EFFICACY AND SUBLETHAL EFFECTS OF MOSQUITO COILS ON *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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Abstract—Mosquito coils with the active ingredient d-allethrin (0.2% w/w), and d-trans allethrin (0.1% w/w) together with blank coils were tested in the laboratory against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* Say of different ages and physiological condition, using a test method of the Standard and Industrial Research Institute of Malaysia (SIRIM). Results indicated that *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus*. The blank coil caused neither significant knockdown nor mortality of mosquitoes, while coils containing either d-allethrin or d-trans allethrin provided adequate knockdown of mosquitoes, but with low mortalities for both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes below 10 days of age. The susceptibility of mosquitoes increased with age as indicated by a decrease in KT_{50} and increase in mortality of female mosquitoes tested for both species. This study also indicated that blood-engorged mosquitoes were more difficult to be knocked down, but easier to kill than non blood-engorged mosquitoes. However, susceptibility of mosquitoes to coils was not affected by oviposition. The survivors of mosquito coil exposure were used for sublethal effects studies, concentrating on the longevity and fecundity of treated individual and progeny survival. The longevity of both species decreased more than 40% after exposure to mosquito coils containing either d-allethrin or d-trans allethrin. The percentage of blood-engorgement activity for *Ae. aegypti* and *Cx. quinquefasciatus* was reduced to less than 55% and 15%, respectively after a 20 minutes exposure to coils containing either one of the active ingredients. Furthermore, *Cx. quinquefasciatus* also showed a decrease in blood-engorgement ability (≤40%) after being exposed to a blank coil. Nevertheless, the number of eggs oviposited by the treated females, egg hatchability, pupation rate and adult emergence rate were not affected if the mosquitoes were still able to blood-engorge.

INTRODUCTION

Information from industrial sources indicates that the mosquito coil is the most commonly used household insecticide product in Asia. As of 1994, annual worldwide consumption of mosquito coil was estimated at 22,000 million pieces with a total retail price of approximately US$650 million. Consumption in Asia constituted about 90% of this figure. The popular usage of coils in this area is due to their low cost (cheapest among existing major household insecticide products), ease of usage (without electricity requirement) and high consumer accceptance (traditional and cultural practice of using smoke to repel mosquitoes). Yap & Foo (1984) showed that the highest usage of mosquito coils is in rural areas (71%) compared with its usage in urban (46%) and suburban areas (47%) on Penang Island, Malaysia.

Mosquito coils have been shown to repel and disrupt the host-seeking activity of mosquitoes, followed by knockdown (MacIver, 1964; Fales *et al.*, 1968; 1971; Mace, 1969; Teshima, 1993), although the mortality of mosquitoes caused by mosquito coils may be low (Smith & Chadwick, 1964; Smith & Obudho, 1967; Smith *et al.*, 1973; Mosha *et al.*, 1989). Field trials on several formulations of mosquito coil in a suburban squatter area in Penang, Malaysia, demonstrated that the mosquito biting activity can be reduced by more than 70% when using coils containing either d-allethrin (0.20% and 0.30% w/w) or d-trans allethrin (0.10% and 0.15% w/w) (Yap *et al.*, 1990).

Although numerous studies have been reported on the performance of mosquito coils, there has been no study on the sublethal effects on mosquitoes induced through the use of these products. This study was initiated to determine the efficacy of two mosquito coil formulations containing either d-allethrin or d-trans allethrin against female mosquitoes of *Aedes aegypti* and *Culex quinquefasciatus* of different age groups, nutritional and physiological conditions. Further studies were conducted using the survivors from mosquito coil exposure to determine the sublethal effects on blood-engorgement activity, egg production, egg hatchability, pupation, progenies adult emergence and longevity.

