Impact of fertilizers and green algae (*Spirogyra*) on the survival and development of immature malaria vector, *Anopheles gambiae sensu lato* Giles (Diptera: Culicidae)

Wondmeneh, J. *, **Solomon, T. and Raja, N.**

ABSTRACT

Anopheles gambiae is one of the important malaria vectors in Ethiopia. The survival, development and multiplication of the immature stages are determined by different interdependent factors. The application of manures and fertilizers in agro-ecosystem is one of the important factors. A laboratory study was conducted to evaluate the impact of inorganic fertilizer (DAP and UREA 1:1/2 ratio), organic fertilizer (cow dung) and green algae (*Spirogyra*) against immature *An. gambiae*. The experiment includes three doses of inorganic fertilizer, cow dung and green algae in 1 L of deionised water prepared in a square tray and 1 L of deionised water alone was considered as control. In the experiment and control group twenty five I, II, III and IVth instar larva was released into individual tray and monitored every 24 h till the completion of their life cycle. The numbers of surviving larval stage was recorded continuously until all the larvae died or emerged into adults. The experimental result revealed that in the case of the tray mixed with 0.75g/L of inorganic fertilizer, the number of adults that emerged from four different larval instar stages was statistically significant ($p < 0.05$). The number of adults that emerged in high turbidity green algae filament treatment was 95.61 ± 3.86, 93.85 ± 3.30, 90.5 ± 5.43 and 86.84 ± 4.78 from I, II, III and IVth instar larvae respectively. This study concludes that inorganic fertilizer significantly suppressed the survival of *An. gambiae* immature stage whereas, green algae supported the larval development.
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Keywords: *Anopheles gambiae*, inorganic fertilizers, cow dung, green algae, survival, developmental rate.

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INTRODUCTION

Agricultural practices are one of the potential sources for ecosystems alteration and substantially change the nature of malaria vector risk proximal to their location (Ijumba and Lindsay, 2001, Van der Hoek *et al*., 2003). In Ethiopia, malaria epidemiology is variable and unstable, although high and stable transmission was reported in western lowland river basin. According to WHO (2004), mortality and morbidity due to malaria was decreased but the exact factors for the reduction were not well defined. *An. gambiae* is the primary malaria vector and their immature preferred small, clear, sunlit pools of water such as foot or hoof prints, edges of bore holes and burrow pits, roadside puddles formed by tire tracks, irrigation ditches and other man-made shallow water bodies (Imbahale *et al.*, 2011, Mutuku *et al*., 2006; Kenea *et al*., 2011) and also polluted water with a rich source of organic matter (Awolola *et al*., 2007).

Immature mosquito distribution and abundance are associated with different factors including the use of inorganic fertilizer and pesticides. Application of organic and inorganic fertilizers in rice fields increases the larval productivity (Service, 1997). Sunish and Reuben (2002) reported that *Cx. vishnui, Cx. tritaeniorhynchus* and *Cx. pseudovishnui* population was increased in a dose-dependent manner of inorganic nitrogen fertilizers application. The study of Mutero *et al*. (2004) in Kenya demonstrates that a pond treated with ammonium sulphate facilities increase in the population of *Anopheles arabiensis*. Kibuthu *et al*. (2016) observed significantly influenced oviposition preference and adult emergence rate of *An. arabiensis* while exposed to ammonium sulphate. Muturi *et al*. (2007) and Olayeme *et al*. (2012) reported mosquito larval development and decreased of survival in inorganic fertilizer treatment compared to control in a dose dependent manner. The survival of *Culex pipiens pipiens* mosquitoes was reduced in 2, 4 dichorophenoxyl acetic acid (2,4-DAA) herbicide application under laboratory condition (Olayeme *et al*., 2014).

