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Studies on Biological Attributes of *Culex (Culex) univittatus* Mosquito (Diptera: Culicidae) Population From Saudi Arabia

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ABSTRACT

A deep understanding of vector biology enables us to discover mechanisms of disease transmission and to design modern and effective strategies for vector control. Although *Cx. (Cx.) univittatus* is one of the main disease vectors in Saudi Arabia and worldwide, little information on its biology and population dynamics are available. Herein we evaluated many biological and reproductive attributes of *Cx. univittatus* mosquito from Saudi Arabia, under semi-field conditions. We found that female *Cx. univittatus* spent half of its longevity in digestion of blood meal and oviposition. To that end, a mortality rate of 44.5 % was recorded due to feeding and oviposition. Survival rates of different life stages of *Cx. univittatus* mosquito were assessed, too. The fecundity of female *Cx. univittatus* was estimated 157.4 eggs/ female. Fertility was evaluated 65%. 35% of eggs failed to hatch. Only 41 % of the hatched larvae developed to pupae and 43 % of pupae developed to adults. *Chi-square* test indicated that sex ratio is biased in favor of female to compensate the loss in generations. Developmental period from egg to adult was 20 days (2.1 for egg, 15.3 for larva, 1.5 for pupa). These findings support the conclusion that the environmental factors and habitat conditions have major impact on the vector biological attributes and in turn, on the vector competence for a disease.

INTRODUCTION

Acceptable strategies of vector control require insight and deep knowledge of its population dynamics and life span parameters. Understanding the parameters such as feeding behavior, preoviposition period (POP), oviposition period (OP), longevity of blood-fed females, mortalities after feeding and oviposition, developmental duration, survival rates and fecundity is very important (Aida *et al.*, 2008). Studies on such biological attributes provide basic knowledge on population growth and inhibition. Concise information on reproductive capacity, survivorship and inheritance of a population characters under different ecological conditions can be very helpful in interpreting how a definite species proves fitness in a particular environment (Maharaj, 2003).

Little has been published on the biological attributes and bionomics of *Cx. (Cx.) univittatus*, especially, in Saudi Arabia. *Cx. (Cx.) univittatus* is considered a cosmopolitan species and distributed worldwide (Stone *et al.*, 1959). Despite of recording this species in different regions of Saudi Arabia (Ahmed *et al.*, 2011; Alahmed, 2012; Sallam *et al.*, 2013; Alikhan *et al.*, 2014; Hassan *et al.*, 2017), little information on its ecology and bionomics are available.

Furthermore, *Cx. univittatus* mosquitoes are considered the main vector of Sindbis virus in Saudi Arabia (Wills, 1985). This species is involved in transmission of West Nile virus (McIntosh *et al.*, 1976; Miller *et al.*, 2000), Rift Valley Fever virus (Sang *et al.*, 2010) and other arboviruses (Xiong *et al.*, 1989). Additionally, this mosquito has been associated with *Wuchereria bancrofti* (Brengues *et al.*, 1969) and avian *Plasmodium* parasite (Santiago-alarcon *et al.*, 2012). The major work on taxonomy, morphology and biology of *Cx. univittatus* has been done by Jupp (Jupp, 1971; Jupp *et al.*, 1980; Jupp and Harbach, 1990).

The present study aims to introduce a detailed report on several biological attributes of *Cx. (Cx.) univittatus* mosquito from Saudi Arabia. This report will be very helpful in intervention strategies of vector control and in disease transmission in nature.

Materials and methods

Larval collection and laboratory maintenance of mosquitoes

Cx. (Cx.) univitattus Theobald, was originally collected on October and September 2014 from a breeding site in East Gara, Sakaka, Aljouf, Saudi Arabia and maintained under controlled laboratory conditions (12L: 1twilight: 10D: 1dawn, 24-25°C and RH 75-85%) according to Jupp and Brown (1967). A tightly restrained domestic pigeon (*Columba livia domestica*), as a blood meal source, was exposed to the mosquito cages twice a week.

Experimental design

Twenty fully engorged female mosquitoes (3-5-days old) were placed singly into cups (diameter 6 cm and height 10 cm) covered with thin nylon cloth and supplied with a small plastic container (diameter 3 cm and height 3 cm) half-filled with de-chlorinated water as oviposition media and a cotton wick soaked in sucrose solution. Egg patches were removed daily and reared separately in new white-enameled pans (diameter 25 cm and height 10 cm) half-filled with de-chlorinated water. Larval hatching and pupation were followed up. Pupae from each pan were counted, transferred into rearing cage and followed up for adult emergence and sex ratio. Larval, pupal and adult survivals were monitored too. The experiment was repeated thrice.