MATERIALS AND METHODS

Mosquito coil formulations

All mosquito coils used in this study were provided by Fumakilla Malaysia Berhad, Butterworth, Penang, Malaysia with the following formulations: coils containing d-allethrin (d 1-3-allyl-2-methyl-4-oxo-2-cyclopentenyl d-cis trans chrysanthemate) (0.20% w/w), coils containing d-trans allethrin (d 1-2-allyl-4-hydroxy-3-methyl-2- cyclopenten-1-one ester of d-trans chrysanthemate monocarboxylic acid) (0.10% w/w) and blank coils (without active ingredient). The choice of the amount of two active ingredients used in this study reflects the normal amount used in mosquito coil formulations in Malaysia and Southeast Asia (SIRIM 1986a). The inert ingredients in the coils were essentially similar to those listed by the Standard and Industrial Research Institute of Malaysia (SIRIM 1986b), i.e., wood powder (34.5%), coconut shell powder (34.5%), incense powder (30%), malachite green (0.5%) and sodium benzoate (0.5%).

Before and after the completion of this study, chemical analysis using gas chromatography (Shimadzu GC 12A) was performed to determine the exact amount of the active ingredient in the coil samples.

Mosquitoes

The two species of mosquitoes used in this study were *Ae. aegypti* and *Cx. quinquefasciatus*. Both species, originating from Penang, Malaysia, have been reared in our laboratory since the 1980s. The techniques for rearing these two species follow those of Chong *et al.* (1995). All adult female mosquitoes were collected within 24 hours of emergence and reared separately. These mosquitoes were then divided into three groups and reared under different physiological/nutritional conditions before being tested at the following ages: (1) sucrose fed group: 1–2 days, 4–5 days, 9–10 days, 14–15 days and 19–20 days. (2) sucrose-fed and blood-engorged group: 4–5 days (3) sucrose-fed, blood-engorged and oviposited group: 9–10 days. The mosquitoes were allowed to blood-engorge at day 4 and oviposited at day 8.

Bioefficacy test

The bioefficacy test used in this study follows exactly those of SIRIM (1986b). It was conducted in a glass chamber with aluminium frames (70×70×70 cm) under the conditions of 26±2°C and 65±10% R.H. A small window (18×20 cm) with a sliding closure at the mid-bottom of the chamber door was used for the introduction of coils and mosquitoes into the chamber. A portion of 0.50±0.003 g of the formulated coil was fixed on a stand and ignited at both ends inside the chamber. A small battery-operated fan was placed and switched on in the chamber while the coil was burning to ensure uniform dispersion of the smoke emitted from the coil inside the chamber. Immediately after the coil was completely burnt out, twenty mosquitoes were released into the chamber. Knockdown of the mosquitoes was recorded at 0.5 minute followed by 1 minute intervals for up to 5 minutes for *Ae. aegypti* and up to 20 minutes for *Cx. quinquefasciatus*. Longer exposure time was needed for *Culex* mosquitoes to achieve total knockdown of the introduced mosquitoes. Criteria for knockdown were that the mosquitoes no longer maintained normal posture and were unable to fly or were on their backs.

At the completion of each test, the inner wall surfaces of the chamber were thoroughly cleaned with detergent and water to remove any toxic residue present. The chamber was then air dried with an electrical fan before the onset of the next test.

After the exposure, the mosquitoes were collected from the chamber and placed in a clean polyethylene cup and provided with a wet cotton bung containing 10% sucrose solution. The mosquitoes were kept under the environmental conditions mentioned above, and mortality was recorded at 24 hours post-exposure. For each experiment, tests involving all formulations and control were repeated 10 times for each species and age group.

Sublethal effects of coil exposures

On the second day after the bioefficacy test, the survivors at each age groups were collected and kept separately in cages (30×30×30 cm). On the third day, a white mouse restrained within a piece of wire-netting was placed in each cage for 12 hours for blood-engorgement. Due to different biting behaviour, the mouse was provided to *Ae. aegypti* during the day, while the animal was placed in the *Cx. quinquefasciatus* cages at night. The number of mosquitoes which blood-engorged was recorded at the fourth day. Twenty female blood-engorged mosquitoes were then transferred into individual polyethylene cups (height 8.9 cm×diameter 4.5 cm) and provided with 10% sucrose solution. Each individual was allowed to lay eggs up to the seventh day. For *Ae. aegypti*, a wet cotton bung lined with filter paper was provided as the oviposition site, while 40 ml of distilled water was introduced into each polyethylene cup for *Cx. quinquefasciatus*. The egg production of each mosquito was recorded daily.

