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Toxicity of Benzaldehyde and Propionic Acid against Immature and Adult Stages of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae)

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ABSTRACT

The larvicidal, pupicidal and knockdown effects of benzaldehyde, propionic acid and their blend (1:1 ratio) were evaluated against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) mosquitoes in laboratory trials to find out the possibilities of using these compounds in mosquito control programmes. Larvicidal and pupicidal activities were tested at six different concentrations and mortality was recorded after 48 h. Knockdown bioassay was performed inside a cage at single concentration. Lethal concentrations (LC_{50} and LC_{99}) and knockdown time (KT_{50} and KT_{99}) were calculated. Benzaldehyde treatment killed maximum larvae and pupae and its knockdown effect was also significantly high. LC_{50} values of benzaldehyde for *Ae. aegypti* and *Cx. quinquefasciatus* were recorded as 30.39 and 40.48 ppm, respectively. Fifty % populations of *Ae. aegypti* and *Cx. quinquefasciatus* were knocked-down in 9.44 and 12.08 min, respectively by benzaldehyde treatment. Knocked-down *Ae. aegypti* adults could not recover from the fumigant effect of benzaldehyde treatment and 100% adult mortality was noticed within 24 h. Hundred percent mortality of knocked-down *Cx. quinquefasciatus* was recorded in both benzaldehyde and propionic acid treatments. The blend of benzaldehyde and propionic acid (1:1 ratio) was not as effective as benzaldehyde treatment. *Ae. aegypti* larvae were more susceptible than *Cx. quinquefasciatus* to all the three treatments tested. But the adult stage of *Ae. aegypti* was less susceptible to propionic acid treatment than *Cx. quinquefasciatus*. The aromatic compound benzaldehyde can be used in mosquito control programme.

Key words: Organic compounds, mosquito larvicidal activity, pupicidal activity, median knockdown time (KT_{50}), median lethal concentration (LC_{50})

INTRODUCTION

Mosquitoes are major public health pests throughout the world. Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases to humans and other vertebrates (Rueda, 2008). Many devastating diseases such as malaria, West Nile virus (WN), dengue, filariasis, yellow fever, Japanese encephalitis and chikungunya are transmitted to humans by vector mosquitoes. Also mosquito bite causes considerable pain and leads to loss of sleep. Mosquito attack on farm animals can cause loss of body weight and decreased milk production (Nour *et al*., 2009). Mosquito menace is particularly high in South East Asian countries (Rao *et al*., 2008) and in recent years global warming has lead to the spread of mosquitoes into temperate countries and higher altitude regions and the people in these regions are severely affected (Nerio *et al*., 2010).

At present several synthetic larvicides and adulticides such as temephos, methoprene, pyriproxyfen, diflubenzuron, petroleum oils, malathion, chlorpyrifos, permethrin and Resmethrin and biological products obtained from *Bacillus thuringiensis israelensis* and *B. sphaericus* are used in mosquito control programmes (Brattsten *et al*., 2009). Eventhough synthetic mosquitocides are very effective, most of them are environmental pollutants and cause lethal effects on humans and non-target organisms. A major concern with the synthetic mosquitocides is that they are responsible for the development of pesticide resistance in mosquitoes (WHO, 1992; Hemingway *et al*., 2002; Ahmad *et al*., 2007). Hence, researchers are aiming at discovering alternate mosquitocidal agents from natural products to avoid the development of pesticide resistance, environmental pollution and other side effects.

Several investigators have reported that volatile oils have the potential to kill mosquito larvae adults (Cavalcanti *et al*., 2004; Choochote *et al*., 2005; Lucia *et al*., 2007; Pitasawat *et al*., 2007; Dua *et al*., 2010) and these oils are generally safe to non-target organisms. Benzaldehyde, also known as artificial almond oil, is the simplest aromatic aldehyde and the primary component of bitter almond oil. It is used to flavor many food products. Propionic acid is a naturally occurring carboxylic acid. It is used as a preservative since it inhibits the growth of mold and some bacteria. Benzaldehyde and propionic acid were found to be potential fumigants against *Tribolium castaneum* (Nattudurai *et al*., 2010). Already benzaldehyde has been reported as larvicide against *Ae. albopictus* (Cheng *et al*., 2009). There is no report on the larvicidal, pupicidal and knockdown effects of benzaldehyde and propionic acid against *Ae. aegypti* and *Cx. quinquefasciatus*. Hence, the present work was carried out to screen benzaldehyde and propionic acid for their larvicidal, pupicidal and knockdown effects against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes.

