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EFFECT OF DIFFERENT PLANTS EXTRACT ON ACETYLCHOLINESTERASE ACTIVITY OF *AEDES AEGYPTI* AND *MUSCA DOMESTICA* ADULTS

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ABSTRACT: The present study was conducted to evaluate AChE inhibitory effect of three plants (*Calotropis procera*, *Eucalyptus globulus* and *Mentha spicata*) extract using *Aedes aegypti* and *Musca domestica* as model insects. The effect caused by plants was also compared with commonly used insecticide i.e., Chlorpyrifos. WHO recommended protocol was used for conducting bioassay tests against selected insects. Three different doses of plants extract were used. The mortality rate was assessed after 24 hour post treatment. The AChE activity was determined by spectrophotometry at 412nm wavelength using Ellman's assay. There was a marked decline in the enzyme activity of treated groups as compared to control group. *M. spicata* treated groups proved to have high AChE inhibition power among three plants extract. While, *C. procera* extract effectively control both *Ae. aegypti* and *M. domestica*. It is concluded that plants extract not only inhibit the AChE activity of insects but also control them very proficiently.

Keywords: AChE, *Aedes aegypti*, *Musca domestica*, Chlorpyrifos, plant extract

INTRODUCTION

A number of insect pests are damaging the human health badly because they act as vectors for certain disease causing agents. Of these *Aedes aegypti* and *Musca domestica* are well known examples. Mosquitoes are playing a role of vector for various pathogens causing numerous diseases like dengue fever, yellow fever, malaria, chikungunya and filariasis posing a great threat to human life (Jang *et al*., 2002). Similarly, house fly (*Musca domestica* Linnaeus) is closely associated to human, poultry as well as with livestock throughout the world (Rahul, 2013). These flies are playing a major role as vector of disease causing agents (Macovei *et al*., 2008) and become a root cause of more than 100 diseases in human (Mian et al., 2002) and animals (Tian *et al*., 2011). House flies are also reported to be a carrier of bird flu virus thus posing a great threat to human, poultry and live stock (Iqbal *et al*., 2014). House flies spread many diseases in humans, poultry, and livestock (Iqbal *et al*., 2014). In poultry forms, poultry manure having high humidity and temperature becomes a suitable environment for reproduction of house flies (Khan *et al*., 2012). High densities of house flies not only cause annoyance to hens and the poultry workers but also affect the quality of their products (Acevedo *et al*., 2009).

A variety of pesticides are used by farmers against the insect pest populations that destroy crops yield (Popischil *et al*., 2005; Aktar *et al*., 2009). The field use of these pesticides not only kill the target organisms but also pose a great threat for human life (Igbedioh,1991; Jeyaratnam, 1985; Forget, 1993). These pesticides contaminated-fruits and vegetables are eventually consumed by humans and damage their organs. Pesticides may affect the various sites in target organism, one of which is Acetycholinesterase (AChE) (Soreq and Zakut, 1993). Pesticides act as toxins even at low concentrations and inhibit AChE activity (Varó *et al*., 2002).

AChE is an important enzyme involved in hydrolysis of acetylcholine (ACh) and thus completing the cholinergic neurotransmission (Colovic *et al*., 2013; Tripathi and Srivastava, 2008). In insects and humans it causes termination of cholinergic neurotransmission at synapses (Carlier *et al*., 2008). This enzyme is also found in many types of conducting tissues. However, its major concentration is found in brain muscles (Massoulie, 2002). It is also present in liver, in red blood cells and muscles (Chen *et al*., 2010). The activity of AChE is higher in motor neurons than in sensory neurons (Koelle, 1954; Massoulie *et al*., 1993; Srivastava and Tripathi, 2007).

AChE activity is considered the major resistance mechanisms against the organophosphrous and carbamate in insects (Hemingway *et al*., 1986; Yoo *et al*., 2002). Along with the target organisms, pesticides also affect non-target organism including humans. Approximately 200,000 deaths are recorded world-wide due to pesticide poisoning (Attia, 2006; Dawson *et al*., 2010).

