

**AN ATTEMPT TO ESTIMATE HONEYDEW PRODUCTION OF DIFFERENT STAGES OF *Ommatissus lybicus* DE BERG. (HOMOPTERA : TROPODUCHIDAE) IN VITRO**

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**ABSTRACT**

Honeydew produced by the mobile stages of *Ommatissus lybicus* De Berg, indirectly affects the physiology of date palm leaves leading to reduction in the fruit yield and quality. The capacity of each stage of the insect producing honeydew is assessed as the number of droplets produced per hour. The nymph instars produced higher number of droplets per hour (from 6.4 to 7.6), while the adult produced the less number of droplets (2.3 for female and 2.9 for male). However, the female adult produced the largest droplet size (734 microns in diameter) while the first nymphal stage produced the smallest (diameter 157 microns). The calculated volume of produced honeydew obviously showed that the fifth nymphal instar is producing 73 times of honeydew more than that produced by the first nymphal instar within one hour. The obtained results suggested another possibility to determine the critical level of *O. lybicus* infestation on which the control methods should be considered.

**INTRODUCTION**

*Ommatissus lybicus* De Berg, is a serious pest of date palm in the Sultanate of Oman. It has been recorded in date palm plantations in Iraq, Iran, Egypt and N. Africa (Hill and Waller, 1990) and in many Arab countries (El-Haidari and Al-Hafidh, 1986). *O. lybicus* was referred to in earlier literature as the "lybicus" variety of *O. binotatus* it was raised to species status by Asche and Wilson (1989).

*Ommatissus lybicus* is known in Arab area as "Dubas bug", and in some literature it is known as the "Old world date bug". This species is restricted to date palm where it complete its life cycle on it. The infested date palm with *O. lybicus*, affected directly through the feeding of the mobile stages of this insect, and indirectly by producing copious amounts of honeydew, which then covers the fronds of the date palm. The deposited honeydew accumulates, which support sooty mould growth, and under the rainless conditions of the desert (where date palm usually grow) a thick layer of dust may accumulate on honeydew-coated surfaces. After sometimes following the infestation, the frond surfaces tend to become chlorotic. In Iraq, losses of 50% of the date crop have been attributed to this insect (Kranz *et al.* 1978). Dates of infested palms are reported to be smaller and to ripen slowly, with high percentage of reducing sugars and sucrose (Hussain 1985).

Successful pest management requires estimating the population of the insect precisely. The population of any pest could be estimated directly by counting the number of that pest in the habitat or indirectly by assessing its products or its effects (Southwood 1978; Ruesink and Kogan 1982). Honeydew production is one of the parameters that indicate changes in *O. lybicus* population in date palm groves (Mokhtar and Al-Mjeni, 1999).

The honeydew production and its impact on the host plants has been reported for some other Homopteran insects (Jacob and Evans 1998, Henneberry and Jech 2001, Slosser *et al.* 2002), while this information was lacking for this serious date palm feeder *O. lybicus*. The present contribution provided new knowledge for the honeydew production of the mobile stages (fifth nymphal instars and adult) of *O. lybicus in vitro*.

Due to the adverse effect of the honeydew on the physiological function of the date palm leaflets was suggested in this study, another concept for the economic injury level assessment was suggested in this study depending on the produced honeydew droplets that cover 10% of upper surface of the leaflet. Then the economic threshold has been calculated accordingly as the number of insects those produce enough honeydew droplets to cover 10% of the upper leaflet area of date palm within one week.

## **MATERIALS AND METHODS**

A culture of *O. lybicus* was reared in the laboratory on date palm seedlings grown in plastic pots for a period of one year (two complete generations). The newly hatched nymphs in February 2000 were collected to investigate the honeydew production of the different insect instars as follows: Individual date palm seedlings were grown in the laboratory in plastic pots (15 cm diameter) for about seven months. One vertical leaf of each seedling was left standing while all the other leaves were removed. On this leaf the insect was reared under the laboratory conditions at  $25 \pm 1$  °C mean temperature, and 40-50% RH.

