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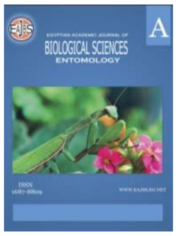

Influence of the aquatic plant, *Lemna minor* on the development and survival of *Culex pipiens* mosquito immature.

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ABSTRACT

Many environmental factors, biotic and abiotic interact to influence organismal development. Aquatic plants are considered as important environmental factors. Duckweed considered plants and grasses aquatic that most widely used water in the world for its ability to grow and reproduce greenery in aquatic habitats and various breeding sites of mosquitoes. Effect of the aquatic *Lemna minor* on the development and survival of *Culex pipiens* mosquito immature was investigated under laboratory conditions. Duckweed plants were used as low (15 plants/cm^2), medium (25 plants/cm^2) and high (35 plants/cm^2) density on the surface of the water. The results showed the presence of aquatic plants duckweeds was affected larval development and survival of *Culex pipiens*, where they reach 11.4, 12 and 19 days of larval duration at non-plant, low and high plant density, respectively. Survival of larval and larval-adult stages of mosquito shortened as the plant density interval increased (%: 47.16±2.84 and 32.93±0.87) compared to the absence of plant (94.83±0.83 and 95.87±1.35). Analyses data indicated a high density mats of duckweed plant was marked inhibitory effect on mosquito breeding. The attractiveness of oviposition site for vector mosquitoes is dependent upon a number of physical and chemical factors. Cage tests in the laboratory indicated that the plant could be a suitable breeding place for gravid females especially at polluted sites (F= 19.705, P= 0.0001). The suggestion is made that the aquatic plant might be considered as a natural vector control measure in certain types of breeding places.

INTRODUCTION

Methods of mosquito control are diverse and include chemical, biological, microbial, genetic, and physical control. Physical control "nature control" is considered an eco-friendly method. Currently, the new trend of mosquito control researches uses alternative methods to fight mosquitoes away from pesticides for environmental safety (Ibrahim, 1993; Kittayapong *et al.,* 2012).

Mosquitoes are usually preferred the stagnant water with aquatic plants to lay their eggs. Although aquatic plants provide facilities for mosquitoes; nutrition, sheltering, oxygen and reproduction habitat they can be used as physical barriers against mosquito development (Angerilli, 1978; WHO 2013).

Aquatic plants can be influenced the behavior and survival of mosquito adult and immature stages (Angerilli and Beirne, 1974; Rowbottom *et al.,* 2017). It may reduce the wave action of the water and thus provide a suitable substrate for oviposition and may also restrict oviposition by rendering the water surface inaccessible to ovipositing female. However, aquatic plants may conceal and protect mosquito stages from predators but they also may provide both an oviposition site for predators. Also, aquatic plants can influence mosquito stages through chemical compounds; toxic metabolism that inhabit the development of mosquito larvae (Angerilli and Beirne, 1974).

The influence of aquatic plants on mosquito breeding and development has been observed and studied for many years (Hall 1972; Hobbs and Molina, 1983; Dua *et al.,* 1988; Eid *et al.,* 1992; Mwangangia 2007; Chirebvu and Chimbari, 2015). Aquatic plants are growing partially or completely in water and they may be classified into four groups: emergent, floating-leaved, submersed, and free floating. Duckweeds (F: Lemnaceae*)* are small free-floating aquatic angiosperm plants which do not have distinct stems and leaves. Duckweed family comprises four genera, *Lemna, Spirodela, Wolffia*, and *Wolfiella*, and of 34 species (Iqbal 1999; Gupta and Prakash, 2013).

Duckweeds may form such dense mats on the water surface that act as mechanical barriers to mosquito breeding (Hobbs and Molina, 1983). Ibrahim (1993) stated that mosquito larvae were rarely found in otherwise suitable breeding places when the water surface is densely covered with the duckweed, *Lemna* species. The present work aims to assess the influence of the aquatic plant, *Lemna minor* on the development and survival of mosquito immature in the laboratory.