MATERIALS AND METHODS

Benzaldehyde, propionic acid and their combination treatments were evaluated for their larvicidal, pupicidal and knockdown effects against *Ae. aegypti* and *Cx. quinquefasciatus* from 5th December 2010 to 20th January 2011.

Test mosquitoes: Eggs, larvae, pupae and adults of *Ae. aegypti* and *Cx. quinquefasciatus* were obtained from laboratory culture raised from larval instars collected from different places in Chennai and surrounding areas during 2007. They were maintained at 27-30°C and 75-80% relative humidity under a photoperiod of 11±0.5 h in the insectarium of Entomology Research Institute, Loyola College, Chennai. Larval stages were reared on powdered commercial dog biscuit and yeast (1:3). Adults were fed on raisins and 10% sucrose solution. Female mosquitoes were periodically blood-fed on restrained albino rats principally for egg production.

Treatments and concentrations: Benzaldehyde and Propionic acid were procured from Ranbaxy fine chemicals, Mumbai. Three different treatments namely benzaldehyde, propionic acid and benzaldehyde+propionic acid (1:1 ratio) were tested. Larvicidal and pupicidal activities of all three treatments were tested at six different concentrations viz., 10, 20, 30, 40, 50 and 100 ppm. Knockdown activity was tested at only one concentration (1.28 mL) inside a cage (40x40x40 cm). The concentration used in knockdown experiment was derived on the basis of 2 μL/100 mL air which was standardized previously in the laboratory.

Larvicidal and pupicidal bioassay: For larvicidal bioassay third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were used (Ansari *et al.*, 2000). Preparation of different concentrations (ppm) of test compounds was based on the calculations of Dharmagadda *et al.* (2005). To prepare the test medium with different concentrations, 1 mL of appropriate dilution of compounds and their mixtures in ethanol was mixed with 249 mL of tap water taken in plastic cups (175 mL volume). Newly molted 25 larvae (third instar) were collected from the stock culture and transferred to the test medium. Along with each experiment, a set of control using 1 mL ethanol dissolved in 149 mL water was also run for comparison. Chlorpyriphos (20% EC), at different concentrations viz., 0.001, 0.002, 0.004, 0.006, 0.008 and 0.01 ppm, was used as positive control in larvicidal bioassay. Different concentrations of Chlorpyriphos were prepared from a stock solution of 1 ppm in water. Percent larval mortalities in benzaldehyde, propionic acid and benzaldehyde+propionic acid treatments were calculated 48 h after treatment (Autran *et al.*, 2009). In Chlorpyriphos treatment the larval mortality was recorded 24 h after treatment and the instructions given by WHO (1981) was followed to calculate the larval mortality. Larvae were considered dead when they failed to move to the surface of the medium when provoked with a needle. The pupicidal activity was also tested by following the same method as described above using 3-5 h old pupae of *Ae. aegypti* and *Cx. quinquefasciatus*. The treatments and concentrations were the same as in larvicidal bioassay experiments. Chlorpyriphos treatment was not included in pupicidal activity. Pupal mortality was recorded till eclosion. Each treatment and control in larvicidal and pupicidal bioassay experiments was replicated 25 times. Abbott's formula (Abbott, 1925) was used for the correction of larval and pupal mortality when the control mortality was within 5-20%.

Knockdown activity: Knockdown activity was tested inside a cage (40x40x40 cm) enclosed by glass and wooden material on all sides. The bottom of the cage was covered with white paper. About 1.28 mL of benzaldehyde solution was impregnated in cotton taken in a glass vial and placed inside the cage through a sliding door. Twenty five female adult mosquitoes (3-4 day old) were released inside the cage. The number of mosquitoes knocked-down at every 5 min interval was recorded and the study lasted for 60 min. The experiments were repeated seven times. After the study period all mosquitoes were taken out and put inside 500 mL plastic boxes and the number of mosquitoes recovered or died after 24 h was recorded. The same experiment was repeated with propionic acid and benzaldehyde+propionic acid combination. The median knockdown time (KT_{50}) and KT_{99} for each treatment against each species were calculated by computer software (US EPA, version 1.5) based on Finney (1971).