Therefore, it is absolutely necessary to discover and develop some alternative pest control strategies that destroy the harmful pests without harming the beneficial organisms. In this context, use of biopesticides is the best solution as they are eco-friendly and cause minimal or no

harm to non-target organisms (Gupta and Dikshit, 2010; Kandpal, 2014; Ortiz and Possani, 2015). In the present study, extracts of some local plants (*Calotropis procera*, *Mentha spicata* and *Eucalyptus globulus*) were evaluated for their potential as substitutes of harmful pesticides to control the *Ae. aegypti* and *M. domestica* population by inhibiting their AChE enzyme activity.

MATERIALS AND METHODS

Plant extraction: The leaves of *Calotropis procera* (Family: Asclepediaceae), *Eucalyptus globules (Family: Myrtaceae)* and *Mentha spicata* (Family: Lamiaceae) were collected. These leaves were collected from plants then washed with water and kept for seven days or more in shady areas for drying. After that dried leaves were grounded to a powder and then stored in glass bottles. Methanolic extract of the leaves were prepared by following the method of Mishra *et al*. (2009).

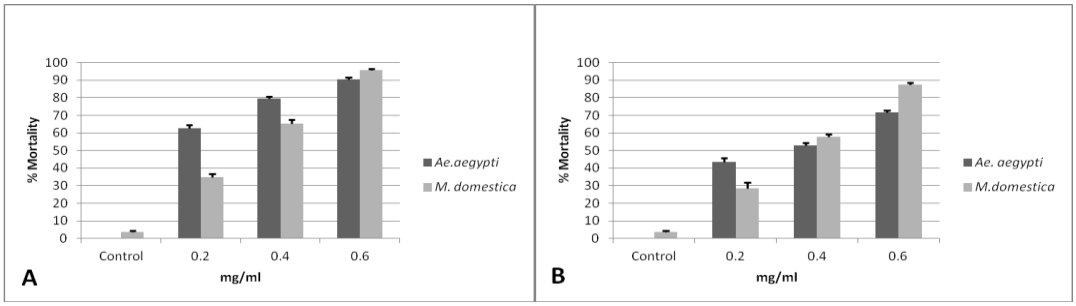
Bioassay against plant extracts: Adult mosquitoes (*Ae. aegypti*) were a gift from the insectory of GC University Lahore, Punjab, Pakistan. The adult house flies (*M. domestica*) were collected from local areas of Lahore. WHO recommended bioassay cones were used for residual bioassay. For this purpose, different dilutions (0.2 mg/ml, 0.4 mg/ml and 0.6 mg/ml), were prepared from stock solution (150 mg/150 ml) of plants extract. A filter paper was cut according to the size of a cone and impregnated with specific dose of each plant extract. The impregnated filter paper was kept for drying for almost 15 minutes. After that, filter paper was adjusted in bioassay cone (bottom and all sides) and 15 adult *Ae. aegypti* (both male and female) were introduced in it. After 1 hour exposure, treated *Ae. aegypti* were removed and transferred to a new bioassay cone without filter paper. Mortality rate was assessed after 24 hours. The experiment was repeated thrice. The same procedure was used for *M. domestica*. After 24 hours, heads of these treated insects were removed and homogenized in sodium phosphate buffer (0.1M, pH: 8.0). The homogenate was centrifuged at 15000rpm for 15 minutes. The resulting supernatant was collected and stored at -20 ºC. In case of control groups, the filter paper was soaked in distilled water and same procedure was followed as described above for experimental groups. Adults of both selected species were considered dead when they were not able to move their legs. Chlorpyrifos impregnated filter paper was used as positive control.

Ellman assay for Acetylcholinesterase estimation: AChE activity was determined by method of Ellman et al. (1961) with slight modifications. The volume of reaction mixture was 3ml. We added 1.5 ml (100mM) sodium phosphate buffer (pH 8.0), 0.3 ml of 5 mM DTNB [5, 5'-dithiobis-(nitrobenzoic acid), 0.3 ml (5 mM) acetylthiocholine iodide (ATI), 0.1 ml homogenate (10%), and 0.8 ml distilled water in reaction mixture. The absorbance was recorded at 412 nm at 28°C for 3 min in spectrophotometer (APELPD-303S). For each tissue homogenate measurements were made in triplicate.