The survival of the adult mosquitoes was monitored by recording the number of mosquitoes that died daily. The longevity of the mosquitoes was calculated according to a modified formula of Fletcher *et al.* (1990): longevity=Σ (number of dead mosquitoes×number of days mosquitoes survived)/total number of mosquitoes.

The larvae that hatched from the eggs produced by treated individuals were counted at the third and fourth day for *Ae. aegypti* and at the fourth and fifth day for *Cx. quinquefasciatus*. A total of 500 larvae from each experiment was taken at random for further study on the pupation rate and adult emergence rate. The adult mosquitoes were also segregated into males and females and counted. All experiments were replicated three times.

Effect of coil exposure time on blood-engorgement activity

As different coil exposure times had been set at 5 and 20 minutes, respectively, for *Ae. aegypti* and *Cx. quinquefasciatus*, a further study was conducted to determine the effect of this factor on blood-engorgement activity. In this study, adult females of *Ae. aegypti* and *Cx. quinquefasciatus* aged 4–5 days (sucrose-fed) were subjected to exposure time of 20 and 5 minutes, respectively and their blood engorgement activity were then assessed based on the method described earlier.

Data analysis

The bioefficacy data were subjected to probit analysis using a computer program developed by Daum (1970) to determine the KT_{50} values. Data from experiments on sublethal effects were analyzed with analysis of variance and means were separated with least significance difference (LSD). Data in percentage were subjected to arc-sine transformation before analysis of variance. All statistical analysis was performed using a statistical analysis computer program, StatGraphics Version 5.0.

RESULTS AND DISCUSSION

Chemical analysis and bioefficacy test

Chemical analysis using gas chromatography showed that the amount of d-allethrin in the coil formulation ranged from 0.2051 to 0.2057% w/w and from 0.1060 to 0.1088% w/w for d-trans allethrin. There were no active ingredients detected in the blank coil. This indicated that the amount of active ingredient did not deviate from what was stated for each formulation.

Results showed that *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* to both coil formulations containing an active ingredient (Table 1). This finding corresponded well with earlier findings by MacIver (1964), Chadwick (1975) and Yap & Chung (1987). MacIver (1964) suggested that the larger body size of the *Cx. quinquefasciatus* when compared to *Ae. aegypti* probably

Table 1. Efficacy of mosquito coils against *Aedes aegypti* and *Culex quinquefasciatus* of different ages and physiological conditions.

Active ingredient	age (d)	physiological/ nutritional condition*	*Aedes aegypti*		*Culex quinquefasciatus*	
			KT_{50}±S.E.[1]	% Mortality±S.E.[1]	KT_{50}±S.E.[1]	% Mortality ±S.E[1]
d-allethrin (0.2% w/w)	1–2	1	2.37±0.13 c	11.67±1.59 a	8.25±0.55 bc	7.33±0.73 a
	4–5	1	2.24 ± 0.02 bc	13.67±2.39 a	8.82±0.39 c	6.00±0.76 a
	9–10	1	2.01± 0.11 b	26.00±5.57 ab	7.91±0.39 bc	18.67±5.04 a
	14–15	1	1.39± 0.09 a	53.33±14.74 bc	7.26±.31 b	58.50±17.88 b
	19–20	1	1.28± 0.09 a	73.00±12.85 c	5.59±0.56 a	84.33±2.68 c
	4–5	2	3.09 ± 0.13 d	26.83±1.59 b	9.03±0.08 c	21.67±5.45 b
	9–10	3	2.08± 0.12 b	27.83±3.92 b	8.72±0.57 c	13.00±2.65 a
d-trans allethrin (0.1% w/w)	1–2	1	2.79± 0.09 c	13.67±2.62 a	8.71±0.73 b	8.17± 2.17 a
	4–5	1	2.44±0.08 b	17.50±4.19 a	9.20±0.75 b	7.67±2.33 a
	9–10	1	2.15±0.13 b	18.17±2.17 ab	8.41±0.31 b	25.17±8.15 a
	14–15	1	1.54±0.07 a	51.17±10.08 bc	7.36±0.75 ab	53.67±16.47 b
	19–20	1	1.38±0.09 a	72.00±15.74 c	5.70±0.42 a	81.83±4.23 c
	4–5	2	3.34±0.08 d	31.10±0.40 b	10.06±0.37 b	21.83±3.53 b
	9–10	3	2.27±0.10 b	25.33±4.48 ab	9.90±0.72 b	16.33±3.76 a
blank	1–2	1	–	1.17±0.17 a	–	1.33±0.44 a
	4–5	1	–	2.17±0.44 a	–	1.17±0.17 a
	9–10	1	–	2.67± 0.88 a	–	1.83±0.44 ab
	14–15	1	–	8.17±3.28 a	–	6.50±2.75 b
	19–20	1	–	27.17±9.10 b	–	16.33±4.47 c
	4–5	2	–	2.33±0.44 a	–	3.67±0.88 b
	9–10	3	–	3.33±0.83 a	–	1.83±1.59 ab
control	1–2	1	–	0.33±0.33 a	–	0.33±0.17 a
	4–5	1	–	0.50±0.29 a	–	0.50±0.29 a
	9–10	1	–	1.00± 0.29 a	–	0.50±0.29 a
	14–15	1	–	3.33±2.03 ab	–	0.67±0.17 a
	19–20	1	–	9.33±3.47 b	–	1.17±0.60 a
	4–5	2	–	0.67±0.17 a	–	1.33±0.33 a
	9–10	3	–	1.33±0.33 a	–	0.33±0.33 a