The growth of certain poisonous algae in mosquito breeding site prevents oviposition behaviour that may be used as a possible biological control agent together with other mosquito intervention tools. On the other hand, some species of filamentous green algae are highly beneficial for rapid growth and survival of mosquito larvae (Bond *et al*., 2004). According to Hegewald and Schnepf (1979) many species of algae in the order *Scenedesmu, Kirchneriella, Dactylococcus, Elakotothrix, Tetrallantos, Coelastrum, Selenastrum* and *Tetradesmus* are indigestible by the larvae of *Culex, Aedes* and *Anopheles.* Ahmad *et al*. (2004) reported that *Aedes aegypti* mosquitoes are killed after 7 days exposure to *Chlorococcum* UMACC-184 and *Scenedesmus* UMACC – 193. Field observation by Kenea *et al*. (2011) found a negative association of algae mat with densities of mosquito larvae. Kachroo (1959) reported that variety of *Anopheles* species easily digest diatoms, desmids, cladophorans, filamentous green algae and planktonic green algae. The presence of filamentous algae in mosquito breeding sites provide nutrition for the development, adult emergence and also protection to immature mosquitoes against predators and water current (Sunish and Reuben, 2002). The current agricultural extension program focuses on productivity and improvement of small holder agriculture by supplying fertilizers, improved seeds and setting up credit schemes (MOARD, 2007). Therefore, the present study was conducted to confirm the influence of inorganic fertilizer, organic fertilizer and green algae on the development and survival of *An. gambiae* under laboratory condition.

MATERIALS AND METHODS

Collection and rearing of *Anopheles*

Anopheles gambiae in their immature stage were collected from breeding sites of four rural localities in Dembia district, North West Ethiopia by using standard dippers (350 mL). Dembia district is known for its successive malaria epidemic with high mortality. The immature mosquitoes collected from the breeding habitat were transferred to a large plastic container and brought to the Entomology laboratory, Maraki campus, University of Gondar. In the laboratory, homogenous immature stages were separated and allowed to acclimatize in deionised water. The four immature mosquito stages namely I, II, III, IV and adults were identified based on morphological characters and identification key (Gillies and Coetzee, 1987; Walker and Lynch, 2007). In the laboratory immature mosquitoes were provided with powdered dog biscuit and yeast powder at 3:1 ratio as a source of feed. The culture of the immature mosquitoes was maintained at 27 ± 1°C, 65–70% RH and a photoperiod of 12 :12 h light: dark cycle throughout the study period.

Preparation of fertilizers and green algae

Three different doses of inorganic fertilizer namely 0.25g/L, 0.5g/L and 0.75g/L was prepared from a mixture of DAP and UREA (1:1/2 ratio). The three doses were mixed individually with 1 litre of dechlorinated tap water in the plastic tray (25 cm x 25 cm x7 cm length, width and height) containing 25 immature *An. gambiae*. Similarly, three different doses of organic fertilizer (2g/L, 4g/L and 6g/L) were prepared by using dried cow dung. The dose of inorganic fertilizer and organic fertilizer were calculated based on the field recommendation (100kg/hectare for inorganic and 1000kg/hectare for organic fertilizer) by the Ministry of Agriculture and Rural Development (MOARD, 2007). For green algae, high, medium and low turbidity was placed into 1 L of dechlorinated tap water in the plastic tray. When the green algal filament covers the entire experimental plastic tray it was considered as high turbidity; covering up of half of the tray indicate medium turbidity and if the filament was scattered in the experimental tray it was considered as low turbidity treatment. The tray containing one liter of deionized water without any treatment was considered as control. The water of the culture media was replaced by every 24 h with the same concentration. The experiment was continued until all the larvae were either dead or emerged into adults. Each treatment was replicated four times.

Statistical analysis

The data collected from the experiment was subjected to statistical analysis by using SPSS statistical software package version 20. One way analysis of variance was carried out subjected to determine the effect of treatment on larval development time, number of individuals surviving in each life stage and the probability of surviving from one stage to the next. Further, significant difference for individual mean of survival was calculated by using post hoc test LSD (Least Significant Difference Test, $p<0.05$).

RESULTS AND DISCUSSION

Inorganic fertilizer on *An. gambiae*

The laboratory findings clearly demonstrate dose dependent effect of inorganic fertilizer on survival and development of *An. gambiae* immature stages. Mean number of survival rate of *An. gambiae* larvae exposed to inorganic fertilizer under laboratory condition is depicted in Fig.1. The results revealed that all the immature stages were susceptible to inorganic fertilizer. Among the four immature stages, early Ist instar larvae were highly susceptible when compared to rather later larval stages. The time taken for the completion of growth rate i.e., larvae emerging into adult stage was 14-18 days (Fig.4). The mean number of survival rate of *An. gambiae* Ist instar larvae exposed at 0.75g/L of inorganic fertilizer and control were 2.58 ± 0.95 and 85.34 ± 1.70 and for IVth instar larvae it was 30.17 ± 1.68 and 93.96 ± 3.59 respectively. The statistical analysis results (Table I) showed significant difference at 5% level ($F= 301.17$, $df= 3, 12$, $p<0.000$ for 1st instar larvae; $F= 27.60$, $df= 3, 12$, $p < 0.000$ for 4th instar larvae). The study proved that

