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KNOCKDOWN OF ADULT MOSQUITOES (DIPTERA: CULICIDAE) EXPOSED TO VAPORIZED ACETONE

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Abstract Inhalation of acetone at concentrations above the permissible exposure level (PEL) has been reported to cause adverse effects on human central nervous system, characterized by nausea, headache, dizziness and motor incoordination. The PEL in most European Union countries is limited to 500 ml/m^3. This study investigates the possibility of vaporized acetone in affecting mosquito nervous systems, characterized by knockdown i.e. inability of mosquito to fly or stand. *Aedes aegypti, Culex quinquefasciatus* and *Toxorhynchites splendens* exposed to vaporized acetone at a dose of one-sixth of the PEL (PEL/6) of in an airtight flask (0.002 m^3) were knocked down in less than 19 minutes. *Culex quinquefasciatus* were significantly ($P < 0.05$) more susceptible to acetone compared to *Aedes aegypti* and *Toxorhynchites splendens.* Differences of knockdown time between male and female among these three species were insignificant ($P > 0.05$).
Key Words Volatile organic compound, neurotoxin, chemical safety, Culicidae

INTRODUCTION

Mosquitoes are well known as vectors of major public health diseases such as dengue fevers, malaria, filariasis, and Japanese encephalitis (Lee et al., 2003). The Southeast Asia region, which is geographically located in the tropical zone, provides a suitable habitat for mosquito breeding throughout the year (Hassan and Salmah, 1990; Lee, 1991; Vythilingam et al., 1992). Hence, controlling mosquito population in this region has become a routine task. Besides large scale control activities carried out by governmental agencies through fogging and ULV sprays of insecticides, community members also take initiatives to protect themselves, which is categorized as personal protection measures. Burning of mosquito coils as a personal protection measure against mosquitoes is being widely practiced by the local community in the Southeast Asia region including Malaysia (Lee et al., 2003; Rozendaal, 1997).

The capability of mosquito coil in providing protection against mosquitoes is due to the content of active ingredient namely d-allethrin, d-trans allethrin, prallethin etc. These active ingredients are chemicals known as insecticides that affect the mosquito's nervous system. Insecticides attack the parasympathetic nervous system of insects either by disrupting the transmission of impulse by acetylcholine at the synapse of neuron or by destroying the kalium-natrium ionic balance at neuron axon (Lee et al., 2003; Tashio and Janice, 1989). The effects on nervous system are manifested through the mosquito's inability to detect human (sensory inhibition), avoidance from treated area (repellence), inability to coordinate movement (paralysis) and death (Lee et al., 2003; Yap and Chung, 1987; Yap et al., 1990). The effect of paralysis is easily observable within 2 to 4 minutes after exposing mosquitoes to a low dose of d-allethrin at 4.4 mg/m^3 (Yap and Chung, 1987; SIRIM, 1996). In view of such high sensitivity of mosquito to chemicals that affects nervous system, one wonders whether mosquito could exhibit similar sensitiveness to other chemicals besides insecticides that also affects nervous system.

Bernier et al. (2003), Kellogg (1970), Schofield and Brady (1997), Takken et al. (1997) and Vale (1980) reported that small quantity of acetone, e.g. 0.01 µg/l in the air was capable to elicit neurophysiological responses separately in adult *Aedes aegypti*, *Stomoxys calcitrans*, *Glossina* sp. and other dipterans. These

reported studies indicate that dipterans nervous system including mosquitoes is sensitive to low concentration of acetone. However, information was lacking on the effects of acetone against mosquito at higher doses, which may paralyze or kill the mosquito. Thus, this study was aimed to explore the response of common species of mosquitoes to acetone at a high dose but within the maximum concentration that is considered least hazardous to human, which is known as permissible exposure limit (PEL). The PEL for inhalation of acetone in work place in most European Union countries is limited to 500 ml/m^3 (Dalton et al., 1997; Merck, 2006). This study postulates the capability of vaporized acetone in affecting mosquito nervous systems would be characterized by the inability of mosquito to fly or stand (knockdown) upon acute exposure (John and Gary, 2001; Lello and Nieri, 1999). As such the objective of this study was to determine the knockdown activity displayed by different species of mosquito upon exposure to acetone at the dose below PEL.