Calculations and statistical analysis

We measured the following population parameters: mortality after feeding, preoviposition period (POP), oviposition period (OP), mortality after oviposition, F_1 female longevity, developmental durations, fecundity, egg hatchability, mean survival rates, percentage adult emergence and sex ratio. POP is defined as the period from the day of blood feeding to the day of oviposition for each individual mosquito. OP is defined as the period from the day of first oviposition to the day of last oviposition for a group of individuals having the blood meal in the same day. Fecundity is calculated as the average number of eggs laid per female. Egg hatchability (fertility) is calculated as the number of eggs hatched per egg patch. Other attributes were determined by observing each pan daily, and all larval skins were removed, scored to stage and counted. For survival analysis, 100 newly emerged larvae were placed in a white-enameled pan covered with nylon netting to prevent any cross breeding insects. The number of dead larvae were observed and recorded daily till pupation. Pupae were collected daily and observed for mortality, adult emergence and sex ratio. Food was provided daily and breeding water was changed every two days. The whole experiment was repeated three times by monitoring larvae from first instar stage to adulthood. The experiment was carried out in semi-field conditions, leaving the windows of the insectarium opened to allow fluctuations of the natural weather conditions. Data were pooled and descriptive statistics of the means, standard errors and ranges were calculated. Sex ratio was subjected to *Chi-Square* test. All statistical analyses were done by using SPSS ver. 19 program (SPSS Inc., Chicago, IL).

RESULTS

The different population attributes resulted from the experiments were carried out under open climatic condition (Varying temperature and relative humidity). The daily mean temperature was 32 ± 3 and the daily mean relative humidity was 55 ± 5 %.

Biological attributes of female *Cx. (Cx.) univittatus* after blood meal

Biological attributes of female *Cx. (Cx.) univittatus* after taking blood meal are depicted in Table (1). The period from taking blood meal to the last egg patch laid by a female in a population was approximately 8 days from the whole female life. The percentage mortality due to blood feeding and before oviposition was approximately 12 % (2.6/ 18.3 ♀) and the percentage mortality due to oviposition was approximately 32.5 % (5.2/ 16 ♀). Then the total female mortality from blood meal to oviposition is 44.5 % (12+ 32.5) of the total female population.

Table 1: Mean biological attributes of female *Cx. (Cx.) univittatus* mosquito from Saudi Arabia. Starting number is approximately 18 females.

Attribute	Unit
POP	4.15 ± 0.21 Days (3- 6)
OP	3.71 ± 0.18 Days (3- 4)
Female longevity	6.70 ± 0.33 Days (2- 10)
Mortality due to feeding	2.60 ± 0.16 Ind. (2-3)
Mortality due to oviposition	5.21 ± 0.72 Ind. (1- 9)

Survival rates of different life stages of *Cx. (Cx.) univittatus*

The mean survival rates of different life stages of *Cx. (Cx.) univittatus* mosquito are summarized in Table (2). The study started with an average of 18 full-engorged females, which produced only 14 egg patches containing a total of 1786 eggs (4 females died before oviposition).

Table 2: Mean survival rates of life stages of *Cx. (Cx.) univittatus* mosquito from Saudi Arabia, based on starting female ovipositions.

Stage x	Surviving I_x	Dead d_x	% mortality	Mortality reason
Number of starting females	18.33 ± 1.67 (15- 20)	4.00 ± 1.53 (2- 7)	21.11 ± 6.96 (13.33- 35)	Mortality after feeding
Number of egg laying females	14.33 ± 1.33 (13- 17)	--------	--------	
Number of egg patches laid	14.33 ± 1.33 (13- 17)	1.33 ± 1.33 (0- 4)	10.26 ± 10.26 (0- 30.77)	Completely failed to hatch.
Total number of eggs produced by all laying females	1786 ± 400.43 (1044- 2418)	583.67 ± 40.45 (512- 652)	35.65 ± 6.76 (26.96- 49.04)	Failed to hatch.
Total number of larvae hatched from all eggs	1202.33 ± 360.20 (532- 1766)	711.33 ± 344.36 (247- 1384)	35.81 ± 10.75 (23.66- 57.24)	Drowned.
Total number of pupae emerged	491.0 ± 159.97 (285- 806)	279.33 ± 111.27 (124- 495)	15.68 ± 5.28 (9.06- 29.11)	Failed to emerge.
Total % mortality of immature stages			86.15 ± 3.74 (80.60- 93.26)	
Emerged adults	211.67 ± 49.67 (161- 311)	---------		
Male ♂	85.67 ± 25.33 (51- 135)	----------		
Female ♀	126.0 ± 25.79 (90- 176)	----------		
Sex ratio ♂: ♀	1.00: 1.47 (1: 1.30- 1: 1.75)	----------		

1786 eggs are the total number of eggs produced by 14 females during first gonotrophic cycle. The largest egg patch contained 319 eggs and the smallest one contained 54 eggs (Table 3). Out of the 1786 eggs, only 1202 eggs hatched to first instar larvae. The 1202 larvae developed to 491 pupae and finally a total number of 211 adults were emerged. The average percentage of egg hatching was approximately 65 % (No. of hatched larvae/ total No. of eggs). Larval-to-pupal survival rate was approximately 64 % (No. of emerged pupae/ No. of hatched larvae) and pupal-to-adult survival rate was approximately 84 % (No. of emerged adults/ No. of pupae). *Chi-Square* test revealed that the sex ratio was significantly higher in favor of female at 95% confidence interval (P= 0.00).