For experimental purposes, twenty newly hatched nymphs were transferred by using fine wet camelhair brush and kept individually on a date palm seedling. Then, the whole set up was caged under a transparent 'whole plant' cage type (Van Emden 1972). Honeydew droplets produced by the insect were allowed to impinge on a water sensitive paper (WSP) made of a white plain paper soaked in a solution of 2 gm bromocresol blue dissolved in absolute ethanol and dried (Van Emden 1972).

WSP was cut into a shape of a table tennis racket with a slit on one side. The circular part of the paper measured 13.2 cm in diameter. The handle-like part of WSP left protruding out side the top of the pot under the cage. The handle-like part of WSP used to move and to record the data on it by pencil. The paper was introduced into the pot using the slit to slip it around the stem of the seedling. Honeydew droplets fall on the paper and cause a blue stain to appear. The seedling was irrigated by placing the pot in a plastic tray containing some water. This method avoided wetting the top surface of

the soil where the WSP was placed. As an additional precaution a piece of plastic sheet (50 micron thickness and 15 cm in diameter) was laid beneath the WSP. The number of honeydew droplets produced by each stage of *O. lybicus* was recorded daily from 0800 hours to 1400 hours. Cages were checked daily in the morning time to remove the casted exuviae and record the date of the new instars if any.

Every one hour the WSP was turned slightly by pushing the handle-like part in clockwise or anticlockwise direction to avoid the accumulation of the droplets on top of one another. At the end of each run date; serial number of the cage; and instars of the caged insect were recorded on the handle-like part of the WSP and stored in a plastic bag to be examined in the next day.

The number and size of the droplets produced in each instars were determined throughout its lifetime where a number of  $\geq 500$  honeydew droplets were examined for the size for each insect instars. The diameter of honeydew droplets was measured (in microns) on the WSP (the blue stained spots) using the scaled eyepiece of a compound microscope. The spread factor of the WSP has been overlooked in this case because of high viscosity of the honeydew.

ANOVA and Duncan' multiple range test were carried out for analysis of variance and separation of the means by using MSTATC-C (1988).

## RESULTS AND DISCUSION

### 1. Honeydew produced by different instars:

A clear pattern of the number of honeydew droplets produced by *O. lybicus* emerged. The adult (male and female) produced significantly fewer honeydew droplets per hour than did the nymphs, while there was no statistical difference in the number of droplets produced among the various nymphal instars of the insect (Table 1).

**Table (1): The number of honeydew droplets produced in one hour by different stages of *Ommatissus lybicus*.**

Stage	Mean number of droplets / h $\pm$ SD	Range
N I	6.5 $\pm$ 1.50 A	5.4 - 8.3
N II	6.4 $\pm$ 1.96 A	4.1 - 9.8
N III	7.4 $\pm$ 1.58 A	5.3 - 10.0
N IV	7.6 $\pm$ 2.12 A	4.0 - 10.9
N V	7.6 $\pm$ 1.21 A	5.6 - 8.8
A ♀	2.3 $\pm$ 1.28 C	0.6 - 3.8
A ♂	2.9 $\pm$ 1.50 C	1.7 - 5.5

N I - N V Nymphal stage. N I, First Instar; N II, Second Instar; N III, Third Instar; N IV, Fourth Instar; N V, Fifth Instar; A ♀ adult female; A ♂ adult male. Means in a column not followed by the same letter are significantly different (Duncan's multiple range test,  $P \leq 0.05$ ).

The mean number of droplets produced by nymphs varied from 6.4 to 7.6 droplets per hour. While the number of droplets produced by the adult was significantly lower as 2.3 and 2.9 droplets per hour for the female and male, respectively. so the nymphs feed more frequently than the adults do, and where the food is utilized for their development. The food is retained longer so as to achieve a more complete digestion particularly of proteins since the plant sap would not contain large quantities of these nutrients.

On the other hand, the adults feed less frequently, retain the food for a longer period of time and channel the food towards their sexual development and maturation.