Statistical analyses: Normality of the data was assessed before analyzing the data. Parametric test was applied on normally distributed data. One way ANOVA followed by Tukey`s test was applied to compare the acetylcholineterase activity among different groups. Probit analysis was used to compute LD_{50} values. Statistical Package for Social Sciences (SPSS version 16) and Minitab (13.4) was used for statistical analysis.

RESULTS AND DISCUSSION

Dose dependent response of mean percentage mortality was observed against each plants extract in both insect species (Figure 1). *C. procera* extract found to be more efficiently control the *Ae. aegypti* and *M. domestica* compared to the other two plants extract. In control group, 3.7±0.9% mortality was observed after 24h period in *M. domestica* adults. The mortalities 90.3±1.45%, 71.67±1.2% and 74.67±1.76% were noted in *Ae. aegypti* adults treated with the 0.6 mg/ml dilution of the extract of *C. procera*, *E. globulus* and *M. spicata* respectively. Similarly, 95.67±0.882%, 87.33±1.20% and 94.33±0.882% deaths were observed in *M. domestica* with 0.6 mg/ml dilution of *C. procera*, *E. globulus* and *M. spicata* respectively (Figure 1).



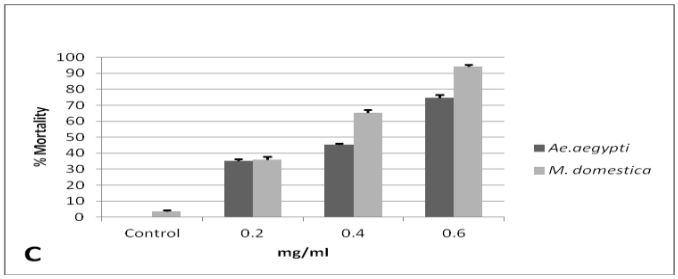


Figure 1: The mean percentage mortality in adults of *Ae. aegypti* and *M. domestica* against different doses of the three plants extract (A= *C. procera*, B= *E. globulus*, C= *M. spicata*)

Figure 2 portrays the comparison of AChE activity in *Ae.aegypti* and *M. domestica* exposed to the methanolic extract of three plants. Dose dependent inhibition of AChE activity was noted in all treated groups compared to control group. However, *M. spicata* extract was observed to be an efficient AChE inhibitor compared to the other two plant extracts in both adults of *Ae. aegypti* and *M. domestica*.

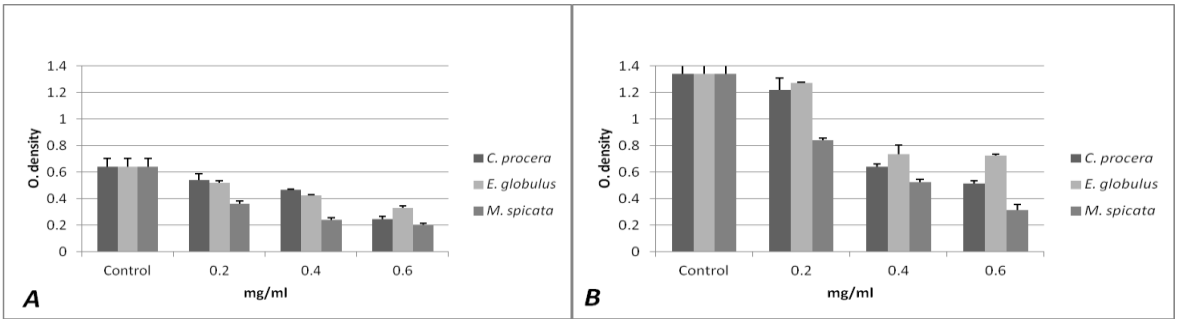
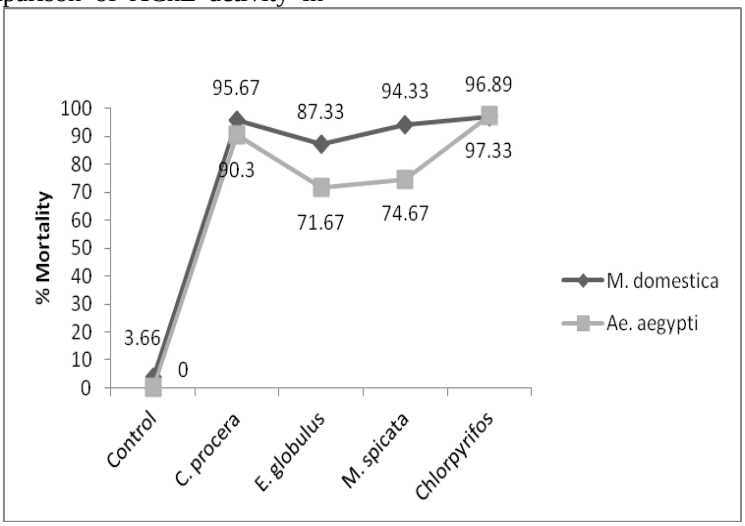