MATERIALS AND METHODS

Plant materials:

Duckweeds, *Lemna minor* were collected from a pool which was located in many village areas, Qalyubiya Governorate. The pool was 6 m long, 0.45 m deep, 21 m^2 water surfaces and contained an estimated 9.45 m^3 of water (Fig. 1a). This site has stagnant water, standing plants floating *Lemna* and partially protected from sun-light. The pool is depressed ground filled with water from flooding irrigated channel or adjacent agriculture lands. The mean values of water temperature, pH, ammonia, nitrate, salinity and dissolved oxygen were 23.8°C, 7.8 pH, 0.88 mg/l, 42.2 mg/l, 1.67 ppt and 6.8 mg/l, respectively. Also, duckweeds were prevalence in ditches and canals as well as drainages (Fig. 1b).

Mosquito culture:

Culex pipiens larvae were obtained from Medical and Molecular Entomology Section, Entomology Department, Faculty of Science, Benha University. They were maintained at 27 ± 2°C and 72-83% RH under a photoperiod of 14:10 h (light/dark) in the insectary. Larvae were fed on fish food (Tetramin®) with grinded bread in the ratio of 3:1. The food was sprinkled once daily in enamel pans and water was changed every day to avoid scum formation which might create toxicity. Pupae were transferred from the enamel pans to a cup containing de-chlorinated tap water and placed in screened cages (35 × 35 × 40 cm dimension) where the adults emerged. The adult colony was provided with 10% sucrose solution and was periodically blood-fed on pigeons. After three days, engorged female mosquitoes oviposited egg rafts on small cups that containing tap water in cages and the eggs were collected and transferred to clean enamel pans. Two developmental stages, larvae and adults were continuously available for the experiments and were maintained at the same laboratory conditions (Baz, 2013).

Laboratory assay:

Duckweeds were collected using stander dipper as in mosquito collection (Abd Elwahab *et al.,* 2011). It was transported green from the field to the laboratory in plastic sacs which are half-filled with water from the breeding sites with plant materials (Fig. 1c). Duckweed was unloaded in enamel plate under laboratory conditions (27±2 ºC, 78±5% RH, and 14L: 10D photoperiod). Each enamel plate was provided with de-chlorinated tap water daily.

- Effect of duckweeds and water quality on mosquito oviposition.

Eighteen plastic trays (10 × 13 × 7 cm) were divided into two groups, each group containing nine plastic trays with different level from duckweed density (low: 15 plants/cm^2, medium: 25 plants/cm^2 high: 35 plants/cm^2) according to Ibrahim (1993) with 500 ml of de-chlorinated tap water and the other group have the same density levels with polluted water from pool (Fig. 1d). After blood-meal, 25 gravid females were introduced in each cage to oviposition for one week. Another three plastic trays were left untreated with duckweed as the control. Three replicates were performed with untreated control groups.

- Effect of duckweeds on mosquito hatchability.

Twelve plastic trays (10 × 13 × 7 cm) were divided into four groups, one has low duckweed (15 plants/cm^2), second has medium duckweed (25 plants/cm^2), third has high duckweed (35 plants/cm^2) and the last has not duckweed. These plastic trays left untreated with duckweed as control with 500 ml of de-chlorinated tap water only. Four new egg rafts oviposited were introduced in duckweed containers for each cage to observation the hatching rate (Fig. 1e). The experiment was replicated three times with untreated control groups**.**

- Effect of duckweeds on mosquito survival and development

Eighteen plastic trays (10 × 13 × 7 cm) were divided into two groups, the first group containing polluted water and the other with de-chlorinated water. The two groups were treated with duckweed (low: 15 plants/cm^2, medium: 25 plants/cm^2 high: 35 plants/cm^2). 25 mosquito larvae 1st instar were added to each cup (Fig. 1f). Three plastic trays were left without duckweed as the control. Three replicates were performed to each group. The larval counts were determined daily until pupation and adult emergence. The survival rate, developmental periods, pupation rates and adult emergences were determined for each group. The experiment was replicated three times with untreated control groups.

