hatched 15 min after submergence, 30 min for 20 days of storage andmore than 4 hours for those stored for 66days [3]. The wild strain eggs stored indoor showed that the fastest time for the eggs to hatch was after 15 minutes, especially the wild strain Ae. aegypti. All eggs were observed to hatch in greater number within 1 hour in the medium and after 24 hours.Christophers[3] reported that eggs kept moist for a few days were necessary for egg maturation so that they can hatch all at once.

5.CONCLUSION

In the wild environment, predators such as fungus, algae, ant, fish, psocids, beetle and other small aquatic insect are present and predate on the eggs/larvae and mayaffect the hatching ability(Christophers 1960; James 1966). The pre-hatching period of 24 hours is very crucial for Aedes egg. The number of eggs submerged in the medium and successfully hatchedis dependent on thiscrucial period. Knowledge on Aedes egg hatching is important for dengue vector control; for example, it is necessary not only to remove water from containers, but also to scrub them thoroughly to ensure that eggs are dislodged. Being desiccation resistant, eggs left in the container will hatch when water is introduced.

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Species	Aedes aegypti (LS)	Aedes aegypti (WS)	Aedes albopictus (LS)	Aedes albopictus (WS)
	(mean ± SD)			
Indoor	51.18 ± 9.74[a] (n = 959)	57.14 ± 9.55[a] (n = 378)	28.80 ± 19.65[c] (n = 802)	41.89 ± 11.40[c] (n = 739)
Outdoor	71.64 ± 8.46[b] (n = 760)	76.98 ± 14.32[b] (n = 224)	53.49 ± 3.12[d] (n = 618)	65.22 ±4.85[d] (n=296)

LS = laboratory strain; WS = wild strain, n= number of eggs used in the study

[1] Mean percentages followed by different letters within the same column are significantly different (df = 60, F = 6.48, p > 0.05)

Table 1 Mean hatchability (%) [1] of Aedes eggs located indoor and outdoor at all storage time

