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Research Article

Toxic effect of *Lantana camara* on *Aedes aegypti* larvae: A scanning electron microscopic study

Author: Dr. Anju R Unnithan[*], Dr. Geetha Unnikrishnan[**]

[*]*Department of Biotechnology, Birla College of Arts Science and Commerce, Kalyan, India.*

[**]*Department of Zoology, Birla College of Arts Science and Commerce, Kalyan, India.*

Corresponding Author: Dr. Anju R Unnithan (anjurunnithan@gmail.com)

Abstract:

Background & Objective: In the present study the morphological changes induced by *Lantana camara* petroleum ether extract on *Aedes aegypti* third instar larvae is analyzed to validate its toxic effect leading to the death of the larvae.

Method: LC_{50} for 24hr exposure time of instar larvae of *Aedes aegypti* against *Lantana camara* petroleum ether extract leaf extract was calculated. SEM analysis was performed by scanning control and extract treated larvae in SEM ' CARL ZEISS EVO 40' at 20 kV.

Results: LC_{50} value of *Lantana camara* petroleum ether leaf extract for 24hr exposure time was estimated as 48.851ppm. SEM analysis of post-treatment larvae showed distortions in the head and thorax region. Mouth region, especially the mouth brushes was disrupted and constrictions were observed on the head. Abdomen (Midgut region) was entirely malformed and the anal papillae were utterly disrupted Siphon was not affected showing normal spiracular valves with little changes on the surface of tracheal trunk and muscle fibers.

Conclusion: Present study provides a scientific rationale to highlight the importance of *Lantana camara* petroleum ether leaf extract as a source of larvicidal agents against *Aedes aegypti.*

Keywords: Aedes aegypti; LC50; SEM; Lantana camara; Dengue Fever

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ISSN: Awaited
Volume 1, Issue 1, 2017

Introduction:

Toxicological efficiency of phytochemicals reflect the potential of plants as a source of insecticidal agents and a number of plants were identified with metabolites having specific lethality on some target organisms that are unwanted burdens to the human health and society. But majority of studies were restricted to preliminary screening without assessing and validating the effects of toxicants on target species. The yellow fever mosquito, *Aedes aegypti*, is a vector for transmitting dengue fever. In the present study an attempt has been made to characterize the morphological changes induced by the *Lantana camara* petroleum ether extract on *Aedes aegypti* third instar larvae to validate its toxic effect leading to the death of the larvae.

Aedes aegypti (Linnaeus) belongs to the family Culicidae and the order Diptera, is a holometabolous insect, meaning that it goes through a complete metamorphosis with an egg, larva, pupa, and adult stage. Larval stages and the pupal stage are aquatic and their adults are aerial [2]. *Aedes aegypti* larvae have three principal body regions, the head, thorax and abdomen. The head is a sclerotised capsule (cranium) bearing the mouthparts, eyes and antennae. The head is connected to the thorax by a membranous cervix (neck). The thorax is globular and it is composed of the fused prothorax, mesothorax and metathorax. In meso and meta thoracic region a pair of thorn like large processes are visible dorsally. Lateral hairs arise from the lateral sides of the pro, meso and meta thoracic segments. The abdomen tapers posteriorly and is 8 segmented, long, cylindrical and dorsoventrally flat. Tufts of lateral hairs arise from the abdominal segments and the number of hairs in each segment varies.

Abdomen bears a respiratory siphon and at the tip of the siphon is the respiratory opening - the spiracle, surrounded by perispiracular lobes [3] .Inside the siphon tube tracheal trunk and strands of muscle fibers are visible. The respiratory siphon bears a row of pecten teeth on both sides laterally and has basal denticles. At the terminal portion of larval abdomen is the anal segment. It has ventral brush with 5 pairs of setae of variable size and four anal papillae which are of equal size. Anal papillae are transparent and boat shaped. Anal segment has incomplete saddle. The identification parameters of *Aedes ae*gypti are smooth antennae, antenna1 hair simple; comb teeth 8-12 in a single row with strong basal lateral denticles; pecten teeth

ISSN: Awaited
Volume 1, Issue 1, 2017

on the siphon 15-20, none widely spaced; pleural hairs of the meso- and metathorax with conspicuous thorn-like processes [4].