Developmental durations of different life stages and fecundity of *Cx. (Cx.) univittatus*

The mean developmental durations of different life stages and fecundity of *Cx. (Cx.) univittatus* mosquito are summarized in Table (3). The developmental period from egg to adult emergence was approximately 20 days. The least developmental time was spent in pupal stage (1.5 days). Meanwhile, the longest duration was spent as larvae (sum of 4 instars). Based on one blood meal, the fecundity of *Cx. (Cx.) univittatus* was calculated as 157 eggs per female (Table 3).

Table 3: Mean developmental duration and fecundity of *Cx. (Cx.) univittatus* mosquito from Saudi Arabia.

Stage	Duration in days
Egg hatching period	2.13 ± 0.11 (1- 3)
Larval duration	15.33 ± 0.14 (4- 23)
Pupal duration	1.52 ± 0.03 (1- 3)
Total immature duration	20.77 ± 0.31 (6- 29)
Largest egg raft	319 eggs
Smallest egg raft	54 eggs
Fecundity (No. of eggs/ female)	157.36 ± 12.53

DISCUSSION

Fluctuations of the environmental conditions have a great impact on biological and reproductive strategies of the biological components of an ecosystem. The present study was carried out in semi-field conditions to fulfill environmental simulation. Our results indicated that female *Cx. univittatus* spent half of its longevity in digestion of blood meal and oviposition. In addition, a total mortality of 44.5 % was recorded due to feeding and oviposition. A semi-field study reported that longevity of female *Cx. univittatus* increased as the incubation temperature decreased, reaching maximum of 114 d at 14 °C (Cornel *et al.*, 1993). The short longevity reported in our study may be due to the higher temperature of Saudi Arabian climate. Mortality after feeding may be attributed to inability of some individuals to digest the blood meal and/ or due to immune response of the host (Phasomkusolsil *et al.*, 2013). The impact of temperature and available nutrition on vector survivorship is also reported (Clements, 1992). The mortality after oviposition may be explained as energy reserve and energy cost issues (Cornel *et al.*, 1993). They studied the effect of environmental temperature (extrinsic incubation temperature) on vector competence of *Cx. univittatus* for WNV. They found that the

vector competence was intermediate between 26 and 30 °C (similar to cycling environmental temperature).

To assess survival rates of different life stages of *Cx. (Cx.) univittatus* mosquito, the oviposition experiment based on 14 females which produced 1786 eggs, was followed up. The mean number of eggs produced by a female (fecundity) was 157.4. This number is comparable to the number of eggs of other *Culex* species (Clements, 1992; Gokhale *et al.*, 2013; Phasomkusolsil *et al.*, 2013; Day, 2016). An average of 35 % from eggs failed to hatch. This proportion is relatively high in comparison to 13.5-20 % for *Cx. quinquefasciatus* (Gomez *et al.*, 1979; Suman *et al.*, 2011; Gokhale *et al.*, 2013), 7 % for *Ae. aegypti* (Phasomkusolsil *et al.*, 2013), 11-26 % for some *Anopheles spp.* (Phasomkusolsil *et al.*, 2013), 12 and 26.5 for *Ae. albopictus* (Tsuda *et al.*, 1994; Aida *et al.*, 2011). Meanwhile, our 35% failure is lower than 41.4 % presented for *An. gambiae* (Olayemi and Ande, 2009). A lot of work has been published to explain causes of hatching failure. In our case, immaturity due to non-insemination and/ or the absence of environmental stimuli are the candidate causes of failure to hatch (Aida *et al.*, 2011). Our results clarified that 41 % of the hatched larvae developed to pupae. Finally 43 % of the pupae developed to adults. The great loss in the progeny may be due to the high temperature in the climate of Saudi Arabia. This loss may explain why sex ratio was biased in favor of female to compensate the loss in generations. The current study presents higher larval mortality than in pupal stage. Similar results were presented in many studies examining different insect species (Gomez *et al.*, 1977; Suleman and Reisen, 1979; Tsuda *et al.*, 2003; Maharaj, 2003; Okogun, 2005; Yurttas and Alten, 2006; Afrane *et al.*, 2007; Olayemi and Ande, 2009; Aida *et al.*, 2011; Suman *et al.*, 2011; Gokhale *et al.*, 2013; Phasomkusolsil *et al.*, 2013; Wang *et al.*, 2016). Contradictory results were presented in a study on Penang strain of *Ae. albopictus* (Aida *et al.*, 2008). This inconsistency in results of larval and pupal mortality is not solved yet. It can be interpreted in the light of the different physiological traits of the two developmental stages (Aida *et al.*, 2011). Larva eats and pupa in non-feeding stage. Thus the greater larval mortality could be explained on the basis of food availability and vulnerability to microorganisms in the environment, too.