MATERIALS AND METHODS

Mosquito Colony

The mosquitoes for the study, namely *Aedes aegypti, Culex quinquefasciatus* and *Toxorhynchites splendens* were sourced from an established laboratory colonies maintained at 27 ± 2°C, 70 ± 10% and photoperiod of 12:12 hours in the insectarium of Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia, a WHOPES regional centre. These mosquitoes were bred using rearing technique described by Chong, et al. (1998). In general, male and female mosquitoes were fed with sugar solution (10% w/w) only and caged together to allow mating. Regularly, female mosquitoes were provided mouse blood for eggs production, except for *Toxorhynchites splendens*.

Method

Acetone of analytical grade (>99.7% purity) was purchased from Merck KGaA, Germany and used in different experiments of this study. The experiment room was protected from sunlight and direct heat source while room air was ventilated as safety precautions in handling volatile organics. Room temperature and humidity were maintained at 26-28°C and 60-80% RH, respectively.

A male and a female of each species of mosquitoes, namely *Aedes aegypti, Culex quinquefasciatus* and *Toxorynchites splendens* aged 2 to 7 days were aspirated from holding cages and transferred into an air-dried 2000 ml volumetric flask. The whole volume of the volumetric flask was approximately 2090 ml. After the transfer, a piece of net (16 mesh) was inserted loosely into the neck of the volumetric flask, to confine the mosquitoes. Then a piece of filter paper sized at 10 x 3 cm was placed next to the net. The flask was air-tightened with a rubber septum that has been inserted with a hypodermic syringe (18G, 1½TW, 1.2 x 38.0 mm, 10 ml). The mosquitoes were left inside for a period of 30 minutes to observe any abnormality that may suggest the unfitness of mosquito or possible accidental contamination of test apparatus.

Next, a total of 157 µl of acetone that is equivalent to one-sixth of human PEL was treated on the filter paper by using a 250 µl gastight microsyringe inserted through the hypodermic needle. The acetone was vaporized in less than 60 seconds with 99% vaporization efficiency. For control experiment, acetone sample was replaced with water. Upon removal of microsyringe, time recording was initiated and mosquito response was observed for a period of 60 minutes. The time taken by the mosquito to exhibit knockdown behaviour was recorded. The knockdown behaviour was characterised by the inability of mosquito to coordinator its normal movement, for example the inability to stand, walk and fly (SIRIM, 1996). At the end of 30th minute, the mosquitoes were disposed accordingly. The remaining acetone inside the glass flasks were flushed at the velocity of 40 ml/s using an air pump (Mark II, NS6200), then both the glass tubes and flasks was submerged in 0.01% chlorhexidine gluconate solution before been rinsed with tap water and dried at room temperature.

The magnitude of acetone affecting the adult mosquitoes was gauged based on the quickness of mosquito to exhibit the knockdown behaviour. In order to ensure the precision of results, the experiment was replicated 20 times.

Data Analysis

Knockdown times recorded in this study were complied according to species and sex. The compiled results were analyzed for precision and normality of distribution using descriptive statistics. Later the data were

also analyzed with one way ANOVA and Student-Newman-Keuls mean comparisons. The statistical analysis was run using the SPSS computer program (Zar, 1974; Kinnear and Gray, 1997).

RESULTS AND DISCUSSION

Descriptive statistical analysis showed the proportion of standard deviation against mean of 20 replicates of knockdown times of *Aedes aegypti* - female*, Aedes aegypti* - male, *Culex quinquefasciatus* - female, *Culex quinquefasciatus* - male, *Toxorhynchites splendens* - female and *Toxorhynchites splendens* - female was 11.6, 21.7, 16.5, 16.7, 25.5 and 16.9%, respectively. Knockdown times displayed by all there species of both sexes were found to be normally distributed with skewness indices (skewness ÷ standard error of skewness) of between -0.25 to 0.64. In general, the method utilized in this study was able to distinguish and quantify the mosquito susceptibility to acetone at a moderate precision.