Statistical analysis: Mean values were derived from replication data for all the parameters tested. Probit analysis was conducted on larval mortality data after 48 h exposure to different concentrations and on adult knockdown data using computer software (US EPA, version 1.5). Lethal concentrations for 50 and 99% mortality (LC_{50} and LC_{99}) values and knockdown time for 50 and 99% adults were calculated along with 95% confidence limits (lower and upper) and chi square values.

RESULTS

Larvicidal activity: Table 1 shows the LC_{50} and LC_{99} concentrations of benzaldehyde, propionic acid and blend of benzaldehyde and propionic acid treatments against *Ae. aegypti* and *Cx. quinquefasciatus* larvae. The results clearly indicated that benzaldehyde was very toxic to both

Table 1: Lethal concentrations of benzaldehyde, propionic acid and their mixture (1:1 ratio) (ppm) against *Aedes aegypti* and *Culex quinquefasciatus* third instar larvae after 48 h of treatment

Species	Treatment	LC_{50} (ppm)	LC_{99} (ppm)	Slope±SE	Intercept ± SE	df	χ^2
Aedes aegypti	Benzaldehyde (B)	30.39 (18.25-44.20)	91.93 (55.97-1479.17)	4.8±1.2	-2.1±1.9	5	11.3
	Propionic acid (P)	38.82 (33.82-44.95)	133.96 (97.26-238.67)	4.3±0.6	-1.8±1.0	5	9.2
	B+P	44.80 (36.38-58.03)	388.57 (208.20-1342.23)	2.4±0.4	0.9±0.6	5	2.9
	Chlorpyriphos[a] (20% EC)	0.03 (0.002-0.005)	0.025 (0.013-0.195)	2.6±0.5	11.6±1.2	5	27.2
Culex quinquefasciatus	Benzaldehyde (B)	40.48 (34.30-48.63)	202.77 (133.05-436.77)	3.3±0.5	-0.3±0.8	5	3.8
	Propionic acid (P)	55.21 (45.15-73.18)	392.35 (214.96-1301.82)	2.7±0.4	0.2±0.7	5	4.2
	B+P	57.38 (45.95-79.73)	509.14 (253.54-2168.44)	2.4±0.4	0.6±0.7	5	2.8
	Chlorpyriphos[a] (20% EC)	0.03 (0.001-0.005)	0.024 (0.011-0.402)	2.5±0.5	11.5±1.3	5	35.3

(Values in parentheses are 95% lower and upper confidence limits); [a]Positive control

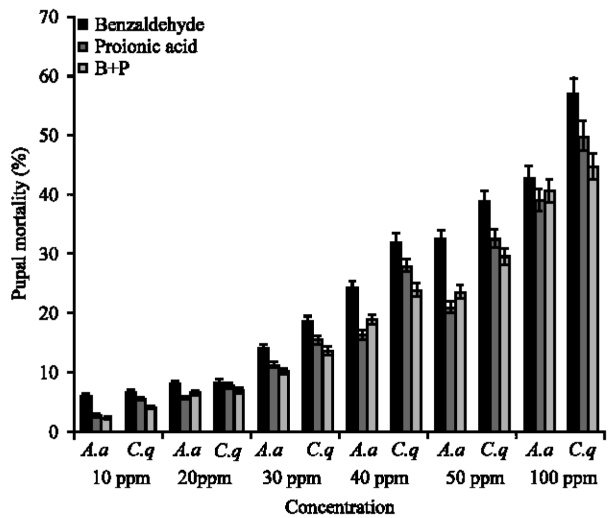

Fig. 1: Pupicidal activity (%) of benzaldehyde, propionic acid and their combination treatments against *Aedes aegypti* and *Culex quinquefasciatus*

mosquito species. The larvicidal activity of benzaldehyde was significantly high compared to propionic acid and combination treatments in both *Ae. aegypti* (χ^2 = 11.3, significant at $p<0.05$) and *Cx. quinquefasciatus* (χ^2 = 3.8, significant at $p<0.05$) and the lethal concentrations of benzaldehyde were much lower than the other two treatments. The LC_{50} and LC_{99} of benzaldehyde were 30.39 and 91.93 ppm, respectively against *Ae. aegypti* and 40.48 and 202.77 ppm , respectively against *Cx. quinquefasciatus* larvae. The combination of benzaldehyde and propionic acid in 1:1 ratio was the least effective against both mosquito species. The reference control Chlorpyriphos recorded a LC_{50} value of 0.03 ppm against both *Ae. aegypti* and *Cx. quinquefasciatus* larvae after 24 h.

