Coagulation-Flocculation: A potential Application for Mosquito Larval Source Management (LSM) with No Insecticide Resistance

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Research

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Abstract

Background: Mosquito control is an essential step to eliminate mosquito-borne diseases. Larval mosquitoes have a more limited home range and lower resistance to adverse environment than adults, thus can be ideal targets for vector control in some cases. Coagulation-flocculation technology, which could be used for water treatment in breeding sites of several vector mosquito species, can significantly change both the distribution of organic particles and surface sediment characteristics in water environment. The aim of this study was to explore the effect, principle and possibility of using coagulation-flocculation technology in immature mosquitoes killing.

Method: In laboratory**,** chlorine-free tap water was treated with Poly Aluminum Chloride (PACl, sewage treatment using). The oviposition preference of gravid *Culex pipiens pallens*, the hatching of mosquito eggs and the survival amount of mosquito larvae were observed, and the pupa amounts were recorded each day.

Results: Coagulation-flocculation treatment could improve the oviposition preference of *Culex pipiens pallens* to some extent (compared with ordinary chlorine-free tap water), but not significantly (p=0.345). After treatment, mosquitoes laid eggs in chlorine-free tap water were 31.88% more than those laid in untreated water. Coagulation-flocculation affected the larvae's survival by physical means: ☒) alum floc layer increases the difficulty of larvae foraging, leads larvae starving to death; (☒) the little floc particles adhere to the surface of larvae, which stops larvae from floating upward to breathe. As a result, the alum floc layer had a good killing effect on the mosquito larvae, presented the half lethal time (LT_{50}) of 2d, the 90% lethal time (LT_{90}) of 8.7±7.3 ~ 14±4.5 d, and the pupation rate of 0 ~ (6.5±0.5)%, respectively.

Conclusions: PACl coagulation-flocculation produced lots of alum flocs, which may attract more gravid mosquitoes for laying eggs, and was shown to be highly active against 1^{st}~2^{nd} instar larvae. The principle of this technology illustrates this method won't develop insecticide resistance. In this study, coagulation-flocculation technology is considered to be a new potential approach to a sustainable, low-impact and low-cost mosquito control method.

Background

Mosquitoes are vectors for many pathogens, millions of people are estimated to be infected through mosquito biting globally and annually [1–3]. Some of these epidemic mosquito-borne diseases could be fatal for lack of licensed vaccines and effective therapeutics [4]. Therefore, increasing attention has been focused on vector mosquito control [5], particularly for now mosquitoes are expanding their scope of residence and extending their activity time under the condition of global warming [6, 7].

Three of four periods through mosquito's life (egg, larva and pupa) are in the water environment, during which their survival depends on various factors such as competitors [8], light [9], temperature [10], food availability [11, 12], predator [13, 14] and characteristics of water environment (such as pH, ammonia, chloride, dissolved oxygen, etc.) [15–18]. Larval mosquitoes have a more limited home range and lower resistance to adverse environment than adults, thus can be ideal targets for vector control in some cases [19, 20].

Larval Source Management (LSM), one of the oldest tools in the fight against mosquito-borne diseases such as malaria and dengue, has been the main focus of mosquito control programs for decades in the United States of America (US), Canada, throughout Europe, Brazil and Singapore [21, 22]. Despite of now efforts are made to develop new biological or botanical larvicides [20, 23, 24], rather than routine chemical larvicides, in attempts to overcome the common disadvantages of larviciding, however, these weaknesses still remain largely unsolved, including: (⊠) toxic to human and other non-target species; (⊠) polluted to water environment; (⊠) costly in long-term using; (⊠) vulnerability to the evolution of target resistance [20]. Therefore, researches in introducing an existing and mature technology of water treatment into mosquito-control field could be attractive, if it has the possibility to avoid the weaknesses above.

Coagulation-flocculation, a proven technology in surface water remediation and sewage treatment, now has been widely used in plenty of water treatment occasions [25–27]. Addition of coagulants could promote agglomeration of suspended particles (including microorganisms), further deposit the latter to the bottom by gravity. Therefore, coagulation-flocculation technology can significantly change both but not limited to the distribution of organic particles and surface sediment characteristics in water [28]. Based on the roles which coagulation-flocculation played in water environment, it’s convincing to believe that larval mosquitoes would be influenced deeply by it. In this study, coagulant Poly Aluminum Chloride (PACl) was used to treat chlorine-free tap water, through simulating this treatment process,we measured the influence of coagulation-flocculation on: (⊠) oviposition preference of gravid mosquitoes, (⊠) the development and survival of immature mosquitoes.