*1 = sucrose-fed only; 2 = sucrose- and blood-fed; 3 = oviposited.

[1] For each active ingredient, mean values of different ages follow by the same letter within the same column are not significantly different ($P > 0.05$; LSD test).

attributed to its increased tolerance. The blank coil caused neither significant knockdown nor mortality of mosquitoes, while coils containing either d-allethrin or d-trans allethrin provide adequate knockdown of mosquitoes, but with low mortalities (<30%) for both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes at age below 10 days. There was no significant difference between the activity of the two coil formulations containing either d-allethrin or d-trans allethrin, based on the KT_{50} values and mortality of mosquitoes.

Generally, the susceptibility (both KT_{50} and mortality) of both species of mosquitoes increased with age (Table 1). The *Ae. aegypti* aged 1–2 days and *Cx. quinquefasciatus* mosquitoes aged 4–5 days were most tolerant. High mortality was clearly demonstrated in the older groups of mosquitoes, i.e those aged 14–15 days and 19–20 days. Other studies with similar findings had been reported earlier on *Ae. aegypti* (David & Bracey, 1947), *Anopheles stephensi* (Rowland & Hemingway, 1987), house flies (Simanton & Miller, 1937) and German cockroaches (Lee *et al.*, In press).

Ae. aegypti mosquitoes fed with sucrose and also having blood-engorgement were more difficult to be knocked down than those fed with sucrose only, but were easier to be killed by coil exposures (Table 1). Similar findings were reported by David & Bracey (1947) when using pyrethrum. Other studies using different insecticidal formulations by Hadaway & Barlow (1956), Eliason *et al.* (1990) and Rieter *et al.* (1990) demonstrated that the susceptibility of mosquitoes is not only affected by blood-engorgement, but also by the period and stage of blood digestion by the mosquitoes.

No significant difference in KT_{50} and mortality of mosquitoes was found before and after oviposition (Table 1).

Sublethal effects of mosquito coil

The blood-engorgement activity of *Cx. quinquefasciatus* decreased to less than 40% after exposure to mosquito coils with or without active ingredients (Table 2). Blood-engorgement activity for this species was highest when the mosquitoes were aged 4–5 days. This ability later decreased with increasing age. *Cx. quinquefasciatus* aged 14–15 and 19–20 days practically ceased blood-engorgement activity after exposure to coils containing d-allethrin and d-trans allethrin.

By contrast, a five minutes exposure of *Ae. aegypti* to coils did not affect its blood-engorgement ability (Table 2). An earlier study by Liu *et al.* (1986) showed that the blood-engorgement activity of *Ae. aegypti* decreased after being subjected to pyrethroid treatment at high dosages.

When the exposure times for the two species was reversed, it was found that the blood-engorgement activity of *Ae. aegypti* was significantly reduced when subjected to 20 minutes of exposure to both coil formulations while the smoke from the non-active ingredient formulation did not affect this parameter (Table 3). On the other hand, after exposing *Cx. quinquefasciatus* for 5 minutes to coils, its blood-engorgement activity was not affected. It can be concluded here that the time of exposure to a coil affects the blood-engorgement activity in mosquitoes.