Statistical analysis

The statistical analysis was carried out using ANOVA with **a** significance level of 0.05 for the whole results using SPSS (ver. 22). Data were treated as complete randomization design according to Steel *et al,.* 1997. Multiple comparisons were carried out applying LSD.

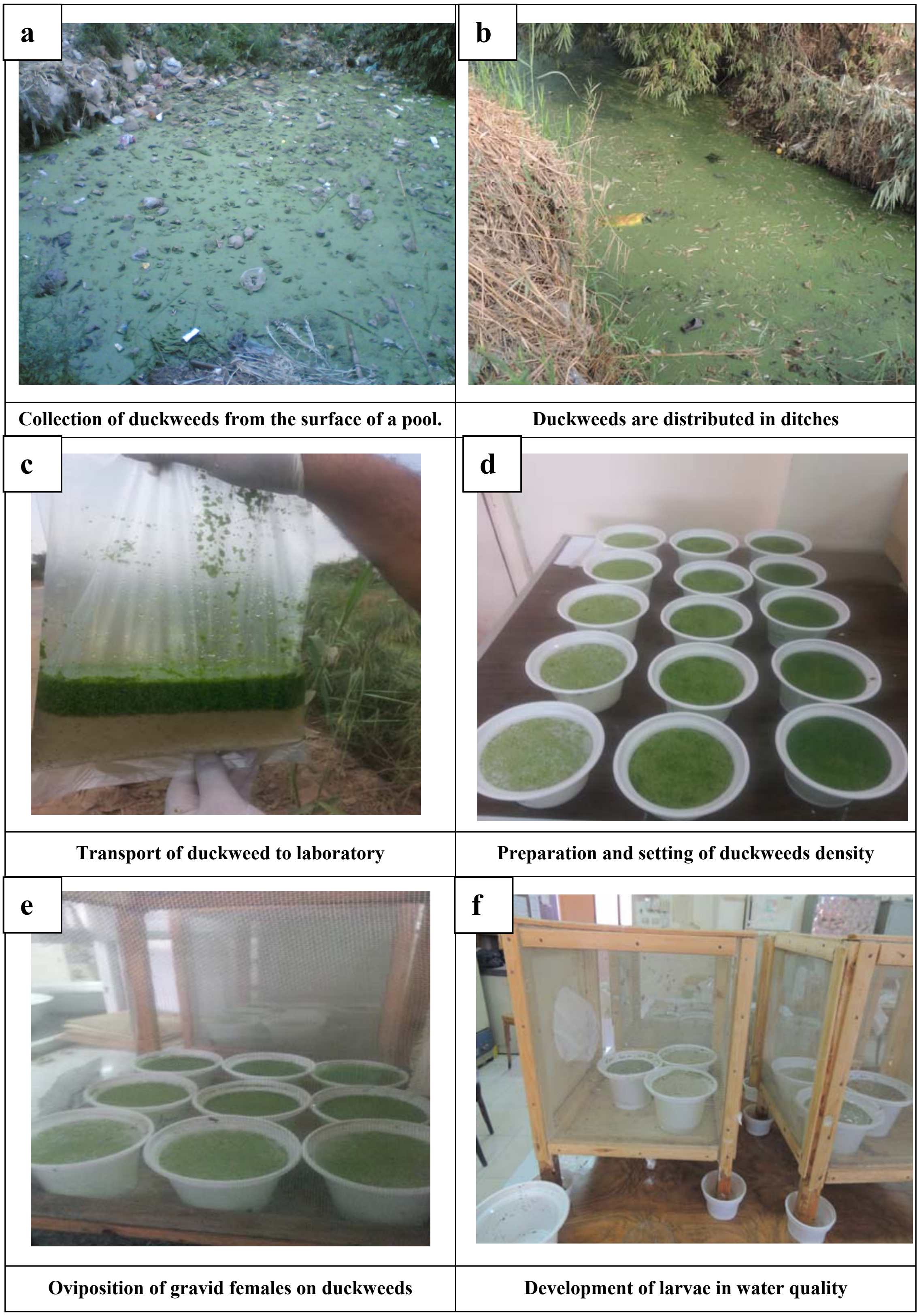

Fig. 1: Collection of duckweed from natural mosquito breeding sites **with experimental design.**