Materials & methods

Plant Material Preparation

Leaves of *Lantana camara* were collected from local regions of Kalyan, Mumbai and get authenticated at Blatter's herbarium; St. Xavier's College, Mumbai. The leaves were washed and shade dried and pulverized to a coarse powder in a mechanical grinder and passed through a sieve. The powdered materials were stored in airtight, dark, glass container to prevent photochemical reactions.

Preparation of Leaf Extracts

Plant powder was suspended in petroleum ether, the mixture was kept for 30 min in sonicator and then in rotary shaker for 24 hr. The mixture was filtered using a Whatmann filter paper no. 1, evaporated and kept in cool temperature.

Raring of *Aedes Aegypti* Larvae

The eggs of *A. aegypti* were procured from Haffkine Institute at Mumbai, India and authenticated at Department of Entomology, NIV, Pune. The egg rafts of *Aedes aegypti* were kept in the tray containing tap water at laboratory condition. After incubation, the eggs were observed to hatch out colonized and they were maintained continuously in the laboratory with controlled conditions of 28^0C - 30^0C temperature and 70-80% relative humidity. Larvae were fed on finely ground dog biscuit. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages for adult emergence. The adults of *Aedes aegypti* were reared in the glass cage provided with 10% sucrose solution in cotton pads and it was periodically blood-fed on restrained rats.

Preparation of stock and test concentrations

The plant extract was dissolved in DMSO to prepare the stock solution. These stocks were further diluted by adding water to the working concentration.

ISSN: Awaited
Volume 1, Issue 1, 2017

Lethal Concentration

The LC_{50} of the plant extract was determined according to WHO protocol with modifications [5]. The 3rd instar larvae of *Aedes aegypti* was treated with plant extracts of 25ppm, 50ppm, 100ppm, 200ppm, 300ppm concentrations. Based on the percent mortality values, LC_{50} of leaf extracts for an exposure time of 24 hrs were obtained separately by calculating the regression line employing Probit analysis [6] using SPSS software [7].

Morphological characterization of larvicidal effect

Treatment of larvae

Five larvae of the early 3rd instars were placed in bumper tubes containing test solution of 100ppm concentration. A corresponding control was maintained with solvent (DMSO). After the exposure of 6 hrs, the larvae were removed to monitor the effects of the extract.

Primary fixation of larvae

Primary fixation was done in 2% paraformaldehyde made in 0.1 M sodium phosphate buffer (pH 7.2) for both control and treated larvae.

SEM analysis

Primary fixed specimens were washed twice with phosphate buffer, distilled water and in graded acetone series finally in dry acetone. Samples were dried in 'HMDS' and dried samples were mounted on SEM stub with Carbon tape and then coated with thin layer of gold in a sputter coater Model: Polaron SC 7640 (UK). The preparations were scanned in SEM ' CARL ZEISS EVO 40' at 20 kV.

Results

Larvicidal screening of Extracts

Petroleum ether leaf extract of *Lantana camara* showed least LC_{50} and LC_{90} values of 48.851 and 83.392 at 95% confidence interval confirming the presence of bioactive compounds in the extract inducing highest larval mortality in a short period of time.

Morphological characterization of larvicidal effect: SEM analysis

The normal morphology for the whole body of the control third instar *Aedes aegypti* larvae showed the common appearance of the typical structure with well-developed distinguished head, thorax, and abdomen region(Figure 1 A). Although SEM analysis showed

ISSN: Awaited
Volume 1, Issue 1, 2017

significant shrinkage in the overall morphology after treatment with *Lantana camara* petroleum ether extract (Figure 1 B). Post-treatment larvae showed distortions in the mouth region, especially with mouth brushes (Figure 2B) and constriction on the back portion of the head (Figure 3B). Significant changes were not observed in the antenna region. Thorax region of control larvae shows the thorn like structures, the identification characteristic of *Aedes aegypti*, but in the treated larvae thorax region was completely deformed (Figure 4B) with numerous wrinkles. Midgut was entirely malformed and the anal papillae were utterly disrupted (Figure 5, 6, 7, 8). Prominent changes were not observed in the siphon with normal spiracular valves in the post treatment larvae (Figure 9). Surface of tracheal trunk and muscle fibers was also normal.