Culex pipiens pallens from *Culex pipiens* complex was selected. Mosquitoes in this complex prefer slightly or moderately contaminated water as breeding habitats [29], and these breeding habitats are the application objects of coagulation-flocculation technology. Species in the *Culex pipiens* complex are world widely distributed [4, 30, 31], meanwhile important vectors to plenty of virus and parasites, including *West Nile virus*, *Wuchereria bancrofti* and *Dirofilaria immitis* [29, 32, 33]. *Culex pipiens pallens*, commonly found north of 37°N in China, Japan and Korea, is one of the major mosquito species natively responsible for biting human and disease transmission [34].

Method

Biological bioassays

Culex pipiens pallens adults and eggs in this research were obtained from Department of Vector Control, Shanghai Municipal Center for Disease Control & Prevention. Adults were maintained under conditions of 27.5 °C ± 1.5 °C, 13 h light/11 h dark (photoperiod), and continuously provided with a 10% glucose solution. Mosquito larvae were reared in chlorine-free tap water with the same temperature and photoperiod. Since eggs were hatched, immature mosquitoes were fed with powdered, sterilized rat diet (QianMin SiLiao, China). 0.01 g of sterilized rat food was added within 24 hours after the hatching of mosquito eggs, and 0.03 g of sterilized rat food was added every 48 hours, increasing or decreasing appropriately according to the age and quantity of larvae. During the feeding and experimenting, larvae were removed with a straw after pupation and put into a mosquito cage (L:30 cm, W:30 cm, H:30cm) for cultivation. Female adults were fed on blood for 3 ~ 4 h, and then after 24 h mosquito spawning test was taken within the same cage. Blood source: SPF KM mouse (Shanghai Jiesjie laboratory animal co. LTD).

Coagulation-flocculation treatment

Poly Aluminum Chloride (PACl) for sewage (Shanghai Ruixin industrial co. LTD, China), contained 28% of Al_2O_3, was used as coagulant. Coagulant was added as a suspension of 10 g/L.

The procedure of coagulation-flocculation was performed as follows: a predetermined amount of coagulant (60 ml/L, determined by pre-experiments) was dosed into the reactor (1L jar), after the water quality reached steady state, then the suspension was stirred at 300 rpm for 15 seconds, followed by 100 rpm for 2 min. JJ-1 digital speed measuring electric mixer (Changzhou Jintan Youlian Instrument Research Institute) was used to provide stir conditions.

Data collection, processing, and statistical analysis

The results of mosquito oviposition preference test were expressed by number of valid oviposition times. Mann-Whitney U non-parametric test (IBM SPSS Statistics 20) was used for data analysis, and $p < 0.05$ was taken to indicate statistical significance. To evaluate how coagulation-flocculation take effect on the development and survival of immature mosquitoes, the mean number of survival mosquito larvae (± SE), the mean number of pupation (± SE), the half lethal time (LT_{50}) and the 90% lethal time (LT_{90}) were used to describe the results.

Results

Oviposition response of Culex pipiens pallens

Adult mosquitoes chosen for mosquito oviposition preference test were mosquitoes which had been 72 h after eclosion. 24 hours after blood feeding, ovitrap 1 (filled with 200 ml chlorine-free tap water + 20 ml PACl suspension, denoted as O1) and ovitrap 2 (filled with 220 ml chlorine-free tap water, denoted as O2) were placed in the cage. Every 24 hours O1 and O2 were observed, mosquito eggs in O1 and O2 were removed as well.

In the experiment, female mosquitoes laying eggs in two cups (d = 11 cm, h = 5 cm, transparent plastic material) were considered as valid oviposition. After each biting, all of the eggs laid by one single female *Culex pipiens pallens* would gather together as an egg raft and float on the water [35], thus we can recognize the number of valid oviposition times easily. Each observation and removal of all mosquito eggs in O1 & O2 is considered as an independent observation.

In order to exclude the influence of the placement order of ovitrap on the experimental results, the experiment was divided into two parallel control groups which only differed in the placement of O1 and O2, remaining other environmental factors consistent. Apparatus is shown in Fig. 1.

Table 1 Results of two replications in mosquito oviposition preference test

In this test, O1 and O2 had equal oviposition opportunities for female mosquitoes, and finally with a total of 320 valid oviposition times (Table 1). Mosquitoes showed the same oviposition preference in the two replications, so we take the results of R1 and R2 calculated in combination. Of the two replications, O1 had 182 valid oviposition times, which is 31.88% more than that of O2 (138 valid oviposition times), indicating that O1 was more attractive to oviposition, but not significant ($n = 15$, $p = 0.345$, $p > 0.05$).

Among the 15 independent observations with a duration of about 30 days (data not shown), there were 11 times to prove mosquitoes prefer O1 as their breeding habitat, only in 3 times there were more mosquitoes chose O2 rather than O1 to lay eggs, remaining one time equally. This suggests that the attraction of O1 to female mosquitoes' oviposition was persisted and didn't decrease over time.