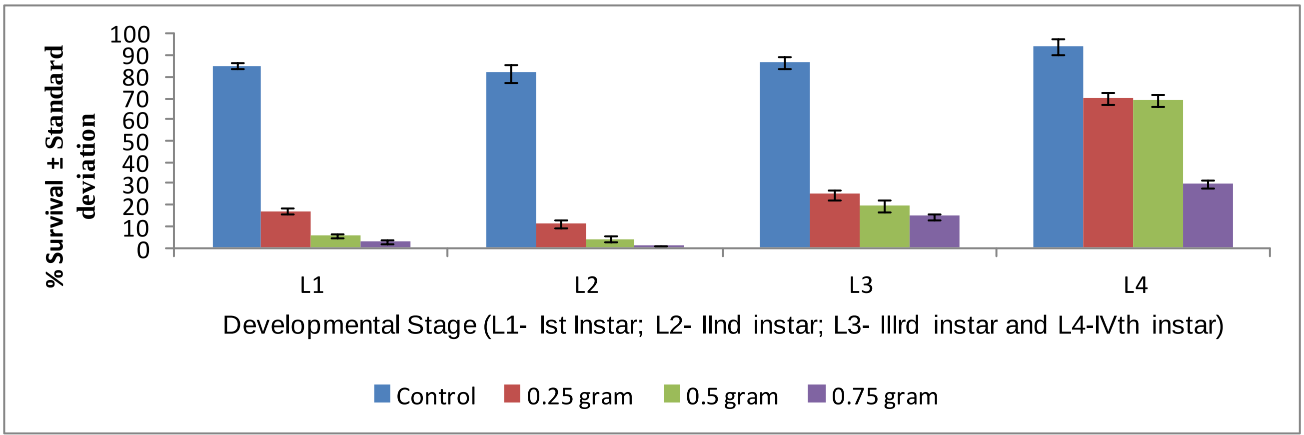


Fig. 1. Mean percentage of survival of *Anopheles gambiae* larvae exposed to inorganic fertilizers

the mixture of UREA and DAP was highly toxic to immature *An. gambiae.* The results are in agreement with those of earlier studies conducted by Muturi *et al*. (2007) and Olayeme *et al*. (2012). They have reported that the application of inorganic fertilizer had a significant impact on development and survival of aquatic stage of *Culex quinquefasciatus* and *Culex pipiens pipiens* respectively. However, mortality rate with the application of mosquito larvae exposed to inorganic fertilizer was higher in current study. The difference may be attributed to the geographical location and laboratory condition and also the species selected for investigation. The field application of nitrogenous fertilizers tends to increase *An. arabiensis* larvae productivity (Mutero *et al*., 2004). Mwangangi *et al*. (2006) also reported the presence of inorganic fertilizer in rice field that has attracted gravid *An. arabiensis* mosquitoes for oviposition. Similarly, Victor and Reuben (2000) also observed higher oviposition of *Culex tritaeniorhynchus* and *Culex vishnui* in inorganic fertilizer treated rice fields. This may be associated with the amount of inorganic fertilizers applied to the rice field and the influence of other environmental factors. Once the adult mosquitoes emerged, they may easily identify the areas with high concentration of fertilizers with their sensory structure. Later they move to areas with low concentration for oviposition.

At low concentration, nitrogenous fertilizers may not be toxic to the mosquito larvae. In addition, low concentration of inorganic fertilizer may support growth of phytoplankton and alter the physicochemical properties of rice field that may be suitable for development and survival of mosquito larvae. The availability and abundance of decomposing microorganism in the field may break down and decompose inorganic fertilizer that may enhance the productivity of mosquito larvae in the rice field. Therefore, concentration of inorganic fertilizer applied in this study proved to be toxic to immature *An. gambiae* mosquitoes. The mosquito larvae were maintained in more or less constant concentrations of inorganic fertilizer throughout study period. In addition, the rate of fertilizer decomposition and utilization in the laboratory may be much slower than that in field condition.