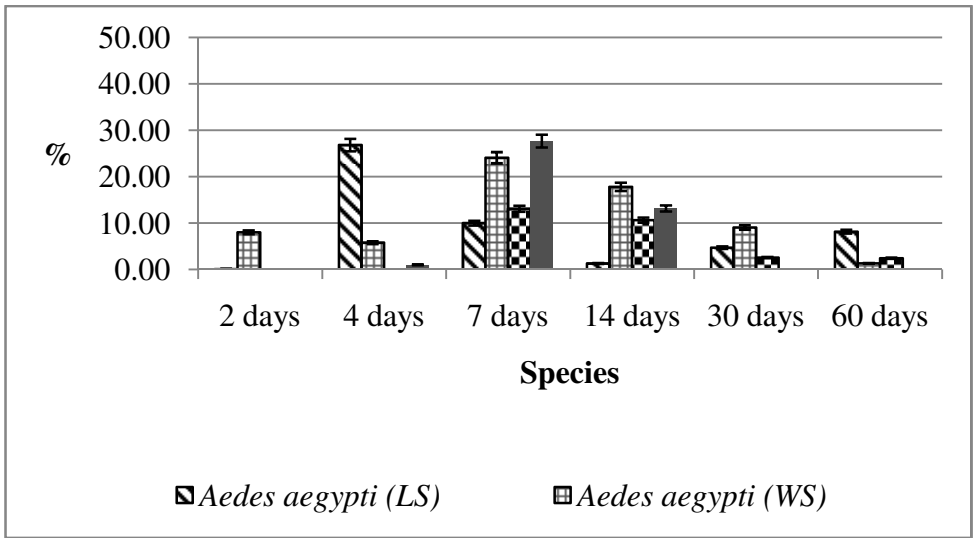

Figure 1 The hatchability of Aedes eggs stored indoor at various time intervals

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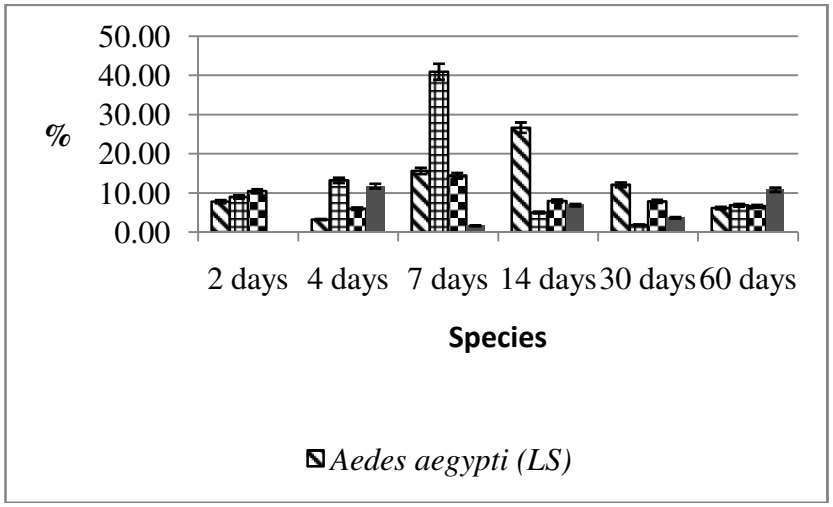

Figure 2 The hatchability of Aedes eggs stored outdoor at various storage intervals

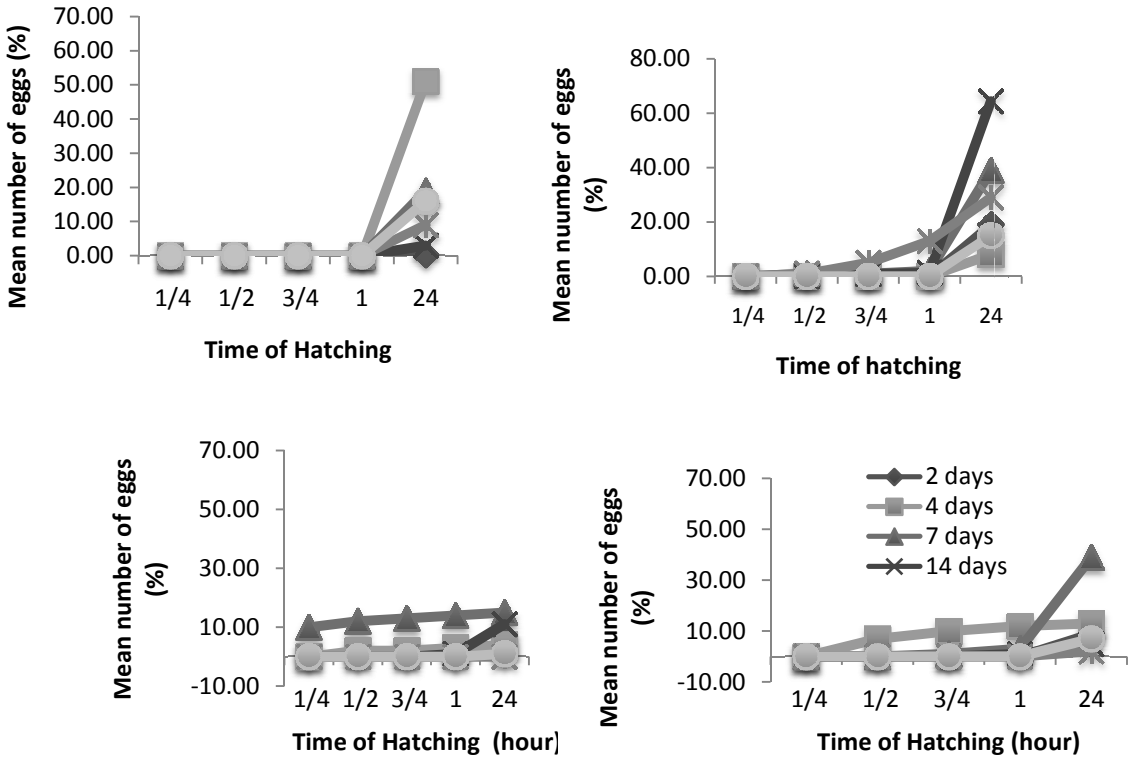

Figure 3 The hatching time for (a) laboratory strain Aedes aegypti eggs stored indoor (b) laboratory strain Aedes aegypti eggs stored