All three species of mosquitoes were found to display the inability to fly or stand after been exposed to vaporized acetone at PEL/6. For the control experiments, none of the mosquitoes were knocked down. The mean knockdown times of the studied mosquito species are shown in the Figure 1. As such the postulate on the capability of vaporized acetone in knocking down mosquito is seems to be true. Though this study was not designed to ascertain the cause of knockdown but visual observation of sudden aggressive movement, rubbing of antenna and rapid knockdown activity suggest the effects on mosquito nervous system.

Females of *Aedes aegypti, Culex quinquefasciatus* and *Toxorhynchites splendens* were knocked down within 15.7, 11.9 and 15.2 minutes, respectively. Whereas the male mosquitoes were knocked down in 14.2 minutes (*Aedes aegypti*), 10.9 minutes (*Culex quinquefasciatus*) and 15.1 minutes (*Toxorhynchites splendens*). Both females and males of the respective species displayed same susceptibility level ($P < 0.05$). This finding seems to differ from the common believe that male mosquitoes are more susceptible than female mosquitoes. Comparisons of the results among species showed *Culex quinquefasciatus* to be most susceptible ($P < 0.05$) to acetone while *Aedes aegypti* and *Toxorhynchites splendens* displayed a similar trend and of knockdown time ($P > 0.05$). This finding also goes against the common understanding that *Aedes aegypti* is most susceptible to insecticide. Perhaps these two unique findings tell us that the acetone mode of action could be different from the typical insecticidal mode of action.

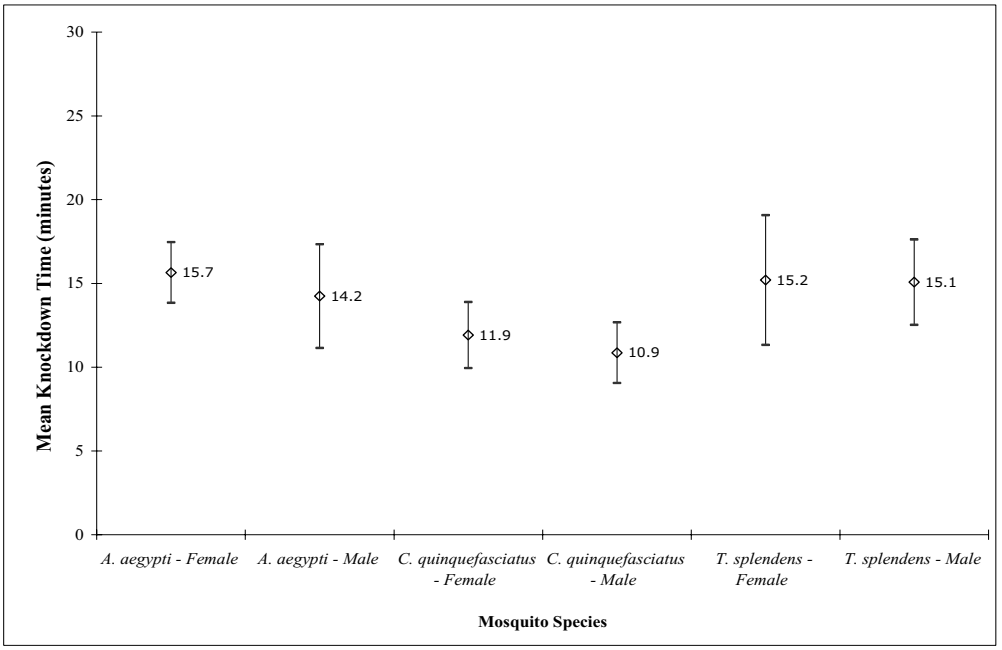

Figure 1. Mean knockdown time of different mosquito species upon exposure to acetone at the dose of PEL/6 in 0.002 m^3 volumetric flask.

CONCLUSIONS

The findings of this study conclude that vaporized acetone at the dose of one-sixth of PEL has capability to knockdown both males and females of *Aedes aegypti, Culex quinquefasciatus* and *Toxorhynchites splendens.* Visual observation on the behaviour of post treatment mosquitoes, strongly suggest the possibility of acetone affecting the mosquito nervous system. However, the findings of this study are not comprehensive and unable to answer many questions pertaining to the knockdown of mosquito by acetone, further investigation will be required.

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