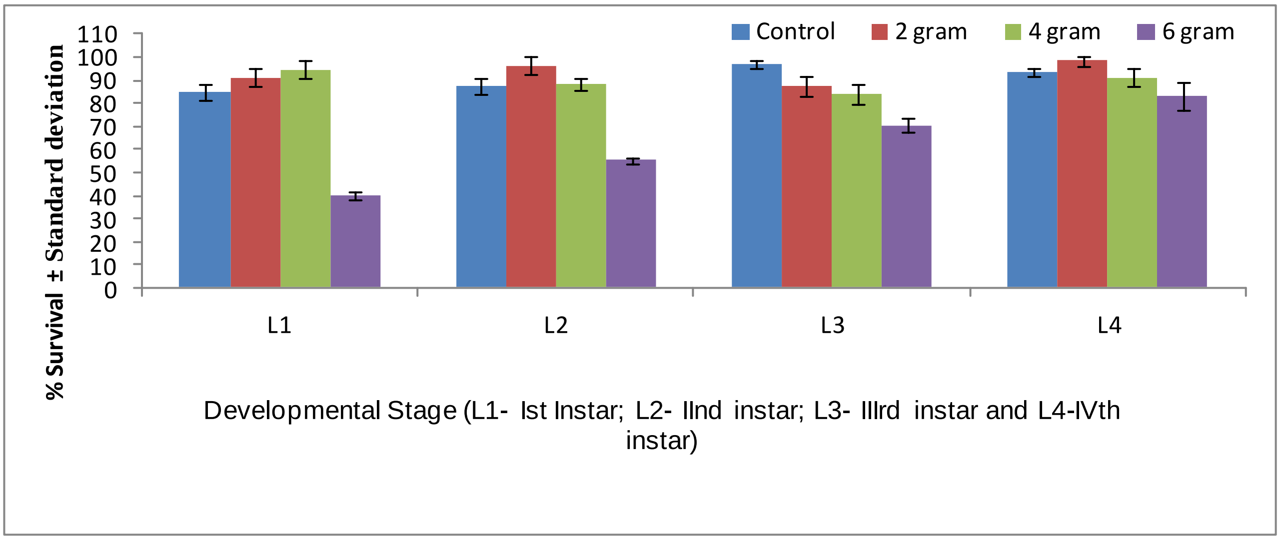


Fig. 2. Mean percentage of survival of *Anopheles gambiae* larvae exposed to organic fertilizer

The survival rate of later instar larvae was increased in all concentrations of inorganic fertilizer treatment. An opposite trend was recorded by Olayeme *et al.* (2012). This may be associated with tolerance limit of different species of immature mosquitoes.

Table 1. One way ANOVA results for the effect of inorganic, organic fertilizer and green algae on survival and development of *Anopheles gambiae* larval stage.

Response variable	Sum of square	Degrees of freedom	Mean square	F-value	P-value
Effect of inorganic fertilizer					
L1-Instar	1544.93	3, 12	1.68	301.17	<0.000
L2-Instar	1553.75	3, 12	5.05	97.88	<0.000
L3-Instar	1221.75	3, 12	5.25	73.57	<0.000
L4-Instar	802.00	3, 12	8.45	27.60	<0.000
Effect of organic fertilizer					
L1-Instar	737.00	3, 12	11.12	18.08	<0.000
L2-Instar	416.93	3, 12	8.89	11.62	<0.001
L3-Instar	263.00	3, 12	12.29	3.13	<0.066
L4-Instar	221.75	3, 12	15.29	0.83	<0.501
Effect of green algae					
L1-Instar	248.43	3, 12	18.64	0.44	<0.728
L2-Instar	257.75	3, 12	21.37	0.01	<0.996
L3-Instar	253.00	3, 12	18.04	0.67	<0.584
L4-Instar	246.93	3, 12	18.39	0.47	<0.706