It is interesting to note that honeydew production is not constant within each instars. At the time of molting the insect ceased to produce any honeydew for a few hours and sometimes even for days. This is doubtless due to the fact that the insect does not feed at this time. However, after the skin is cast, there is a profuse production of honeydew reflecting an increase in intake of plant sap by the insect.

## 2. Size of honeydew droplets:

The size of honeydew droplets produced by the various instars of the insect was measured (Table 2). First nymphal instar produced tiny droplets that measured  $157 \pm 45$  microns in diameter. The size of the droplets increased as the insect developed. The fifth instar nymph produced honeydew droplets about 4.0 times larger in diameter than that produced by the first instar. The largest droplet was  $734 \pm 165$  microns produced by the adult female.

**Table 2. Diameter (in micron) of droplets produced by different stages of *Ommatissus lybicus*.**

Stage	Mean of diameter ( $\mu$ ) of droplets $\pm$ SD		Range
N I	157 $\pm$ 45	D	88 - 220
N II	256 $\pm$ 35	D	198 - 308
N III	481 $\pm$ 112	C	330 - 660
N IV	545 $\pm$ 135	BC	418 - 770
N V	622 $\pm$ 133	AB	550 - 836
A ♀	734 $\pm$ 165	A	550 - 1056
A ♂	622 $\pm$ 148	AB	308 - 748

N I - N V Nymphal stage. N I, First Instar; N II, Second Instar; N III, Third Instar; N IV, Fourth Instar; N V, Fifth Instar; A ♀ adult female; A ♂ adult male. Means in column not followed by the same letter are significantly different (Duncan's multiple range test,  $P \leq 0.05$ ). \* Figures in that column are rounded to nearest integer

The size of the droplets coincide with the size of the insect, the larger nymphal instars consume more plant sap and produce more honeydew which means they are more harmful to the plants in the field. Hussain, 1985, has reported the body length of NI, NII, NIII, NIV, NV, A ♀ and A ♂ of *O. lybicus* as 1.0-1.25, 1.75-2.25, 2.0-2.5, 3.0-4.0, 3.5-4.0, 5.0-6.0 and 3.0-3.5 mm, respectively.

Each nymphal instar was produced a quite vast range of droplet diameter which showed overlapping in the size of the droplets among the different stages. This overlap in the size of droplets produced by different stages of the insect, make it difficult to translate such results under field conditions, with experience to determine roughly the composition of *O. lybicus* population in nature.

It is important to realize that the diameter of the droplets does not reflect accurately on the quantity of honeydew produced by the insect. For this reason the volume of the droplets were calculated in (Table 3).

From the diameter of the droplets in (Table 2), the volume of the spherical droplet was theoretically calculated (diameter<sup>3</sup> multiply by 0.5236) to make it easy to compare the quantity of honeydew produced by different stages of the insect. Then, the quantity produced within one hour was calculated (multiplying droplet volume by no. of droplets/h) as shown in (Table 3). The ratio between the honeydew quantity that produced by different stages to that produced by N I is demonstrated as well. So, it is clear that NV is the most active stage in honeydew production where it produces 73 times of the honeydew quantity produced by N I. It is obvious that the volume of the droplets produced increases with the stage of the insect and the adult produces the largest droplet size in diameter as 734 μ (Table 2) but NV produces larger quantity (0.958 mm<sup>3</sup>) per one hour (Table 3). This apparent discrepancy is explained by the fact that the adult female produces 2.3 droplets/hour while NV produces 7.6 droplets during the same period of time (Table 1). However, the quantity of honeydew produced by the adult sharply declines. The fifth instar nymph produce 73 times of honeydew quantity as the first instar, while the adult produce 36 and 24 times only for male and female, respectively (Table 3).

Again, the volume of honeydew produced, in the time unit, by the various stages of the insect would be of more relevance to compare the honeydew quantity that produced by the different stages of *O. lybicus*.