Numerous studies indicated that the process of selecting breeding sites for female mosquitoes is mediated by multiple factors such as vision [36], smell [37], and pheromone [38]. We speculated in this study mosquitoes' oviposition behavior is mainly influenced by vision. Studies have shown that yellow is more attractive to *Culex pipiens pallens* for laying eggs than white [39], which in this experiment after coagulation-flocculation, yellow alum floc deposited at the bottom of the ovitrap, which is considered to be nutrient-rich for larvae growing, therefore more female *Culex pipiens pallens* mosquitoes preferred O1 as breeding sites [40].

Effects of coagulation-flocculation on mosquito egg hatching performance and subsequent development of mosquito larvae

This phase was carried out in a jar containing 1L chlorine-free tap water. 0.01 g sterilized rat food was added in chlorine-free tap water in advance, for it would provide basic nutrition for larvae after egg hatching. The surface scums were scoured by deionized water, until most of scums dropped down to the bottom.

Pre-experiment confirmed that the appropriate dosage of PACl suspension was 60 ml/L, which means the mass concentration of PACl to water was about 0.6 mg/L. It was normal dosage in water treatment [41, 42]. The complete coagulation-flocculation operation includes the addition of coagulants and the mixing process, so the experiment was divided into four parallel control groups, group1 ~ 4 (Fig. 2). Groups could be divided into the coagulating groups and the non-coagulating groups, according to the addition of coagulant or not. Experiment started after mosquito eggs were added in Group 1 ~ 4, and then treated with stirring and/or coagulant adding.

Table 2 shows the comparison of water quality parameters of each group before (t = 0) and after (t = 30 min) treatment. No significant changes occurred in water quality parameters within non-coagulating groups before and after the treatment; group 3 exhibited obvious flocculation 30 minutes after the coagulation, while pH decreased slightly (7.36 ± 0.49 to 7.21 ± 0.43), and turbidity increased significantly (1.23 ± 0.18 NTU to 2.99 ± 0.38 NTU), for without stirring, alum floc produced by coagulation didn't have enough opportunity for collision and aggregation [43], resulting in the size of some flocs being too small to settled, these floc fragments suspended in supernatant; the pH of group 4 decreased slightly (7.37 ± 0.48 to 7.11 ± 0.36) after treatment, since other factors were basically unchanged. The addition of coagulant increased the salinity (Sal) in the water, and the Electronic Conductivity (EC) and Total Dissolved Solids (TDS) of the coagulating groups (group 3 & group 4) were significantly improved after treatment.

Table 2 Water quality parameters of each group when t = 0 and t = 30 min

The pupation time of larvae in 4 groups (Fig. 3 (b)) were compared. The first pupa in coagulating groups (group 3) appeared significantly later than that of non-coagulating groups, except for there was no pupa in Group 4. The delay time varied in different batches of experiment, but all exceeded 7 days. Moreover, the pupation time of last larvae in coagulating groups (group 3) was also later than that of the non-coagulating groups, suggests that coagulation-flocculation treatment may delay the development of mosquito larvae. It may be a response of food-lacking, which could delay larvae development [44, 45].

Table 3 Comparison of pupation rate, lethal time of 50% (LT_{50}) and lethal time of 90% (LT_{90}) in 4 groups

In table 3, differences between pupation rate, LT_{50} and LT_{90} in 4 groups indicated what degree could coagulation-flocculation affect larvae survival in this experiment. The pupation rate of non-coagulating groups was up to 50% in average, while in coagulating groups was below 10%, suggests that by treating with PACl coagulation-flocculation, the number of larvae that become pupae would be definitely reduced. In addition, LT_{50} of coagulating groups were 2d and LT_{90} were over 7d, indicating that PACl coagulation-flocculation had a stronger killing effect on newly hatched 1st instar larvae and a limited killing effect on late instars.

7d-continuously cultivation to explore the main reason coagulation-flocculation cause 1st ~ 2nd instar larvae death

To explain why PACl coagulation-flocculation could definitely kill 1st ~ 2nd instar larvae, there are two possible reasons: 1) *Culex pipiens pallens* larvae may be sensitive to the water-soluble products produced by PACl coagulation, and died due to toxicity; ([illegible]) alum floc layer hide the food from immature mosquitoes, increases the difficulty of larvae feeding, results in larvae starving to death, moreover, these little floc particles could adhere to the surface of larvae, which prevent larvae to float upward to breathe.