Developmental period from egg to adult was 20 days. This period is relatively long in comparison to its correspondences of *Cx. quinquefasciatus* (15-16 d) (Gomez *et al.*, 1977; Suleman and Reisen, 1979; Suman *et al.*, 2011; Gokhale *et al.*, 2013), *Anopheles spp.* (11-23 d) (Olayemi and Ande, 2009; Phasomkusolsil *et al.*, 2013) and *Aedes spp.* (5-11 d) (Tsuda *et al.*, 1994; Aida *et al.*, 2011; Phasomkusolsil *et al.*, 2013). After hatching, larvae grow by converting food to biomass (Briegel, 2003). The larval duration length relies on many factors such as habitat conditions and environmental factors with major roles for food and temperature (Briegel, 2003; Yurttas and Alten, 2006; Afrane *et al.*, 2007; Suman *et al.*, 2011; Wang *et al.*, 2016). Elongated duration period and higher percentage of mortality in our study may be explained in the light of habitat conditions and extended vulnerability to environmental factors. The present results evidenced that environmental factors and habitat conditions have major effect on biological attributes of mosquitoes.

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ARABIC SUMMERY

دراسات عن الصفات البيولوجية لبعوضة *كيولكس* (*كيولكس*) *يونيفيتاتوس* (ثنائية الأجنحة: كوليسيدى) و المجمعة من المملكة العربية السعودية

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١- قسم الأحياء- كلية العلوم- جامعة الجوف- سكاكا- المملكة العربية السعودية.

٢- قسم علم الحشرات- كلية العلوم- جامعة القاهرة- الجيزة- مصر.

إن الفهم العميق لبيولوجيا ناقلات الأمراض، يساعدنا على اكتشاف آليات انتقال المرض، ومن ثم تصميم استراتيجيات حديثة وفعالة لمكافحة ناقلات الأمراض. و على الرغم من أن بعوضة *كيولكس* (*كيولكس*) *يونيفيتاتوس* هي واحدة من ناقلات الأمراض الرئيسية في المملكة العربية السعودية وفى العالم، إلا أن المعلومات المتاحة عن بيولوجيا وديناميات الآهلات، قليلة جدا. و من هنا تبرز أهمية هذه الدراسة، حيث قمنا بتقييم العديد من الخصائص البيولوجية والإنجابية لبعوضة *كيولكس* (*كيولكس*) *يونيفيتاتوس* المجمعة من سكاكا، المملكة العربية السعودية، تحت ظروف شبه حقلية. و قد أوضحت النتائج أن إناث هذه البعوضة تقضى نصف فترة عمرها في هضم وجبة الدم، ووضع البيض. ولتحقيق هذه الغاية، سجلت الحشرة معدل وفيات يبلغ ٤٤.٥٪ بسبب التغذية، ووضع البيض. و قد تم تقييم معدلات البقاء على قيد الحياة لأطوار الحياة المختلفة للبعوضة. و قد تم تقييم خصوبة إناث هذه البعوضة و التي قدرت ب ١٥٧.٤ بيضة/ أنثى. و قد تم أيضا تقييم البيوض الخصبة و التي قدرت بنحو ٦٥٪ من إجمالي البيوض التي وضعتها الإناث، بينما فشلت ٣٥٪ من البيوض في الفقس. هذا و قد تحورت نسبة ٤١٪ فقط من اليرقات إلى طور العذراء، وتحورت نسبة ٤٣٪ من العذارى إلى حشرات بالغة. و قد أوضح اختبار *Chi-Square* أن النسبة الجنسية للحشرات البالغة منحازة بفرق معنوي لصالح الإناث، و ذلك لتعويض نسبة الوفيات الكبيرة، و الفقد في الأجيال. و قد كانت فترة النمو من البيض إلى الحشرة البالغة نحو ٢٠ يوما (٢.١ يوما للبيضة حتى تفقس، ١٥.٣ لنمو اليرقات و تحورها للعذارى، ١.٥ للعذارى لخروج الحشرات البالغات). و هذه النتائج تدعم الاستنتاج القائل بأن العوامل البيئية، وظروف الموئل، لها تأثير كبير على الصفات البيولوجية لنواقل الأمراض، وبالتالي على كفاءة هذه النواقل في نقل المرض.