Figure 2: Comparison of AChE (O.D) in adults of *Ae. aegypti* (A) and *M. domestica* (B) against different doses of each plant extract.

Figure 3A shows the comparison of mean percentage mortality in *Ae. aegypti* and *M. domestica*. *E. globulus* and *M. spicata* ehad greater effect on *M. domestica* compared to *Ae. aegypti*. Overall, chlorpyrifos caused greater mortality in both insects followed by *C. procera* extract. Figure 3B dpicting the comparison of AChE activity in both selected insects. *M. spicata* extract was more efficient AChE inhibitor after chlorpyrifos. The AChE activity differed significantly between control and experimental groups and among experimental groups.



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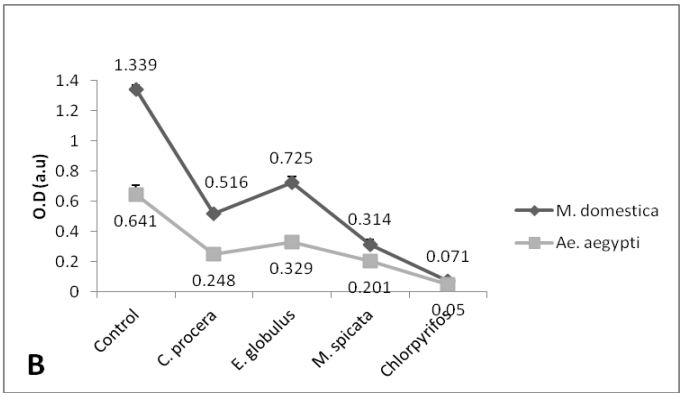

Figure 3: Comparison of mortality (A) and AChE activity (B) in *M. domestica* and *Ae. aegypti* against 0.6mg/ml dose of each plants extract and WHO recommended dose of chlorpyrifos.

Discussion: Biopesticides are the chemical substances obtained from natural organisms influencing the target organisms only (Kandpal, 2014). Plants are the nature's blessing with their numerous biologically active molecules that possess biopesticidal potential (Kandpal, 2014; Testai *et al*., 2002; Shahat *et al*., 2015). In the present study AChE inhibitory potential of was evaluated in the laboratory using *Ae. aegypti* and *M. domestica* as model insects. The mortality rate increased in both *Ae.aegypti* and *M. domestica* with increase of plant extract concentration. This result was not surprising as it was expected and many other researchers have reported similar results Rashid and Ahmad (2013) and Alam *et al*. (2009).