**Table(3): The calculated volume (mm<sup>3</sup>) of the honeydew droplets produced by different stages of *O. lybicus* per one hour; and the ratio between the honeydew quantities that produced by different stages to the first instar nymph.**

Stage	Droplet diameter (mm)	Volume (mm <sup>3</sup> )	Total V/h (mm <sup>3</sup> )	Ratio to N1
NI	0.157	0.00203	0.013	
NII	0.256	0.00878	0.056	4
NIII	0.481	0.05827	0.431	33
NIV	0.545	0.08476	0.644	49
NV	0.622	0.12600	0.958	73
A ♀	0.734	0.20706	0.476	36
A ♂	0.622	0.12600	0.365	27

NI – NV Nymphal stages. NI, First Instar; NII, Second Instar; NIII, Third Instar; NIV, Fourth Instar; NV, Fifth Instar; A ♀ adult female; A ♂ adult male. Means in column not followed by the same letter are significantly different (Duncan's multiple range test, P ≤ 0.05). \* Figures in that column are rounded to nearest integer

### 3. The area of honeydew droplets:

Honeydew *per se* is not injurious. However, it can cause two deleterious effects indirectly: (1) – cover the fruit and thereby reduce its quality, and (2) – provide a substrate on which soot mould can grow. When leaves are covered with sooty mould, their physiology, particularly photosynthesis, is interfered and consequently reduced in yield and poor quality of the fruits.

Since various instars of *O. lybicus* produce different amounts of honeydew, it would be useful to get a measure of the area the honeydew droplets cover on a surface of the leaflet. The area of the droplet on the water sensitive paper was calculated as circle (diameter<sup>2</sup> multiply by 0.7845) as shown in Table 4. The honeydew droplets produced by NI, NII, NIII, NIV, NV, A♀ and A♂ cover an area of 0.126, 0.329, 1.343, 1.771, 2.307, 0.972 and 0.880 mm<sup>2</sup> within one hour. This means that NII, NIII, NIV, NV, A♀ and A♂ produce honeydew would cover an area of 2.6, 10.7, 14.1, 18.3, 7.7 and 7.0 times larger than the droplets produced by NI.

While the leaflet surface is the block unit of the plant food preparation where the carbohydrates and sugars are processed, so it is important to maintain the maximum of this surface area to insure good yield. One mature frond produces about one pound of sugar per year (Ebeed and Khulaif, 1998). The importance of the leaf-bunch ratio has been expressed by many researchers, they stated ratio around 10:1 in many different date palm cultivars is necessary to keep good quantity and quality of date fruits yield (Aldrich *et al.* 1942, Hasan 1993).

So, the coverage of 10% of the upper surface of the leaflet by honeydew was suggested to be as the critical level of the infestation with *O. lybicus*, or with another word it should be the economic injury level (EIL) before which the control measures should be initiated. In this case the economic threshold (ET) should be the lower than EIL, or it should be the number of insect individuals per one leaflet that produce honeydew droplets cover 10% the upper surface of the leaflet within one week (Table 4).

**Table (4): The area of honeydew droplets cover and the number of insects needed to produce enough honeydew to spread over the upper surface of a date palm leaflet.**

Stage	Droplet diameter (mm)	Area of droplet (mm <sup>2</sup> )	Area of droplets produced / hour (mm <sup>2</sup> )	Suggested economic Threshold (ET)
NI	0.157	0.019337	0.126	10.9
NII	0.256	0.051413	0.329	4.2
NIII	0.481	0.181503	1.343	1.0
NIV	0.545	0.233016	1.771	0.8
NV	0.622	0.30351	2.307	0.6
A♀	0.734	0.422654	0.972	1.4
A♂	0.622	0.30351	0.880	1.6

NI – NV Nymphal stage. NI, First Instar; NII, Second Instar; NIII, Third Instar; NIV, Fourth Instar; NV, Fifth Instar; A♀ adult female; A♂ adult male. \*Area of upper leaflet surface averaged 2306.21 mm<sup>2</sup>.