Discussion

Investigations on the modes of action and the resistance mechanisms of plant-based biocides are of practical importance because they may provide useful information on the most appropriate formulations to be adapted for future commercialization and future resistance management. Also, they may contribute to the development of selective mosquito control alternatives with novel target sites and low toxicity. Several studies have demonstrated plant extracts as a source of insecticides and for several plants the plant metabolites with toxic activity have been isolated. Although to the present, limited knowledge is explored about the mode action of plant extracts, it is believed that it involves different targets and mechanisms in the different organisms due to the fact that they contain great variety of components.

Petroleum ether leaf extract of *Lantana camara* showed least LC_{50} value of 48.851 at 95% confidence interval confirming the presence of bioactive compounds in the extract. This data shows the effectiveness of extract even in low concentration, as an evidence for considering it as a source of toxic metabolites to formulate a phytolarvicide. But indepth analysis of the affected target is needed as supporting evidence to validate the effectiveness of the extract. This paper describes for the first time the morphological changes induced by the toxic effect of *Lantana camara* petroleum ether extract on treated larvae. *Lantana camara* petroleum ether extract has significant toxic effect to change the intact morphology of *Aedes*

ISSN: Awaited
Volume 1, Issue 1, 2017

aegypti larvae leading to the death of the larvae. Severe damage was observed in the midgut region. Since midgut portion is known to have functions such as ionic and osmotic regulation [8], lipid and carbohydrate storage [9], [10] and the secretion of digestive enzymes and absorption of nutrients [11], [12] the principal mode of action may be by inducing disruption in any of these activities. The anal gills were also completely destroyed. These results are supported by earlier studies regarding the developmental deformities induced by *A. annua* extract in *Anopheles stephensi* and the growth inhibitory effect of the extract on the developmental profile of *Culex. Quinquefasciatus* [13] where body wall and larval tissues were found ruptured and degenerated in both the larval species. In most cases the mode of action is the obstruction of air passage through the two tracheal trunks of the larvae. In mosquito larvae all air enters the insect through two spiracles at the tip of the siphon. These spiracles are surrounded by perispiracular valves regulated by muscles. But the siphon was normal in the treated larvae eliminating the chance of obstruction of respiration as a reason leading to the death of organism.

Conclusion

The present study provides a scientific rationale to highlight the importance of Lantana camara petroleum ether extract as a source of larvicidal agents against Aedes aegypti which could possibly be exploited for the isolation and identification of active principles present in the extract to formulate a phytolarvicide.

