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The influence of ambient temperature on the insecticide resistance of *Aedes aegypti* larvae to the temephos: An organophosphate insecticide

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Abstract

Insecticides are the most common management strategy used for the control of mosquitoes. The changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms. We examined the influence of temperature on the development of resistance against temephos of larvae *Aedes aegypti* (Diptera: Culicidae). LC_{50} was estimated for larvae *Ae. aegypti* when exposed to six concentration of temephos (0.004, 0.006, 0.009, 0.012, 0.015, and 0.020 ppm) and the temperature 23, 26, 30, 32, and 34°C for 24 hours in larvae assays. If mosquito populations are expanding in space and time because of increased temperatures due to global warming and cannot be managed as effectively with organophosphate, the spread of mosquito-borne diseases may pose considerable risk to public health.

Keywords: Insecticide, organophosphate, temperature, mosquitoes, temephos and public health

Introduction

Many of the emerging diseases that threaten public health are transmitted by arthropod vectors such as mosquitoes (malaria, dengue fever, yellow fever and chickungunya). It has been well documented by world health organization (WHO) and in numerous scientific investigations and reports that the use of synthetic insecticides can dramatically reduce the risk of insect borne diseases, particularly in the case of dengue. *Aedes aegypti* (Linn) is an important urban mosquito vector of dengue fever in India. *Ae. aegypti* has been found responsible for the outbreak of dengue fever (DF) and dengue hemorrhagic fever (DHF) in many urban areas of the country. *Ae. aegypti* thrive in environments closely associated with humans and the majority of their breeding sites result from outdoor pots and containers around human dwellings (Vontas *et al.*, 2012) [21]. Insecticides are the most common management strategy used for mosquito control (Alves *et al.* 2002, Swain *et al.* 2009) [1, 19]. Over the past decade, the use of organophosphate insecticides for the control of vector-borne diseases has increased (Gratz 2004, Vontas *et al.*, 2012) [7, 21]. The use of synthetic organophosphate rapidly increased during the 1970's (Schleier and Peterson 2011) [18] as they have a quick knockdown effect, high insecticidal potency, low mammalian toxicity, and do not bio accumulate (Swain *et al.* 2009, Casida 2010) [19]. However, due to the extensive use of organophosphate insecticides, resistance has evolved in many insect species (Swain *et al.* 2009) [19]. Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms (Andersson and Forlin 1992) [2]. The temperature-dependence relationship in insecticides is of great importance, as from 1948 to 1991 there was a shift to warmer temperatures in the late afternoon hours when compared to other parts of the day for all seasons of the year in the United States (Knappenberger *et al.*, 1996) [13]. Globally, it is projected that by the year 2010, there will be approximately at 1.0 to 3.5°C increase in temperature (Karl *et al.*, 1995, USGCRP 2009, Johnson and Sukhdeo 2013) [9, 11, 20]. This is important because a majority of current mosquito management strategies utilize insecticides in environments shown to have increasing annual minimum, maximum and late afternoon temperatures (Knappenberger *et al.*, 1996, Vose *et al.*, 2005) [13, 22]. Increased temperature due to global warming has also been shown to positively affect mosquito population growth, development, survival, the biting rate of mosquitoes and the incubation/replication time of a virus within mosquitoes (Moudy *et al.* 2007, Kilpatrick *et al.* 2008, Johnson and Sukhdeo 2013, Anyamba *et al.*, 2014) [3, 9, 12, 16]. All of the above mentioned scenarios may pose considerable risk to public health and the spread of mosquito-borne diseases. Studies have shown that changes in ambient temperature can alter the rate of chemical uptake, metabolism, depuration and pesticide toxicity in many ectothermic organisms (Osterauer and Kohler 2008, Harwood *et al.*, 2009, Weston *et al.* 2009, Laetz *et al.*,

2014) [8, 15, 17, 23]. For insect orders, studies have shown that temperature directly affects the toxicity of insecticides to insects (Devries and Georghiou 1979) [6]. Organophosphates are known to exhibit a positive correlation between ambient temperature and mortality for many insect species. The carbamates are known to exhibit a slightly negative to positive correlation between ambient temperature and mortality for many insect species (Devries and Georghiou 1979) [6]. Organophosphate is known to exhibit a clearly negative correlation between increasing ambient temperature and mortality for many insect species.

Materials and methods

Insect rearing

Aedes aegypti was the model organism used because of its medical importance as a major vector of pathogens causing significant disease (Vontas *et al.* 2012, Powell and Tabachnick 2013) [21] rearing room (7.65 m²) with temperature of 27 ± 2°C, relative humidity 60-80%, and photoperiod 14:10 [L/D]. The eggs were housed in plastic containers (27.94 cm x 16.83 cm x 6.99 cm or 35.56 cm x 22.23 cm x 10.16 cm) containing 27 ± 2 °C de-ionized water. *Ae. aegypti* larvae were fed 2.46 ml (1/2 teaspoon) of dog biscuit and yeast At pupation, they were transferred to 27 ± 2 °C deionized water. Each container was placed with a netted top. The assay was done with 125 to 150 fourth instars larvae of *Aedes aegypti* maintain with five replicate and without insecticides are served as a control.