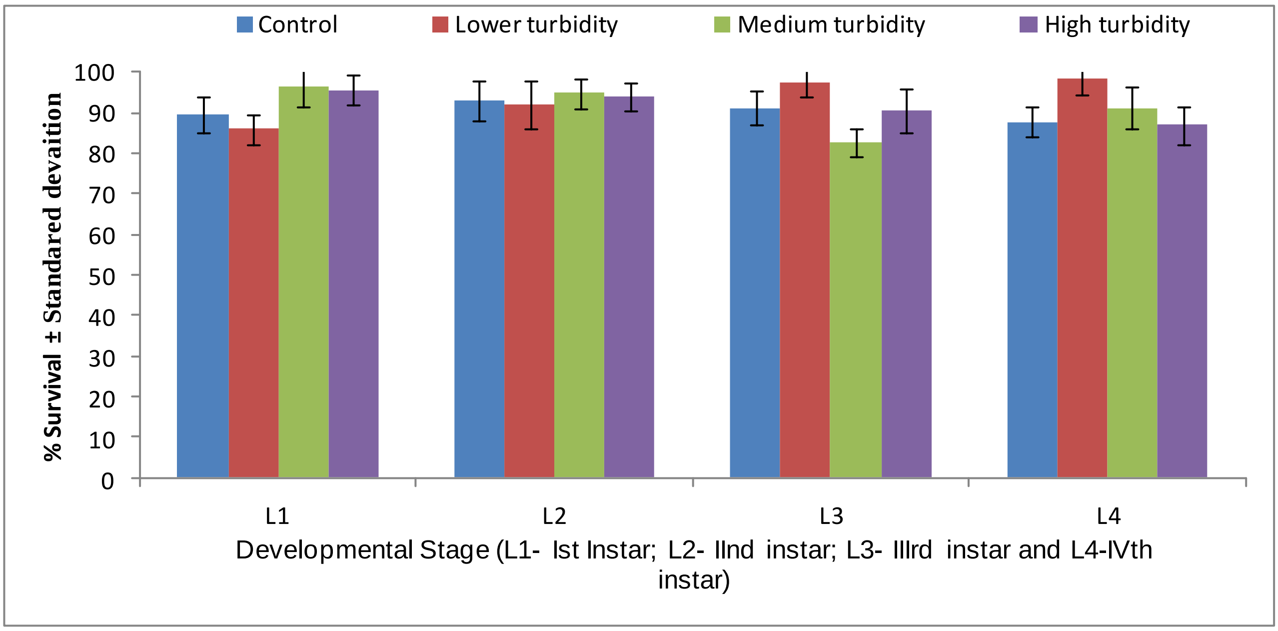


Fig. 3. Mean percentage of survival of *anopheles gambiae* larvae exposed to green algae

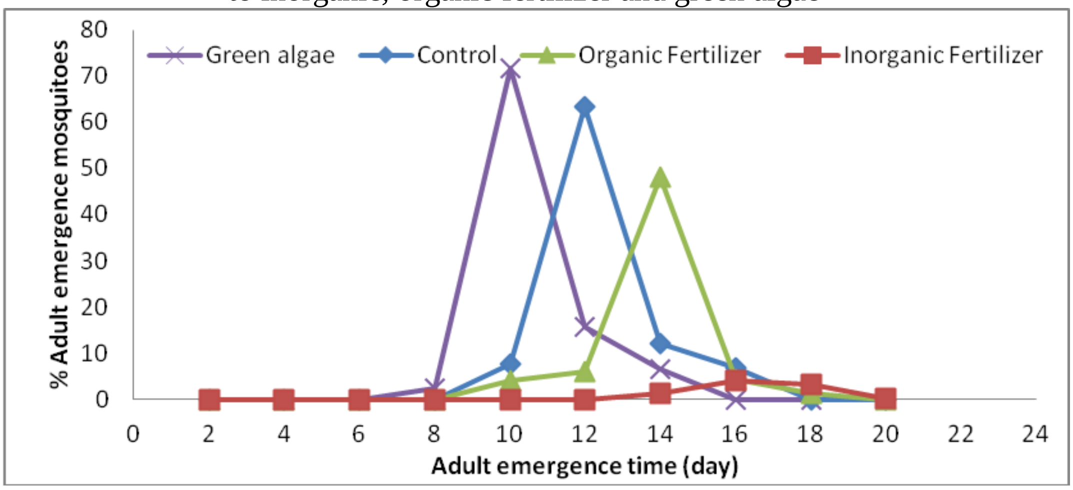
Organic fertilizer on immature *An. gambiae*
The effect of organic fertilizer on survival rate of immature *An. gambiae* revealed that the I[st] instar larvae were highly susceptible when compared to latter instars (Fig.2). Total time taken for the completion of larval stage into