outdoor (c) wild strain Aedes aegypti eggs stored indoor (d) wild strain Aedes aegypti eggs stored outdoor

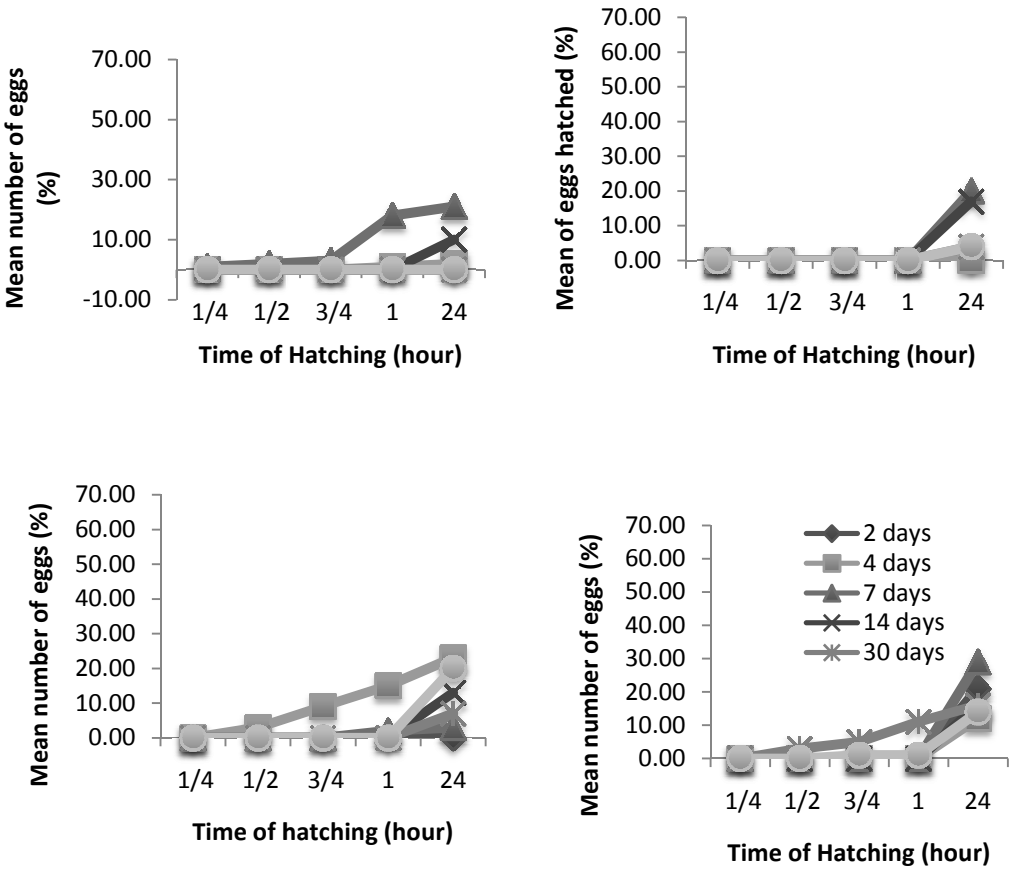

Figure 4 The hatching time for (a) laboratory strain Aedes albopictus eggs stored indoor (b) laboratory strain Aedes albopictus eggs stored outdoor (c) wild strain Aedes albopictus eggs stored indoor (d) wild strain Aedes albopictus eggs stored outdoor

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