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Volume 1, Issue 1, 2017

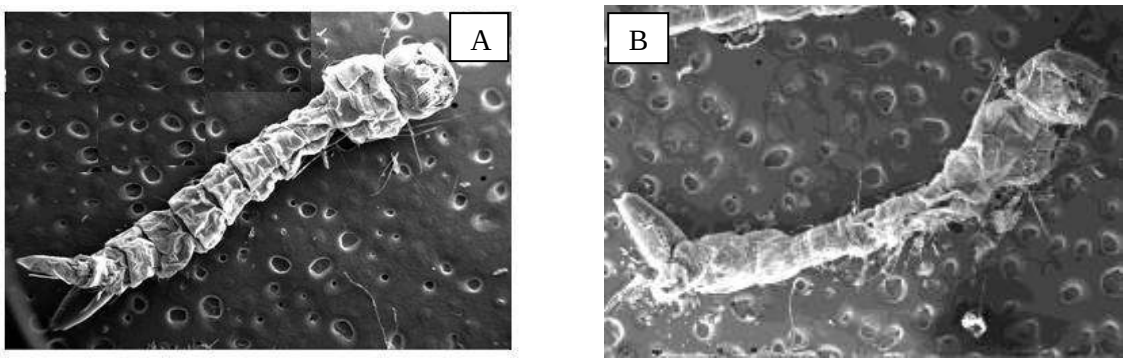

Figure 1: SEM analysis of *Aedes aegypti* larvae A. control B. treated

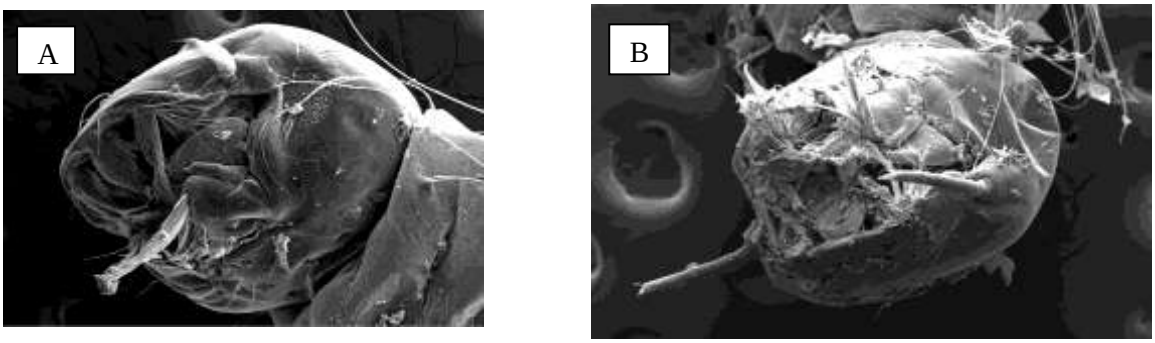

Figure 2: SEM analysis showing distortions in the mouth region of *Aedes aegypti* larvae A. control B. treated

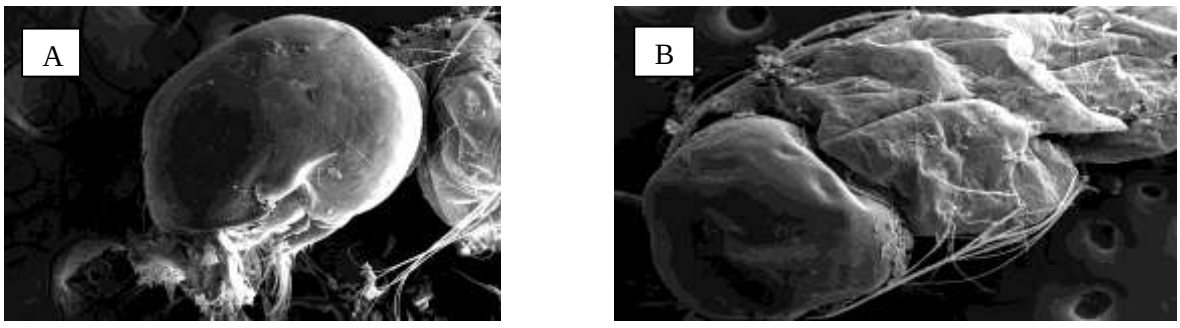

Figure 3: SEM analysis showing constriction on the back portion of the head region of *Aedes aegypti* larvae A. control B. treated

ISSN: Awaited
Volume 1, Issue 1, 2017

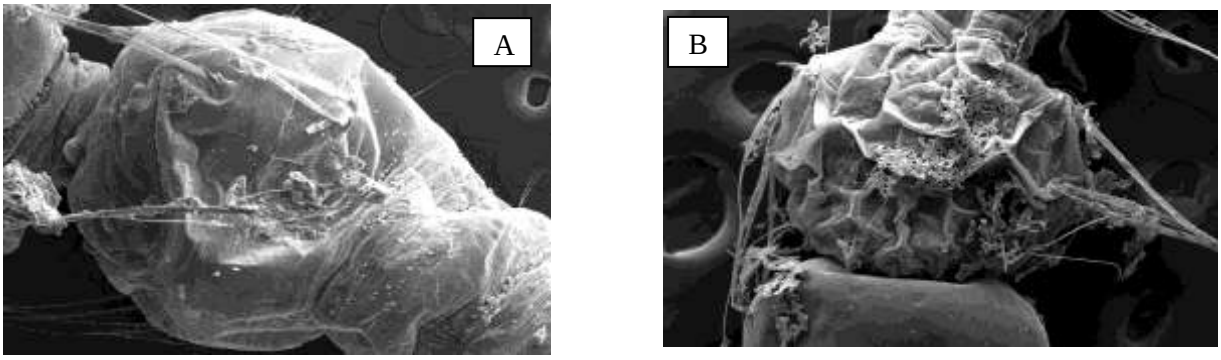

Figure 4: SEM analysis showing deformed thorax region of *Aedes aegypti* larvae A. control B. treated