RESULTS

Effect of duckweeds and water quality on mosquito oviposition

Ovipositing *Culex pipiens* females have a great ability to discriminate the suitable sites to oviposit. Data are given in table (1) and illustrated in figure (2) indicate that, the highest number egg rafts oviposited was observed in high plant cornering container ($p < 0.05$ was significantly higher) either in polluted or unpolluted water with high *Lemna* plant density (11.00±1.53 and 7.33±0.33), respectively. On the other hand, the lowest number of egg rafts oviposited was recorded in low density of *Lemna* plant either in unpolluted or polluted containers (4.00±0.58 and 4.67±0.33), respectively. Also, the results showed that female mosquito was more attractant to polluted water container with highly oviposited than fresh water container (Table 1). The results showed that the cage oviposition tests in the laboratory with *Lemna* plant may provide a good oviposition substrate for *Culex pipiens*, where a positive correlation was found (F= 19.705, P= 0.0001) between the number of egg rafts deposited and the density of plant cover.

Table (1): Effects of duckweed plant and water quality on mosquito female oviposition under laboratory conditions.

Plant density	No. of egg rafts deposited			Mean	F	*P* value
	Fresh water	Polluted water	Polluted and Fresh water			
Control	4.67±0.67b^{aB}	5.67±0.88^{cA}	4.00±0.58dB	4.78±0.43c	19.705	0.0001
Low	4.00±0.58bB	4.67±0.33cB	6.33±1.20cA	5.00±0.53c		
Medium	6.00±1.00aB	8.33±0.33bA	7.00±0.58bB	7.11±0.48b		
High	7.33±0.33^{aB}	11.00±1.53^{aA}	10.00±1.15aA	9.44±0.78a		

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter for plant density.

A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter for water type.

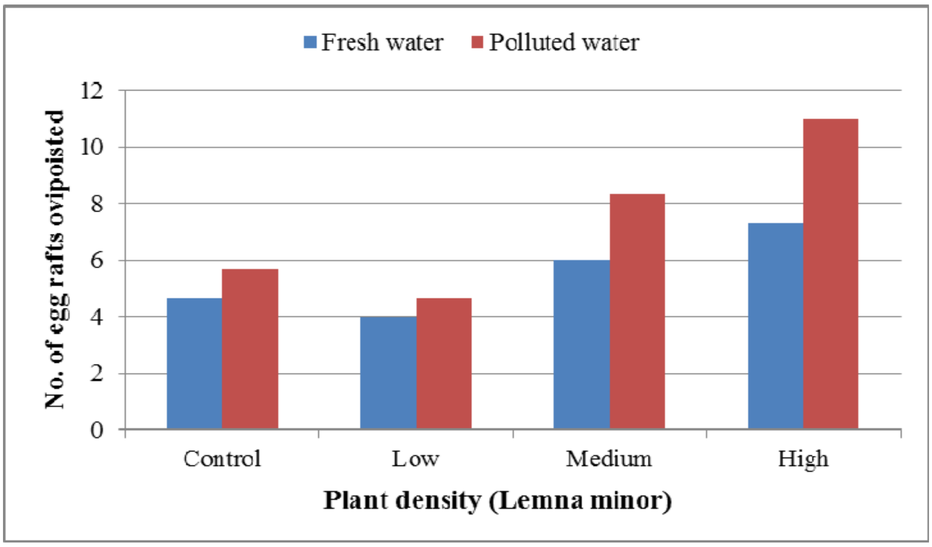

Fig. 2: Effects of duckweed plant and water quality on mosquito *Culex pipiens* female oviposition.

Effect of duckweeds on egg hatchability.