In order to find out which from above the main reason is caused the high mortality of 1st ~ 2nd instar mosquito larvae, a 7d-continuously cultivation of *Culex pipiens pallens* was conducted. From the egg stage, *Culex pipiens pallens* were raised in ordinary chlorine-free tap water (control group, recorded as "group C"), chlorine-free tap water after coagulation-flocculation (coagulation-flocculation group, recorded as "group C-F"), and filtered supernatant of group C-F (recorded as "group SC-F"), respectively.

Results revealed that survival of the larvae in group C and group SC-F was consistent and in good condition, while the number of larvae in group C-F reduced by 75% within 2d after the hatching, and made zero within 5d (Table 4). Therefore, the possibility of *Culex pipiens pallens* larvae died for the water-soluble products produced by PACl coagulation can be excluded, similarly the changes of pH, for larvae favor a neutral pH or slightly alkaline environment [46]. By observing the distribution of the dead larvae in group C-F, it can be found that the location of dead larvae is mainly in the surface and upper part of the floc sediments, and there is no food remains in the digestive tract of larvae (Fig. 4). This phenomenon illustrates that alum floc layer is the main cause of 1st ~ 2nd instar larvae's death.

Table 4 Results of 7d-continuously cultivation in different groups

Discussion

Coagulation-flocculation technology is widely used in waste water treatment and water environment remediation, even in drinking water treatment [47, 48], relevant theories are abundant. To our knowledge, this is the first study to raise the possibility that coagulation-flocculation could be applied to the vector mosquito control. In this study, we found that under laboratory conditions, PACl coagulation-flocculation could promote oviposition preference of female *Culex pipiens pallens* to some extent (compared with ordinary chlorine-free tap water), and has a very high lethal effect on the newborn mosquito larvae after hatching. The best chance what coagulation-flocculation could achieve the highest lethal effect, is a lentic environment to keep the alum flocs at the bottom as long as possible, and it is exactly the environment what mosquito larvae need [17, 49–51].

It is worth nothing that coagulation-flocculation could efficiently kill 1st ~ 2nd instar larvae. Firstly, this treatment reduces the amount of larvae's food (organic suspended solids, bacteria and algae) in the water phase, impels larvae to forage from sediment, where is hidden by alum flocs; besides, coagulation-flocculation produces a large number of alum flocs with different size and irregular shapes, not only could it adhere to the surface of larvae and prevent them from floating upward to breathe, but also reduces the opportunity for larvae to get fed.

According to this study, the killing of immature mosquitoes by coagulation-flocculation is mainly achieved by physical means, which makes coagulation-flocculation have obvious advantages over the commonly used chemical insecticide: (☒) non-toxic, coagulation-flocculation could be applied in drinking water treatment, that means its environmental impact is very weak; (☒) it won't develop insecticide resistance when put into long-term use; (☒) it could trap gravid mosquitoes to spawn and kill the larvae simultaneously.

According to the principle of killing mosquito larvae by coagulation-flocculation technology,we believe there is a failure time of coagulation-flocculation to kill larvae, at this time the alum floc layer is compressed to dense enough by gravity, larvae cannot enter it. In a lentic environment, factors which influence the failure time of coagulation-flocculation include coagulation process (type of coagulant, and how it is used), parameters of water environment (pH, salinity, temperature, etc.), and the moving ability of mosquito larvae (“strong” larvae may rescue themselves from alum flocs). We believe that, on the premise that other variables do not change, the adoption of appropriate methods (such as vibrating slightly) to promote the resuspension of alum flocs is helpful to delay the arrival of the failure time of this larvae-killing technology.

The coagulant use in this study was the inorganic coagulant PACl. The dosage of PACl was determined to be 0.06%, according to the best turbidity removal effect in pre-experiment, and it was added in the form of 10 g/L suspension at one time. In the out-door study, not only the dosage of coagulant should be calculated according to the water volume, but also the bottom area should be taken into consider, for alum floc layer need to be thick enough to trap mosquito larvae. The available methods include: adding coagulant at plural points, and increasing (or decreasing) the dosage of coagulant.

Though in laboratory, coagulant PACl could promote oviposition preference of female *Culex pipiens pallens* to some extent, however, considering the limitation of laboratory, and actually female *Culex pipiens pallens* prefer ovitrap in deep color as breeding sites [39], PACl may avoid mosquitoes’ oviposition if used out-doors. Still, coagulant modification may help to solve.

Larval Source Management (LSM) is a strategy to prevent the spread of vector-borne diseases by targeting the larval stage of vectors, including habitat modification, habitat manipulation, larviciding and biological control. World Health Organization (WHO) lists LSM as a supplementary vector control method for malaria vector control [19, 52]. At present, commonly used larvicides are chemical insecticides with certain biological toxicity [53, 54], which are difficult to be accepted by some communities. Therefore, non-toxic technology coagulation-flocculation could help LSM being generalized in these communities.