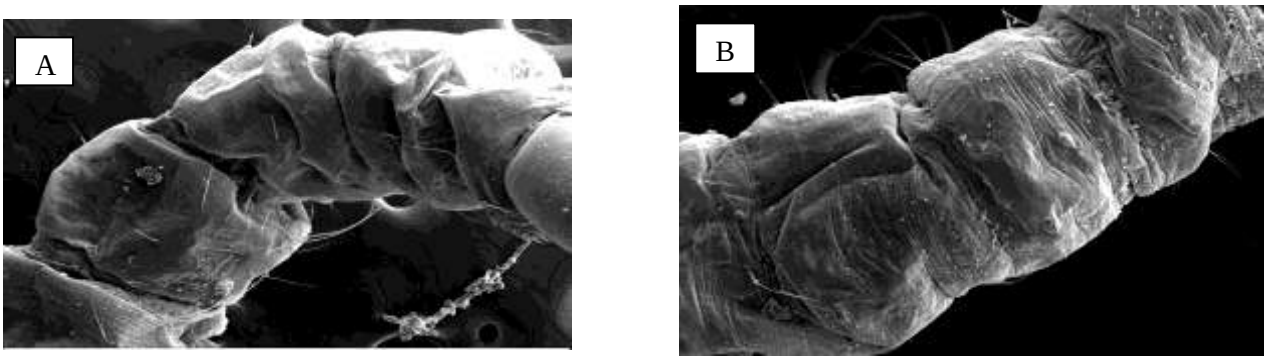

Figure 5: SEM analysis showing normal Abdomen of Aedes aegypti larvae A & B control