One question that arose during this study was whether mosquitoes that had little or no blood-engorgement activity due to exposure to mosquito coil, may recover after some time. A further study showed that after exposure to d-allethrin and d-trans allethrin, *Cx. quinquefasciatus* gradually recovered from day 3 (10–13%) to day 10 (~41%) post-exposure (MP Lim, unpublished).

The number of eggs produced by treated mosquitoes (all coil formulations) that engorged blood were not significantly different from the control. Generally, *Cx. quinquefasciatus* produced more eggs (an average of 160 eggs/female) as compared to *Ae. aegypti* (80 eggs/female). The number of eggs produced however, decreased with increase in age for both species of mosquitoes. In addition, the older mosquitoes only managed to achieve partial engorgement (half-full abdomen) (MP Lim, unpublished).

No significant difference was observed in egg hatchability rate of treated mosquitoes (all coil formulations) when compared with untreated ones. However, the hatchability rate of eggs laid by *Cx. quinquefasciatus* aged 1–2 days (22–45% only) was significantly lower than other age groups (80–91%). Such a finding was not observed for *Ae. aegypti.*

No significant difference in terms of pupation rate, adult emergence and adult female: male sex ratios were observed for the progenies of treated mosquitoes when compared with those untreated.

The longevity of treated mosquitoes (subjected to coil formulations containing either d-allethrin or d-trans allethrin) was reduced by at least 40% and 50% in *Cx. quinquefasciatus* and *Ae. aegypti*,

Table 2. Effect of mosquito coil exposures on blood-feeding activity in *Aedes aegypti* and *Culex quinquefasciatus*.

Species age	physiological/ nutritional condition*	Mean % blood feeding±S.E.M[1]			
		d-allethrin	d-trans allethrin	blank coil	control
Aedes aegypti					
1–2	1	98.38±0.52 a (a)	97.20±1.24 a (a)	98.80±0.74 a (a)	97.14±1.63 a (a)
4–5	1	91.39±5.60 a (a)	93.10±0.60 a (a)	98.59±0.34 a (a)	98.80±0.44 a (a)
9–10	1	95.68±1.60 a (a)	96.36±1.63 a (a)	97.60±1.36 a (a)	97.83±1.37 a (a)
14–15	1	94.69±2.41 a (a)	95.93±1.38 a (a)	98.34±0.65 a (a)	98.80±0.60 a (a)
19–20	1	98.07±1.93 a (a)	94.66±1.56 a (a)	96.98±1.31 a (a)	98.24±1.30 a (a)
9–10	2	97.24±1.64 a (a)	97.82±0.53 a (a)	98.06±1.94 a (a)	97.35±2.39 a (a)
Culex quinquefasciatus					
1–2	1	9.64±4.14 a (a)	6.92±2.55 a (a)	16.91±2.95 ab (a)	94.27±2.75 a (b)
4–5	1	13.10±0.99 a (a)	10.49±4.68 a (a)	40.04±12.92 b (a)	95.27±2.39 a (b)
9–10	1	3.25±0.85 ab (a)	2.64±0.81 a (a)	8.44±4.86 a (a)	95.26±1.95 a (b)
14–15	1	0 ab (a)	0 b (a)	9.23±2.34 a (b)	91.63±5.97 a (c)
19–20	1	0 ab (a)	0 b (a)	4.34±1.76 a (b)	88.86±6.68 a (c)
9–10	2	2.73±1.49 ab (a)	1.15±0.26 ab (a)	10.72±0.70 a (b)	90.17±3.61 a (c)

[1] For each species, mean values follow by the same letter within the same column are not significantly different; mean values in ( ) follow by the same letter within the row are not significantly different (P > 0.05; LSD test).
*1 = sucrose-fed only; 2 = sucrose- and bloodfed and oviposited.