Egg rafts of *Cx. pipiens* were placed on *Lemna* plant covering water surface to determine the hatching rate (Table 2). Data presented in table 2 showed that hatchability of *Cx. pipiens* eggs were affected by the presence of duckweed plants *L. minor*, where the hatching rate was 7% and 15% at polluted and fresh high plant covering, respectively. Results in the same table showed a negative correlation (P = 0.378) between the number of larvae produced and the density of plant cover. The greatest number of larvae produced was observed in low plant density as shown in the same table, where the mean number of larvae found in the water was 746, 604 and 540, 428 larvae in polluted and fresh water containers respectively.

Table (2): Number of *Culex pipiens* larvae obtained from egg rafts at different density of *Lemna minor* under laboratory conditions.

Plant density	No. of eggs		No. of larvae		Larval mortality%		Hatching rate %	
	Fresh	Polluted	Fresh	Polluted	Fresh	Polluted	Fresh	Polluted
Control	572^{bcB}	760^{aA}	540^{aA}	746^{aA}	6^{cA}	2^{cA}	94^{aA}	98^{aA}
Low	496^{cB}	679^{bA}	428^{aA}	604^{abA}	14^{cA}	11^{cA}	86^{aA}	89^{aA}
Medium	720^{aB}	810^{aA}	460^{aA}	406^{bA}	36^{bA}	50^{bA}	64^{bA}	50^{bB}
High	615^{abB}	732^{abA}	91^{bA}	49^{cA}	85^{aA}	93^{aA}	15^{cA}	7^{cA}
Mean	601^{B}	745^{A}	380^{A}	451^{A}	35^{A}	39^{A}	65^{A}	61^{A}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table (3): Mean duration (days) and survival (%) (± S.D) of development phases of immature stages of *Culex pipiens* mosquito at different plant density.

Plant density	Mosquito larval instars							
	1st instar		2nd instar		3rd instar		4th instar	
	Duration	Survival	Duration	Survival	Duration	Survival	Duration	Survival
Control	2.33 ± 0.33^{bB}	96.67 ± 0.67^{aA}	2.67 ± 0.33^{cB}	91.33 ± 6.77^{aC}	2.67 ± 0.33^{cB}	96.67 ± 0.67^{aA}	3.67 ± 0.33^{bA}	94.67 ± 1.33^{aB}
Low	2.33 ± 0.33^{bC}	92.0 ± 4.62^{bAB}	3.00 ± 0.58^{cB}	93.3 ± 3.53^{aAB}	3.00 ± 0.58^{cB}	94.67 ± 4.81^{aA}	3.67 ± 0.33^{bA}	89.33 ± 2.91^{bB}
Medium	3.33 ± 0.33^{aC}	78.67 ± 1.33^{cA}	3.67 ± 0.33^{bC}	73.33 ± 2.67^{bB}	4.67 ± 0.33^{bB}	58.67 ± 8.97^{bC}	5.00 ± 0.58^{aA}	44.67 ± 2.91^{cD}
High	3.67 ± 0.33^{aC}	58.00 ± 2.31^{dA}	4.33 ± 0.33^{aB}	54.67 ± 2.91^{cA}	5.67 ± 0.33^{aA}	34.00 ± 2.00^{cB}	5.33 ± 0.67^{aA}	12.00 ± 4.16^{dC}

Conc.	Larvae (1-4 instars)		Pupae		Larval -Adult	
	Duration	Survival	Duration	Survival	Duration	Survival
Control	11.34 ± 1.32^{bB}	94.83 ± 0.83^{aA}	2.67 ± 0.33^{cB}	100 ± 0.00^{aA}	14.00 ± 0.00^{c}	95.87 ± 1.35^{a}
Low	12.0 ± 1.82^{bC}	92.32 ± 3.96^{aA}	3.00 ± 0.00^{cB}	96.00 ± 2.31^{aA}	15.00 ± 1.00^{c}	93.07 ± 1.73^{a}
Medium	16.67 ± 1.57^{aC}	63.83 ± 3.97^{aA}	3.67 ± 0.33^{bC}	21.33 ± 2.40^{bE}	20.33 ± 0.67^{b}	55.33 ± 2.24^{b}
High	19.0 ± 1.66^{aC}	47.16 ± 2.84^{aA}	5.33 ± 0.88^{aA}	6.00 ± 1.15^{cD}	24.33 ± 1.20^{a}	32.93 ± 0.87^{c}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