Suggested economic threshold calculated as the number of insect individuals that produce honeydew droplets cover 10% of the upper surface of the leaflet within one week

Under field conditions, however, the situation is much more complex. Wind factor can cause drift of the droplets and lose some water through evaporation before they impinge on the leaflet. Temperature, humidity and daily period of sunshine are factors that can influence the results. Finally, the population structure of *O. lybicus* in the field is mixed. The study reported here is an attempt to compartmentalize and get an insight into honeydew production by the various stages of *O. lybicus* under ideal laboratory conditions. So, more investigation is needed to manipulate the output of this work under field conditions.

**On the light of the obtained results of this work the following approaches can be subjected:**

- From the size of the honeydew droplets (Table 2), a rough description for the different stages that composing the population can be obtained. That helps the plant protection decision maker to take action at the right time.
- The control action should be taken in the beginning of the season against the nymphs because they feed more actively and produce larger amount of honeydew.
- Identification of the economic threshold of the infestation in the date palm plantation considering the number of nymphs per leaflet, as shown in Table 4 to avoid the impact of honeydew production on the physiological function of the leaves and the reduction of the fruit quantity and quality.
- Reliable estimation for the population density of *Ommatissus lybicus* in the field could be obtained according to the intensity of honeydew production as one of the products of the insect.

The relation between honeydew production and the insect population in the field should involve the effective field conditions such as temperature; wind; relative humidity (this study under progress). It would be of interest to find out the amount of waste products in the honeydew produced by the various stages of the insect. The nutritional substances that consumed by this insect population can be estimated as one of the damage phases to the date palm tree.

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## محاولة لتقدير إنتاج الندوة العسلية للأطوار المختلفة لحشرة دوبياس النخيل تحت ظروف المختبر

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تقوم الأطوار المتحركة لدوبياس النخيل *Ommatissus lybicus* De Berg بإفراز مادة سكرية لزجة تعرف بالندوة العسلية والتي تسقط على الأوراق والثمار فتقل الكفاءة الفسيولوجية للأوراق وتتدهور نوعية الثمار ويقل المحصول. في هذه الدراسة تم تطوير طريقة لقياس قدرة الأطوار المختلفة على إفراز الندوة العسلية حيث ربيت الحشرة على فسائل نخيل التمر في أصص ووضعت داخل أقفاص بلاستيكية شفافة ثم وضعت أوراق حساسة للماء أسفل الشتلة التي عليها الحشرة لجمع نقاط الندوة العسلية التي تفرزها حيث يتحول لون النقطة التي تسقط عليها القطرة إلى اللون الأزرق. وبذلك أمكن حساب عدد القطرات التي يفرزها كل طور في الساعة، فكانت ٦,٥ و ٦,٤ و ٧,٤ و ٧,٦ و ٧,٦ و ٧,٦ قطرة/ساعة لحوريات العمر الأول والثاني والثالث والرابع والخامس على التوالي بينما كان إفراز الحشرات الكاملة ٢,٣ و ٢,٩ لكل من الأنثى والذكر على التوالي. كذلك تم قياس قطر القطرة بالميكرون فكانت ١٥٧ و ٢٥٦ و ٤٨١ و ٥٤٥ و ٦٢٢ ميكرون لحوريات العمر الأول والثاني والثالث والرابع والخامس على التوالي. بينما كانت ٧٣٤ و ٦٢٢ لكل من الأنثى والذكر على التوالي. تم حساب كمية الندوة العسلية التي تفرزها الأطوار المختلفة فكان العمر الخامس للحورية يفرز كمية من الندوة العسلية أكبر بمقدار ٧٣ مرة من الحورية في العمر الأول عند المقارنة بينما تفرز الحشرة الكاملة الأنثى ٣٦ مرة أكثر من الحورية في العمر الأول.

من النتائج التي تم الحصول عليها تم اقتراح أن يتم حساب الحد الاقتصادي الحرج للإصابة بعدد الحشرات التي تفرز كمية من الندوة العسلية تكفي لتغطية ١٠% من السطح العلوي لوريقة النخيل خلال أسبوع فكان هذا الرقم يتراوح بين ١٠,٩ لحوريات العمر الأول إلى ٠,٦ لحوريات العمر الخامس.