adult stage was 10-16 days (Fig. 4). The mean survival rate of *An. gambiae* Ist instar larvae exposed to 6.00g/L of organic fertilizer and control were 40.17 ± 1.89 and 84.82 ± 3.30 respectively. The results of statistical analysis (Table 1) showed significant difference at 5% level (F=18.08, df= 3, 12, p<0.000). These results are in agreement with the report of Mbuya *et al.* (2014) they have reported that the pond treated with cow dung significantly reduced *Ansopheles* species population. For the IVth instar larvae exposed to organic fertilizer and control it was 83.03 ± 6.23 and 93.75 ± 1.70 respectively. The statistical analysis results did not show any significant difference at 5% level (F= 0.83, df= 3, 12, p > 0.501). This may be associated with the tolerance limit of the mature larvae. However, present findings are controversial with the report of Mbuya *et al.* (2014) they have observed significant reduction in larval population in a pond treated with cow dung. This may be associated with the cow dung condition, dose of the cow dung applied and variation in experimental setup. Present study was conducted in a laboratory with a square tray by using dried powder of cow dung. The dried powder may not cause any significant impact on mature larvae of *Anopheles*.

Spirogyra* on immature *An. gambiae

The mean number of immature *An. gambiae* surviving on green algae treatment under laboratory condition is reported in Fig 3. In general, survival and developmental growth rate for all immature stages was normal. The time taken for completion of growth rate i.e., larval stage in to adult was 8-14 days (Fig.4). The survival rate of *An. gambiae* Ist instar larvae exposed to high turbidity green algae filament and control was 95.61 ± 3.86 and 89.47 ± 4.50 and of IVth instar larvae was 86.84 ± 4.78 and 87.71 ± 3.55 respectively. The maximum survival rate of 98.24 ± 3.65 was observed in IVth instar larvae exposed to low turbidity green algae treatment. In medium turbidity green algae treatment maximum survival rate of 96.49 ± 5.06 was recorded for Ist instar larvae. The survival rate of 95.61 ± 3.86, 93.85 ± 3.30, 90.35 ± 5.43 and 86.84 ± 4.78 was recorded for I, II, III and IVth instar larvae respectively. The results of statistical analysis (Table 1) did not show any significant difference at 5% level (F= 0.44, df= 3, 12, p > 0.728 for 1st instar larvae; F= 0.47, df= 3, 12, p > 0.706 for IVth instar larvae). The lethal effect of some species of algae on *Aedes aegypti* was reported by Ahmad *et al*. (2004). These isolates were found to be resistant to digestion by mosquito larvae. Field observation by Kenea *et al*. (2011) also found negative associations of algae mat with densities of mosquito larvae. Algae may synthesize intracellular toxins, which can remain inside algal cells or extra cellular toxins, which is released into the surrounding water during active algal growth or when algal cells lyses (Ahmad *et al.*, 2004).

Fig. 4. Growth rate and time taken for *Anopheles gambiae* larvae reached to adult stage exposed to inorganic, organic fertilizer and green algae



The toxins produced by algae may be the cause for larval mortality or larvae of mosquito unable to feed, due to increased starvation mortality rate or because some species of algae being indigestible by mosquito larvae. However, in the current study there was a strong association between green algae treatment and larval development, where larvae develop at faster rate and adults emerged first in the green algae treatment than in inorganic, organic and control treatment. In the field wherever these algae were growing, *An. gambiae* larvae were found to be abundant and most of the larvae were observed inside the shade of algal mat.

In conclusion, findings of this study indicated that inorganic fertilizer significantly suppressed the development and survival of *An. gambiae* larvae in a dose dependent manner. However, the green algae and cow dung facilitate development of *An. gambiae* by supplying extra nutrient in the experimental trays. The application of inorganic fertilizer and reduction of green algae is important to suppress the mass multiplication of *A. gambiae,* thereby reducing the risk of transmission of malarial parasites.

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