What’s more, the increasing insecticide resistance of mosquitoes are threatening the results in eliminating mosquito-borne diseases in the past decades, such as malaria and dengue [55]. Issued in May 2012, the WHO Global plan for insecticide resistance management in malaria vectors (GPIRM) is a plan of action for all stakeholders engaged in the fight against malaria. The document provides comprehensive technical recommendations to manage insecticide resistance in different situations [56]. One of the key actions called by GPIRM and experts to be undertaken is to “develop new, innovative vector-control tools” [56, 57], we believe that coagulation-flocculation technology has the potential to be one of them. It requires more researches about coagulation-flocculation applying in Integrated Vector Management (IVM), Larval Source Management (LSM), and attract-and-kill control strategy. These includes the effects on different mosquito species with different ecological habits, the application of other coagulants, the R&D of new coagulants, the optimization of the dosage and way of use, the boundary and potential risks and so on.

Conclusions

This study observed how PACl coagulation-flocculation affect: (☒) *Culex pipiens pallens* gravid female oviposition site selection; (☒) development of *Culex pipiens pallens* from eggs to pupae. Results exhibited gravid adults prefer to choose chlorine-free tap water under treatment as breeding sites, rather than ordinary chlorine-free tap water. And alum floc could kill newly hatched 1st ~ 2nd instar larvae efficiently. The alum floc produced by coagulation-flocculation mainly affected *Culex pipiens pallens* larvae by physical means, which not only reduced the feeding opportunities of the larvae, but also limited the ability of larvae to float on the water to breathe. Thus it won’t develop insecticide resistance. In this study, coagulation-flocculation technology is considered to be a new potential approach to a sustainable, low-impact and low-cost mosquito control method.

Abbreviations

PACl
Poly Aluminum Chloride; LT_{50}:the half lethal time; LT_{90}:the 90% lethal time; IVM:Integrated vector management; LSM:Larval Source Management; WHO:World Health Organization; GPIRM:Global plan for insecticide resistance management in malaria vectors

Declarations

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Availability of data and materials

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Authors’ contributions

Huang-MS proposed the research ideas and assisted in study designing. Li-XR designed the complete experimental scheme, implemented and was responsible for writing the manuscript. Miss Xiao-B assisted in the breeding and caring *Culex pipiens pallens* adults and larvae. Mr Lu-XY assisted in the photograph and making figures. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1 Results of two replications in mosquito oviposition preference test

	number of valid oviposition times	
	O1	O2
R1	71	58
R2	111	80
total	182	138

Table 2 Water quality parameters of each group when t = 0 and t = 30 min

	Pupation rate (%)	**Lethal Time of 50% (d)**	**Lethal Time of 90% (d)**
group1	56.5 ± 23.5	-	-
group2	53 ± 6	-	-
group3	6.5 ± 0.5	2	14 ± 4.5
group4	0	2	8.7 ± 7.3

Table 3 Comparison of pupation rate, lethal time of 50% (LT_{50}) and lethal time of 90% (LT_{90}) in 4 groups

Time (d)	Number of larvae in each group		
	Control (Group C)	Coagulating-Flocculation (Group C-F)	Supernatant of Group C-F (Group SC-F)
1	100	100	100
2	100	25	100
3	100	23	100
4	100	15	100
5	98	0	100
6	95	0	100
7	89	0	94

Table 4 Results of 7d-continuously cultivation in different groups

Group number	pH	Turbidity(NTU)	EC(ms/cm)	TDS(ppm)	Sal(g/L)
Before the treatment (t = 0)					
1	7.42 ± 0.46	1.26 ± 0.22	18.55 ± 0.62	9278 ± 312	10.98 ± 0.40
2	7.37 ± 0.49	1.09 ± 0.18	19.09 ± 1.00	9545 ± 496	11.32 ± 0.63
3	7.36 ± 0.49	1.23 ± 0.18	19.03 ± 0.93	9513 ± 466	11.27 ± 0.59
4	7.37 ± 0.48	1.11 ± 0.15	19.05 ± 0.93	9526 ± 467	11.29 ± 0.59
30 min after the treatment (t = 30)					
1	7.47 ± 0.49	1.09 ± 0.13	18.90 ± 0.87	9452 ± 437	11.2 ± 0.56
2	7.46 ± 0.48	1.68 ± 0.92	19.16 ± 1.04	9583 ± 523	11.37 ± 0.67
3	7.21 ± 0.43	2.99 ± 0.38	23.00 ± 1.07	11503 ± 533	13.87 ± 0.70
4	7.11 ± 0.36	1.18 ± 0.20	23.68 ± 0.88	11840 ± 439	14.31 ± 0.57