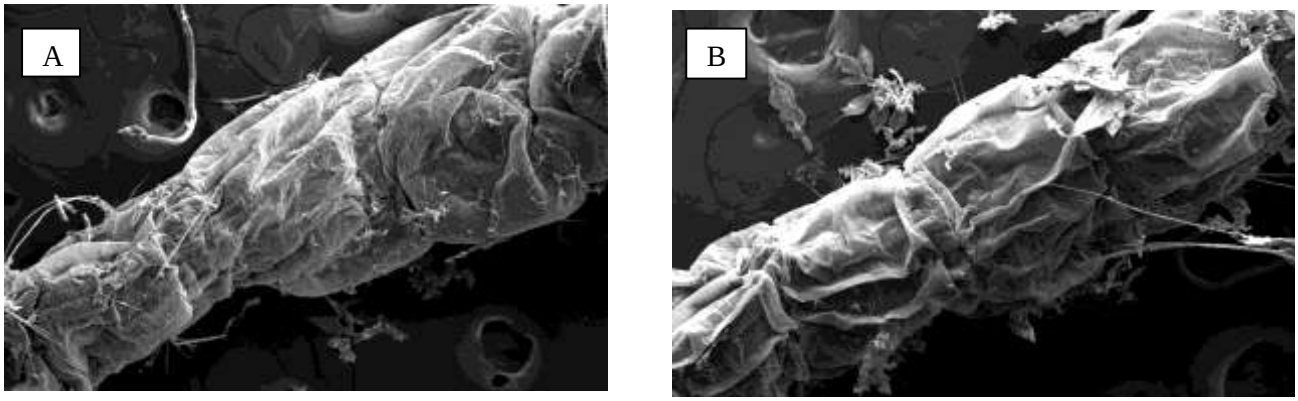

Figure 6: SEM analysis malformed Abdomen of *Aedes aegypti* larvae A & B treated

Sci Journo
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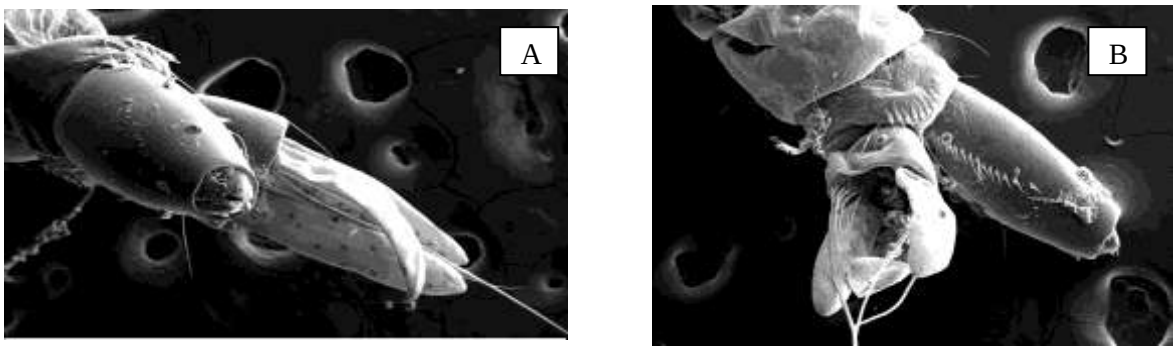

Figure 7: SEM analysis showing normal anal papillae of Aedes aegypti larvae A & B control

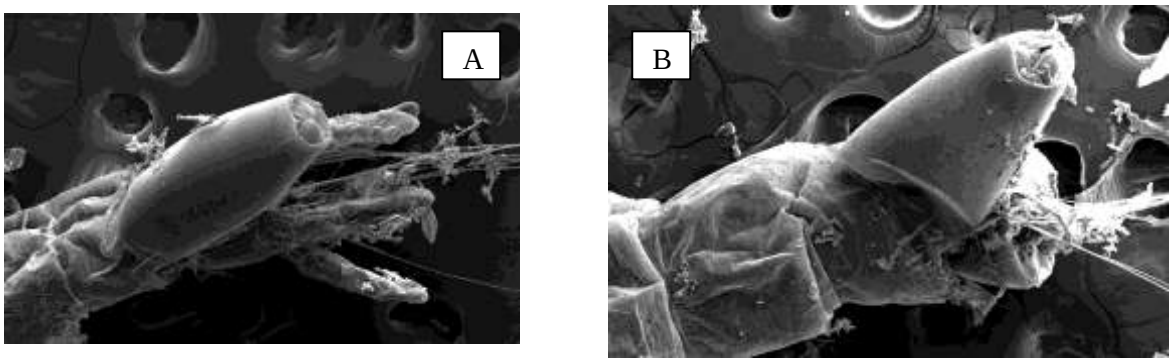

Figure 8: SEM analysis showing deformed anal papillae of Aedes aegypti larvae A & B treated

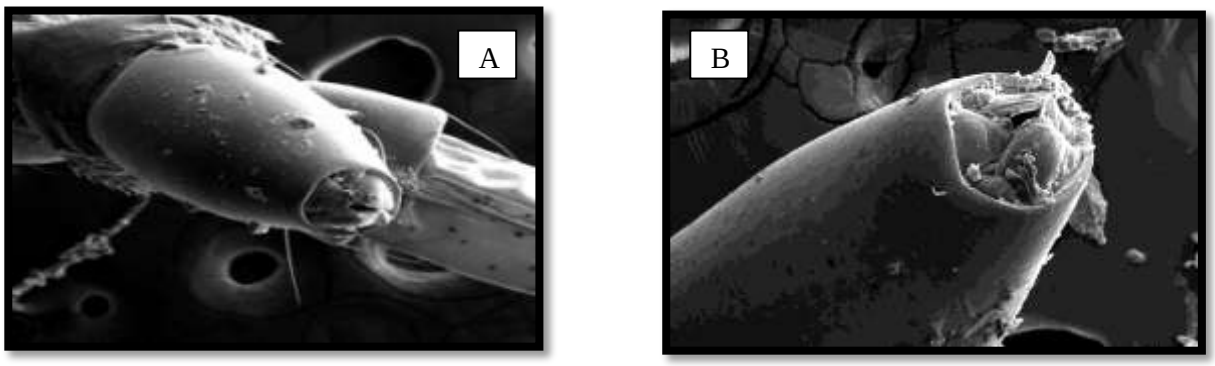

Figure 9: SEM analysis showing siphon with normal spiracular valves of Aedes aegypti larvae A. control B. treated

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ISSN: Awaited
Volume 1, Issue 1, 2017

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