The presence of *Lemna* plant affected larval development and survival. The results showed that development time was faster in containers covered with low plant density and non-plant control containers than in high plant density covering containers (Table 3), where the duration of larval reached 11.34, 12 and 19 days at control, low and high plant density respectively. The duration of the pupal stage reached 2.67, 3 and 5.33 days at control, low and high plant density, respectively. Survival of larval and larval-adult stages of mosquito shortened as the plant density interval increased (%: 47.16±2.84^{aA} and 32.93±0.87^{c}) and it was lengthened at low plant density (%: 92.32±3.96^{aA} and 93.07±1.73^{a}, respectively). Also, the pupal period was influenced by the plant density interval in the same table. The results also indicated that dense plant mates completely inhibited larval breeding apparently.

DISCUSSION

Aquatic plant duckweed (*Lemna* species) and similar floating plants (*Wolffia*, *Azolla*, *Eichhornia* and filamentous algae) may form dense mats on the water surface that act as mechanical barriers to mosquito breeding, although *Anopheles* and *Culex* larvae may be found in abundance when the growth is scattered (Angerilli, 1978).

The presence of high plant density in containers showed that *Lemna* plant may provide a good oviposition substrate for *Culex pipiens*. Based on results a positive correlation was found between the number of egg rafts deposited and the density of plant cover. Where, the highest number of egg rafts oviposited was observed in high densities plant cornering containers either in polluted or unpolluted water. This finding was agreed with Ibrahim (1993) demonstrated that scattered mates of plant *Lemna* were attracted more females to oviposit in containers with *Lemna*.

Initially, the female mosquito must find an aquatic site to lay her eggs and it appears that gravid female mosquito uses visual and olfactory cues to locate and select suitable or potential sites. Also, mosquitoes may be selected oviposition sites based on the availability of larval food (Bentley and Day, 1989).

Gravid females keep track of her visual or olfactory cues to appropriate water breeding collections and are guided by chemical cues and physical factors in the water and the quality of water before deciding to lay their eggs (Bonnets and Chapman, 1956; Muir,, 1988). It is possible that site selection behavior is influenced by aquatic vegetation present in the site. Once the gravid female choice of appropriate oviposition sites, eggs were laid and therefore it has a significant impact on the fitness of progeny. The survival of the species/larvae is dependent on choosing the right one (Millar *et al.,* 1994; Spencer *et al.,* 2002; Overgaard, 2007; Himeidan *et al.,* 2013)

The ability of the female mosquito to discriminate the suitable sites was affected by many environmental factors such as light, moisture, the color of water contamination of water and aquatic plants (Russell and Rao, 1942; Focks *et al.,* 1983; Sayed, 1989; Orr and Resh, 1989; Clements 1999).

A negative correlation was found between the number of larvae survival and the density of plant cover. Where, the little number of larvae obtained in the presence of plant cover may be due to dryness of some eggs separated from water surface by the plant and/or the plant may mechanically obstruct hatching of larvae. Some larvae may be died due to other mortality factors.

Many investigators believed that emergent vegetation as well a floating free/floating wood plants or any matter placed in a manner similar to emergent vegetation may mechanical obstruct egg laying by Anopheline mosquitoes (Angerilli and Beirne, 1980; Jiannino and Walton, 2004). This may be due to the difference in oviposition behavior between anopheline and culicine mosquitoes or due to other factors. Unlike other mosquitoes, the larvae and pupae of *Mansonia* have attached their breathing tubes to floating aquatic plants for development and survive (Rajendran *et al.,* 1989). Removal of such plants via mechanical, biological, or chemical control would therefore effectively prevent the development of *Mansonia* mosquitoes (Chandra *et al.,* 2006).