Figures

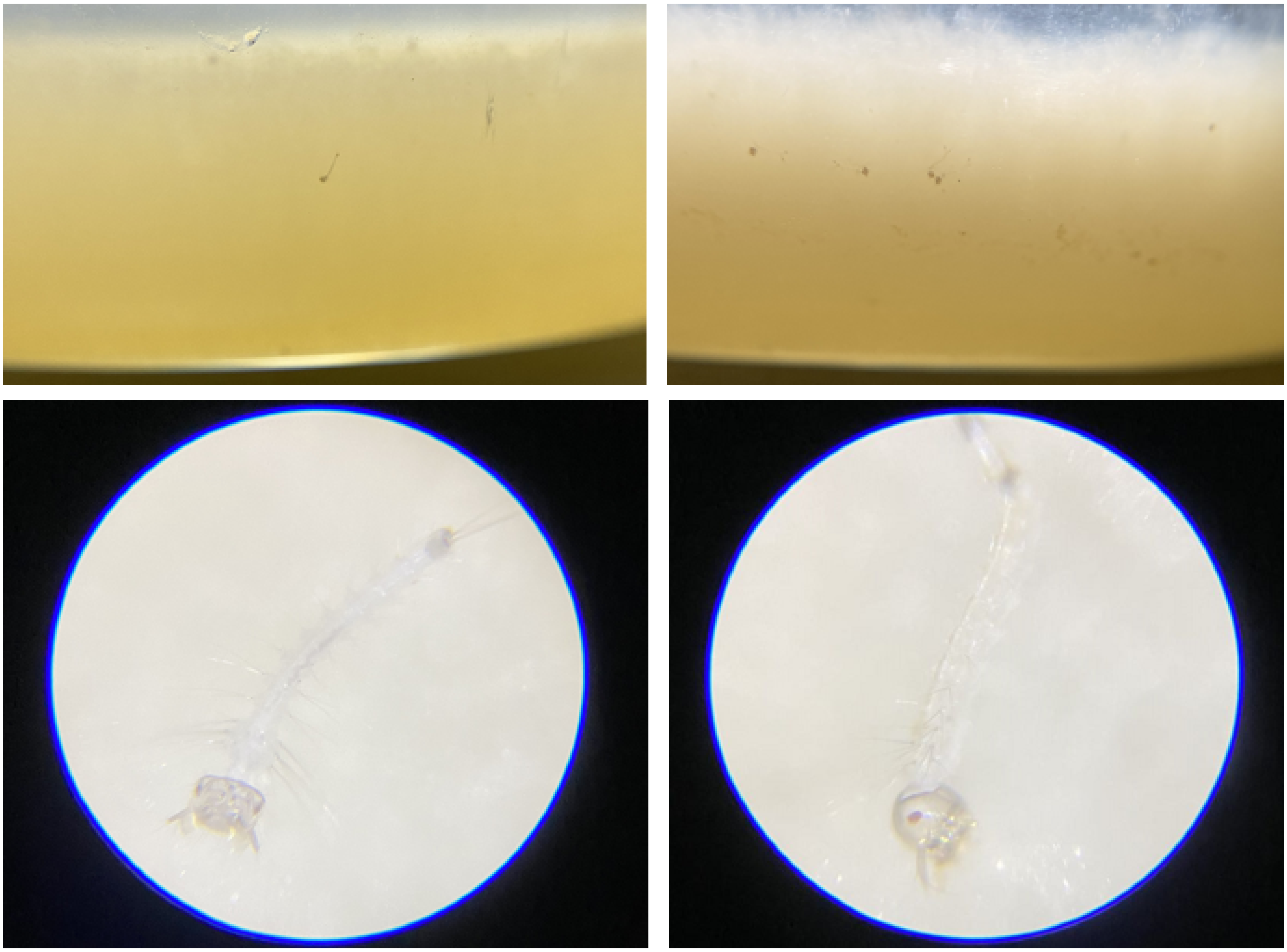

Figure 1

Dead mosquito larvae in alum floc layer of group C-F

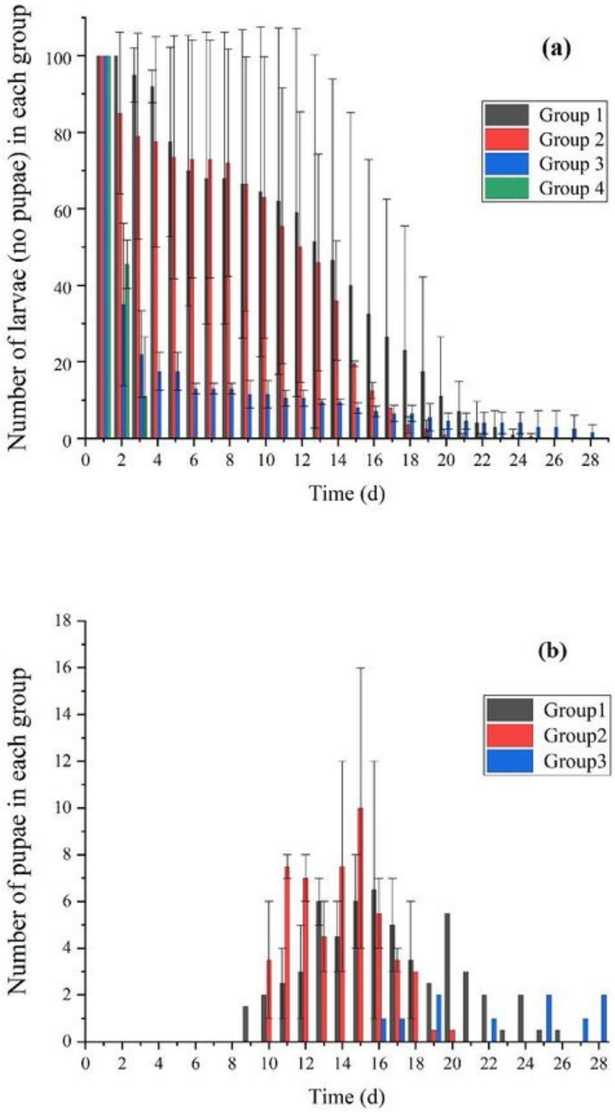

Figure 2

The survival of larvae and the number of pupation over time, group 1~4. (a): number of larvae (no pupae) in each group over time; (b): number of pupae in each group over time.