Chemicals

Temephos was selected for the present studies. Temephos is an insecticide registered by the U.S. Environmental Protection Agency (EPA) in 1988. Temephos belongs to a group of chemicals called organophosphate. It is white crystal in nature. It has low water solubility and is nonvolatile. Temephos in ethanol solution

Bioassays at Different Temperatures

The randomly set a temperatures at 23, 26, 30, 32, 34 ± 2°C with relative humidity 60-80% and photoperiod 14:10 [L/D] h. test tubes were used to done an assay and were diluted with insecticide, ethanol were used. The concentration of temephos was 0.005, 0.01, 0.02, 0.04, 0.08 and 0.16 ppm and an ethanol served as a control. The Treated test tubes were placed in the rearing room (27 ± 2°C, relative humidity 60-80%, and photoperiod 14:10 [L/D] h) overnight and covered to avoid light exposure. The next morning test tubes with mosquito larvae were taken in to the account. Each test tube with mosquitoes was placed in a randomly selected temperature unit/environmental growth chamber and allowed to acclimate for two hours. After 24 hours, the number of alive and dead mosquito larvae was assessed. The experimental design was a complete randomized block with 50 individuals per concentration, five concentrations, and six temperatures (23, 26, 30, 32, 34 ± 1 °C). A minimum of seven replications were conducted at each temperature.

Statistical analysis

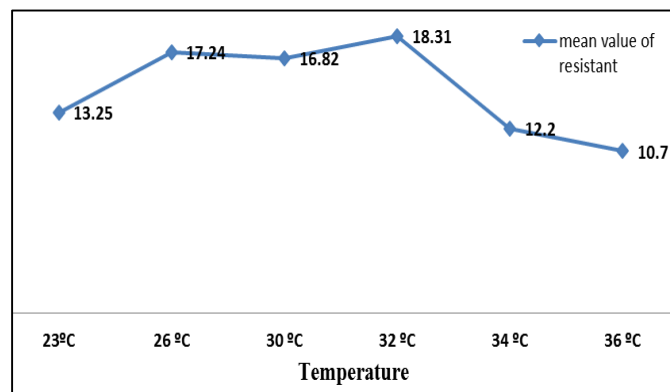
The statistical analysis was made by using SPSS 5.0 of Mean, Standard error and P-value. The t-test showing the level of significance is 0.05% (t-2.44).

Result

The influence of temperature on insecticide resistant of temephos to larvae *Aedes aegypti* was estimated under the

laboratory conditions. This result shows the influence of temperature on resistant on the dengue vector. The temperature of 26°C, 30°C and 32°C shows highest insecticidal resistant (17.24±0.48, 16.82±0.73 and 18.31±0.28). The 36 °C of temperature has a least (10.7± 0.24) mean value than the other temperature, it clearly shows that increasing temperature that simultaneously influences the increasing insecticide resistant activity but in further increasing temperature, the resistant against insecticide decreased.

Temperature influencing insecticidal resistance of Temephos on *Aedes aegypti* larvae



Discussion

The activity of insecticides for different insect species is temperature dependent (Devries and Georghiou 1979; Toth and Sparks 1988) [6]. Studies have consistently reported positive temperature coefficients for organophosphates, and slightly negative to positive temperature coefficient for carbamates (Devries and Georghiou 1979, Johnson 1990) [6, 29]. Susceptible and resistant strains of adult *An. gambiae* were exposed to permethrin for 24 hours, a positive temperature coefficient for toxicity was observed between 16 and 37°C (Hodjati and Curtis 1999). However, as part of the same study, a positive temperature coefficient for toxicity was only observed between 28 and 37°C when resistant *An. stephensi* were exposed to permethrin. Changes in ambient temperature affect the rate of chemical uptake, binding affinity, metabolism, and excretion of insecticides for insects, as they are ectothermic organisms (Osterauer and Kohler 2008, Harwood *et al.* 2009, Weston *et al.* 2009, Laetz *et al.* 2014) [8, 15, 17, 23]. Temperature affects metabolic function and toxicokinetic rates of many ectothermic organisms (Weston *et al.* 2009) [23]. Weston *et al.* (2009) [23] reported greater uptake of permethrin at 23°C when compared with 13°C for *Chironomus dilutes* (Diptera: Chironomidae). As a result at lower temperatures there is less penetration of the parent compound and less metabolite formation in insects. This is supported by the decreased rate of parent compound bio transformed to the less toxic metabolites at 23°C compared with 13°C when *C. dilutus* were exposed to permethrin. Insecticide metabolism is temperature dependent and at lower temperatures the more toxic parent compound persists in insects. For many insect orders, temperature directly affects the toxicity of insecticides to insects (Devries and Georghiou 1979) [6]. Organophosphates are known to exhibit a positive correlation between ambient temperature and mortality for many insect species and carbamates are known to exhibit a slightly negative to positive correlation between ambient temperature and mortality for many insect species (Devries and Georghiou 1979) [6]. Temperature coefficients are often

used in laboratory trials to determine the relationship between temperature and toxicity for an insecticide over a range of temperatures (Ma *et al.* 2012, Glunt *et al.*, 2013) [31].

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