The presence of *Lemna* plant also affected larval development and survival. Development was faster in containers covered with low plant density than in plantless control containers. Faster development may be due to a change in the kind and or amount of particulate matters or micro-organisms eaten by larvae. We noted that survival of larval and larval-adult stages of mosquito was shortened as the plant density interval increased. Also, the pupal period was influenced by the plant density interval. The results also indicated that dense plant mates completely inhibited larval breeding apparently, where the high mortality rate was found at high plant density especially for pupae and the survival rate was low gradually with the increase the plant density.

The results also indicated that dense plant mates completely inhibited larval breeding apparently because larvae failed to reach water surface for respiration. It is needless to say that the results obtained from these laboratory experiments may not necessarily **be** applicable to field conditions where many other factors may interfere with mosquito breeding. As the result of their rapid growth of duckweeds over the water surface, they will prevent the transmission of light and cause oxygen decrease in water to other living organisms, which are hanging on their bodies like mosquito immature. Apart from the mechanical effects of the duckweed, Angerilli and Bryan (1980) stated that the duckweed influenced the survival of mosquitoes concerning the effect of the chemical characteristics of the water on mosquito colonization. Bradley (1932) suggested that floating plants do not provide effective protection for anopheline larvae because their leaves lie on top of the water surface and therefore do not hide the larvae from their enemies.

Various explanations have been offered for the deterrent effect of floating aquatic plants on mosquito breeding. It may be concluded that *Lemna* spices and other similar floating vegetation must be taken **into** consideration in designing mosquito control program. Transplanting the weed to select permanent breeding places, as pools, ditches, wells and old sakia pits that cannot be **a** deal by **another** method may be suitable natural methods of mosquito control.

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ARABIC SUMMARY

تأثير نبات عدس الماء ( ) على نمو وحياة الاطوار غير الناضجة لبعوض

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قسم علم الحشرات، كلية العلوم، جامعة بنها، القليوبية، مصر.

تتفاعل العديد من العوامل البيئية الحيوية وغير الحيوية فى التأثير على نمو وازدهار الكائنات الحية. وتعتبر النباتات المائية من العوامل البيئية الهامة والذى يدرج بعضها ضمن المقاومة الفيزيائية ضد العديد من الحشرات الطبية، ويعتبر نبات عدس الماء (*اليمنا مينور*) من النباتات والحشائش المائية الاكثر انتشارا فى العالم لقدرتة الفائقة على النمو والتكاثر فى البيئات المائية وأماكن توالد البعوض المتنوعة. تناولت هذه الدراسة تأثير نبات عدس الماء على نمو وحياة الاطوارغير الناضجة لبعوض *الكيولكس بيبينز* تحت الظروف المعملية. حيث تم استخدام كثافات مختلفة من نبات عدس الماء (كثافة منخفضة (١٥ نبات / سم^{٢})، متوسطة (٢٥ نبات / سم^{٢}) ومرتفعة (٣٥ نبات / سم^{٢})) على سطح الماء. وأظهرت النتائج أن وجود نبات عدس الماء قد أثر فى نمو يرقات البعوض كما ادى الى تأخير دورة الحياة حيث استمر متوسط نمو الطور اليرقى الى 11.4، 12، 19 يوما في عدم وجود النبات وعند كثافات منخفضة وعالية من النبات على التوالي. كذلك أدت زيادة كثافة عدس الماء الى قصر فترة بقاء اليرقات واليرقات- الطور اليافع (47.16±2.84) ، (32.93±0.87) مقارنة بعدم وجود النبات (94.83±0.83) ، (95.87±1.35). وحيث أن العوامل الفيزيائية والكيميائية تلعب دور هام فى جذب البعوض لوضع البيض، فقد تم دراسة تأثير غطاء من عدس الماء على عدد من كتل البيض التى وضعتها الاناث فى وجود كثافات مختلفة من النبات، ولاحظ أنه بالرغم من الكثافات العالية لعدس الماء التى تؤدى الى تثبيط نمو اليرقات الا إنها عامل جذب مهم للاناث لوضع كمية اكبر من كتل البيض خاصة في المياه الملوثة (P = 0.0001, F = 19.705). ويعتبر نبات عدس الماء من أليات مكافحة البعوض المستخدمة فى أماكن التوالد الطبيعية المختلفة.