According to present study, as the concentration of plant extract increases, the mean absorbance values of the samples that were treated with higher doses of plant extracts were low as compared to the samples treated with lower plant extract dose. This indicates that the AChE activity was declining with the increasing plant extract dose. Similar results were recorded by Talic *et al*. (2014), who observed the dose dependent inhibition activity of the enzymes by different plants extract. Similarly, decreased AChE activity was determined in *M. domestica* when 5% and 10% of *C. procera* extract by Begum *et al.* (2011). Phrompittayarat *et al*. (2014) observed anti cholinesterase activity of *E. globulus* oil containing 1-8 cineole. Begum *et al.* (2011) also discovered that both low and high concentrations of *C. procera* extract inhibit the AChE activity very efficiently. Low AChE concentration was noted in rats when treated with *C. procera* (Malabade and Taranalli, 2015). Miyazawa *et al.* (1998) observed that *Mentha aquatica* (water mint) containing sesquiterpene alcohols showed the most efficient inhibition of AChE. Vladimir-Knežević *et al.* (2014) reported that more than 75% AChE inhibition was shown by ethanolic extracts of *Mentha x piperita, M. longifolia* Linarin.

Similar pattern was observed by Rashid and Ahmad (2013) on mosquitoes when treated with neem extract. Alam *et al*. (2009) also noted insecticidal activity of *Clatropis gigantea* against *Tribolium custaneum*. In the proposed study, anti-insect potential of *C. procera* leaf extract was investigated and it caused more lethality as compared to *M. spicata* and *E. globulus* extract. Marcio *et al*. (2006) also found that the whole latex of *C. procera* shows greater mortality in *Ae. aegypti* larvae. The leaf extract of *C. procera* showed more mortality as compared to its stem extract (Butt *et al*., 2016). The present results indicated that *C. procera* methanolic extract caused 90.3±1.45% and 95.67±0.882% deaths in *Ae. aegypti* and *M. domestica* respectively. The compounds present in the *C. procera* have larvicidal activity and they killed the mosquito's larvae within a short time (Giridhar *et al*., 1987; Markouk *et al*., 2000; Ramos *et al*., 2006; Shahi *et al*., 2010; Tahir *et al*., 2013). As the concentration of *C. procera* extract increases, the mortality rate also exceeds (Butt *et al*., 2016).

In the current study, 71.67±1.2% and 87.33±1.20% mortalities were recorded in *Ae. aegypti* and *M. domestica* against 0.6mg/ml concentration of *E. globulus* methanolic extract respectively. When house fly larva were exposed to *E. globulus* extract, their lethal concentration, LC_{50}, ranges between 2.73 and 0.60µl/cm noted by Kumar *et al*. (2012). The insecticidal activities of *E. globulus* oil on the *M. domestica* third stage larvae were observed by Abdel Halim and Morsy (2005). They recorded 100% mortality in their laboratory experiments. *M. spicata* methanolic extract caused 94.33±0.882% in *M. domestica* during the present study. The increase in percentage mortality with increased concentration of *M. spicata* in *Ae. aegypti* larvae and *Anopheles arabiensis* adults has been reported by Berhe (2017). The essential oil obtained from *M. piperita* showed its potential to greatly affect *M. domestica* larvae,

causing late adult emergence and also cause morphological abnormalities (Hanan, 2013). Chauhan *et al*. (2016) reported that *M. piperita* was very effective against *M. domestica* and *A. stephensi* larvae with LC_{50} values of 0.66 µl/cm^2 and 44.66 ppm, respectively, after 48 h. Patil *et al*. (2016), Morey and Khandagle (2012) also observed the biopesticidal potential of *M. piperita* against *M. domestica*. The studies of Patil *et al*. (2016) exhibited that *M. piperita* extract caused 100% mortality with 40% while its 50% concentration showed almost same mortalities as that of 10% in *M. domestica* adults. The reason may be that an excess of secondary metabolites repel the flies.

Conclusion: It was concluded from present findings that plants extract contain an insecticidal property and effectively killing the *Ae. aegypti* and *M. domestica*. It was also observed that they directly target the acetylcholinesterase enzyme, inhibiting it and thus increasing the acetylcholine level that results in paralytic effects. Overall, *M. spicata* extract efficiently disturbing the level of AChE and *C. procera* extract effectively control the *Ae. aegypti* and *M. domestica*. Further, the effect of these plants extract can be evaluated against other harmful insects as well as on beneficial insects.

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