Table 3. Effect of coil exposure time on blood-engorgement activity of *Aedes aegypti* and *Culex quinquefasciatus*[1]

exposure time (min)	mean % blood engorgement activity±S.E.M[2]			
	d-allethrin	d-trans allethrin	blank coil	control
Aedes aegypti				
5	91.39±5.60 a	93.10±0.60 a	98.59±0.34 a	98.80±0.44 a
20	53.01±3.74 b	50.51±0.57 b	98.38±1.13 a	98.86±0.89 a
Culex quinquefasciatus				
5	96.79±0.90 a	98.12±0.32 a	98.16±0.33 a	98.30±0.91a
20	13.10±10.99 b	10.49±4.68 b	40.04±12.92 b	95.27±2.39 b

[1]Adult females aged 4–5 days (sucrose-fed) were used in this study.
[2]For each species, mean values follow by the same letter within the same column are not significantly different (P>0.05; Student t-test).

respectively (Table 4). However, the longevity of mosquitoes exposed to blank coil was not significantly different from control except in *Cx. quinquefasciatus* aged 9–10 days and in *Ae. aegypti* aged 19–20 days. It was also shown that when the mosquitoes were exposed at a later age, longevity was further decreased. Decrease in longevity of insects treated with insecticides has been reported previously, such as in *Ae. aegypti* (Duncan, 1963) and German cockroaches (Abd-Elghafar & Appel, 1992; Lee, 1995).

Significant sublethal effects of coil formulations against *Ae. aegypti* and *Cx. quinquefasciatus* demonstrated in this study include shorter longevity and reduced blood-engorgement activity. With its proven efficacy and consumer friendly properties, the mosquito coil can be incorporated into overall vector control strategies for major mosquito-borne diseases, especially in the endemic tropical areas.

Table 4. Effect of mosquito coil exposures on the adult longevity of *Aedes aegypti* and *Culex quinquefasciatus*.

Species age	physiological/ nutritional condition*	Mean longevity±S.E.M[1]			
		d-allethrin	d-trans allethrin	blank coil	control
Aedes aegypti					
1–2	1	15.48±5.48 a (a)	15.26±3.02 a (a)	31.72±0.78 a (b)	38.10±1.66 a (b)
4–5	1	13.62±0.57 a (a)	15.09±0.98 a (a)	24.47±3.75 ab (b)	35.85±2.18 ab (c)
9–10	1	11.21±0.87 ab(a)	11.78±1.01 a (a)	22.62±2.13 b (b)	30.82±0.66 b (c)
14–15	1	4.15±1.08 bc (a)	5.17±1.29 b (a)	13.71±2.74 c (b)	13.93±2.75 c (b)
19–20	1	2.28±0.80 c(a)	2.42±0.82 b (a)	8.33±1.14 c (b)	9.30±0.38 c (b)
4–5	2	12.81±3.16 ab (a)	12.95±4.11 a (a)	26.68±1.98 ab (b)	28.87±6.70 b (b)
9–10	3	11.81±2.50 ab (a)	13.07±2.95 a (a)	25.82±2.29 ab (b)	27.99±3.26 b (b)
Culex quinquefasciatus					
1–2	1	33.29±3.19 a (a)	34.19±4.80 a (a)	48.10±0.32 ab (b)	49.98±0.24 a (b)
4–5	1	28.15±0.49 a (a)	28.92±2.62 a (a)	53.72±5.37 a (b)	56.29±4.99 a (b)
9–10	1	11.27±2.47 b (a)	8.87±2.17 bc (a)	38.83±3.16 b (b)	44.38±3.92 a (b)
14–15	1	3.72±1.81 c (a)	3.60±1.62 c (a)	22.31±6.53 c (b)	26.70±7.19 b (b)
19–20	1	1.58±0.06 c (a)	1.71±0.17 c (a)	7.75±1.05 d (b)	16.63±2.17 b (c)
4–5	2	22.46±4.37 a (a)	25.14±5.77 a (a)	44.06±3.92 ab (b)	49.05±2.58 a (b)
9–10	3	16.79±1.23 ab (a)	14.96±3.09 b (a)	38.91±3.11 b (b)	43.44±3.81 a (b)

[1]For each species, mean values follow by the same letter within the same column are not significantly different; mean values in ( ) follow by the same letter within the same row are not significantly different (P>0.05; LSD test).

*1=sucrose-fed only; 2=sucrose- and blood-fed; 3=sucrose- and bloodfed and oviposited.

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