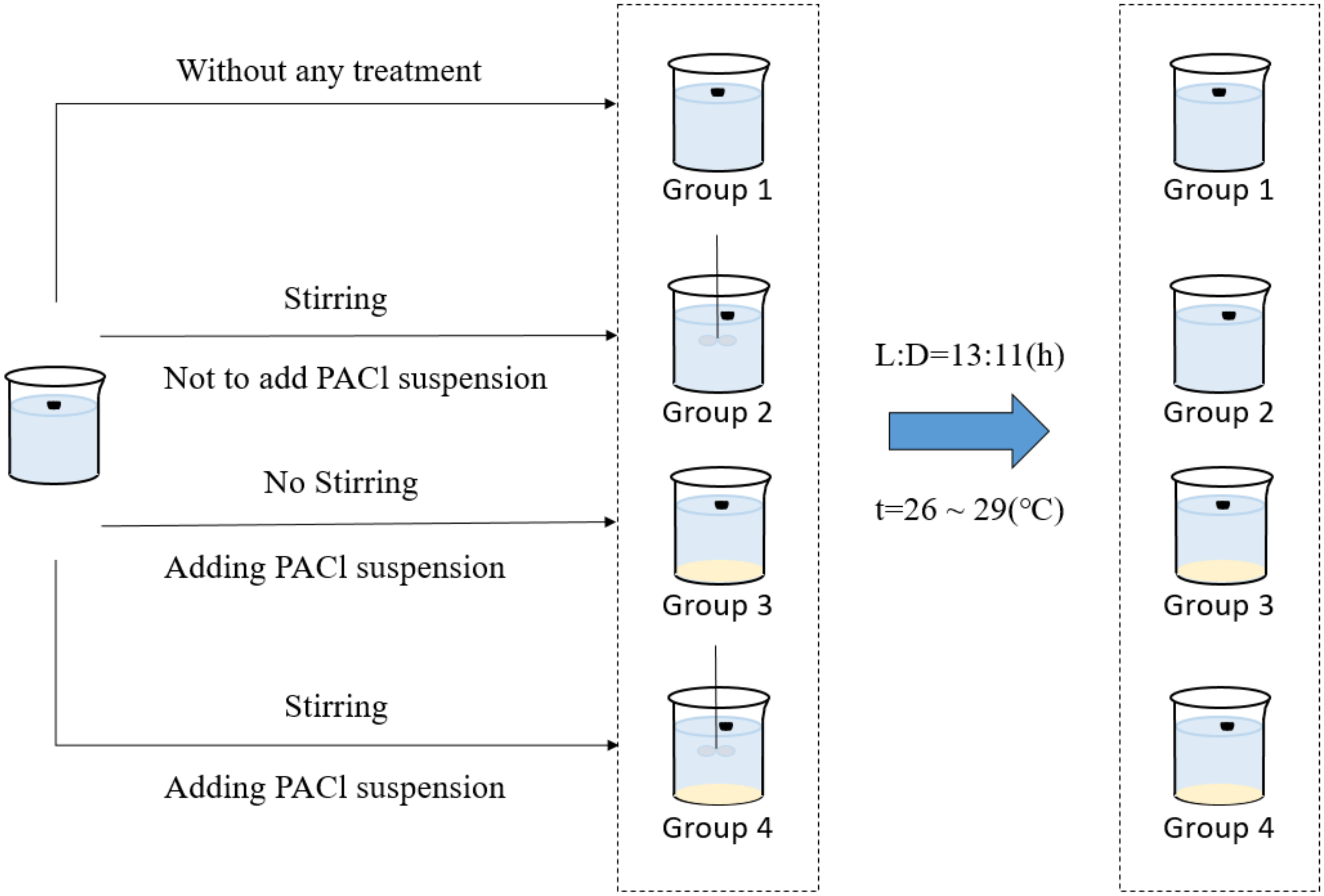

Figure 3

Setting of mosquito egg hatching and larvae developing experimental groups (Stirring was consisted of two stages: 300 rpm rapid stirring for 15s, 100 rpm slow stirring for 2 min)

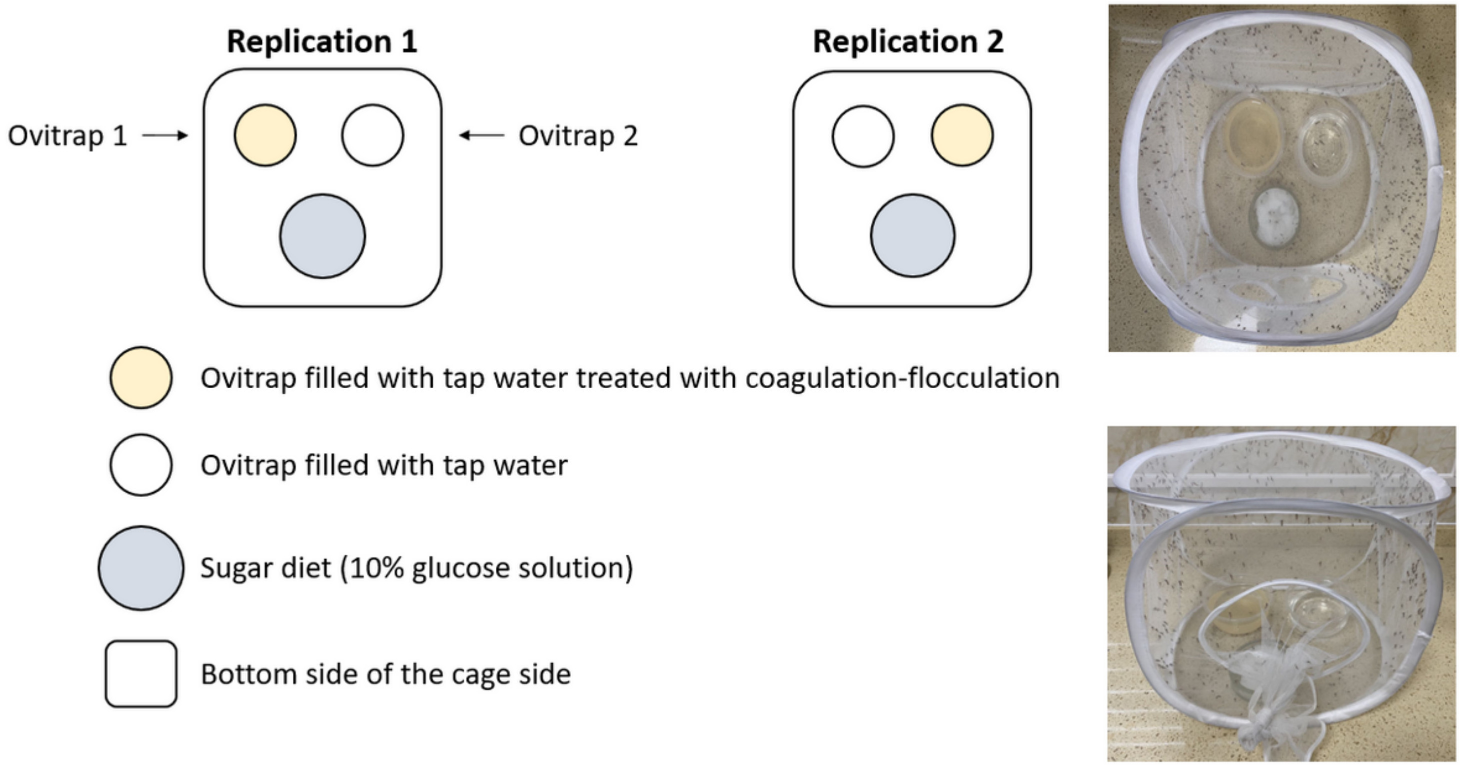

Figure 4

Replication 1 and Replication 2 in mosquito oviposition preference test. (Two replications differed in ovitrap order only).