Pupicidal activity: Benzaldehyde and propionic acid treatments showed pupicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*. The pupal stages of both mosquito species were found to be less susceptible than larval stages to both compounds. The most effective treatment was identified as benzaldehyde which recorded 42.6 and 56.8% pupal mortality against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively at 100 ppm concentration (Fig. 1). At 100 ppm level, benzaldehyde recorded significantly high pupal mortality ($p<0.001$) in *Cx. quinquefasciatus*. At

Table 2: Knock-down time (KT_{50} and KT_{99}) (in minutes) and adulticidal activity (%) recorded in benzaldehyde, propionic acid and their combination treatments

Species	Treatment	KT_{50} (min)	KT_{99} (min)	Slope±SE	Intercept±SE	χ^2	Adult mortality (%) after 24 h
Aedes aegypti	B	9.44 (7.93- 10.85)	28.55 (23.11-39.62)	4.8±0.6	0.3±0.7	2.7	100.0
	P	30.91 (28.31-33.47)	84.42 (71.00-109.04)	5.3±0.5	-2.9±0.8	3.6	96.0
	B+P	42.38 (39.43-45.76)	103.19 (85.16-140.36)	6.0±0.7	-4.8±1.2	5.3	78.6
Culex	B	12.08 (10.44-13.59)	32.01 (26.63-42.55)	5.5±0.7	-0.9±0.8	6.3	100.0
quinquefasciatus	P	21.61 (19.88-23.23)	41.19 (36.37-49.80)	8.3±1.0	-6.1±1.4	2.9	100.0
	B+P	37.22 (34.51-40.06)	92.76 (77.86-121.27)	5.8±0.6	-4.2±1.0	3.9	93.0

(Values are mean of 7 replications; values in parentheses are 95% lower and upper confidence limits)

50 ppm concentration, benzaldehyde registered significantly high pupal mortality ($p<0.001$) in both species. Benzaldehyde+propionic acid combination was less effective than other two treatments in the case of *Cx. quinquefasciatus* pupae, whereas in *Ae. aegypti* this combination killed more pupae at the higher concentrations of 40 (18.7%), 50 (23.4%) and 100 (40.5%) ppm.

Knockdown activity: All the treatments exhibited knockdown activity against *Ae. aegypti* and *Cx. quinquefasciatus* adults. The median knockdown time (KT_{50}) and KT_{99} values of three different treatments are given in Table 2. The data clearly indicated that benzaldehyde had the maximum knockdown effect against both *Ae. aegypti* (KT_{50} = 9.44 min) and *Cx. quinquefasciatus* (KT_{50} = 12.08 min). Benzaldehyde recorded 100% adults in both species after 24 h. In *Cx. quinquefasciatus*, propionic acid also recorded 100% mortality.

DISCUSSION

The over exploitation of chemical pesticides in mosquito control programmes has lead to the development resistance in mosquitoes. Another important problem with current mosquitocides is their non-target effects on various organisms including aquatic animals. These problems have directed the researchers either to invent novel substitutes from natural sources or to develop synthetic mimics of natural compounds. Plant extracts (Thangam and Kathiresan, 1993; Venkatachalam and Jebanesan, 2001; Nathan *et al*., 2006; Ramar *et al*., 2008; Vinayagam *et al*., 2008; Chakkaravarthy *et al*., 2011; Joseph *et al*., 2011) and plant essential oils (Carvalho *et al*., 2003; Odalo *et al*., 2005; Knio *et al*., 2008; Dadji *et al*., 2011) are reported as ecofriendly mosquito control agents. Marine sponges and algae are also reported to possess mosquitocidal properties (Manilal *et al*., 2011; Sujatha and Joseph, 2011). The synthetic mimics of plant compounds like benzaldehyde may give satisfactory control at low concentrations (Cheng *et al*., 2009). In the present study benzaldehyde treatment showed promising results against the immature and adult stages of two common mosquito species.

Mosquito control programmes are largely targeting the larval stage in their breeding sites with larvicides (El Hag *et al*., 1999, 2001). Larviciding is a successful method of reducing mosquito population in their breeding places before they emerge into adults (Tiwary *et al*., 2007). In the present study 100 ppm of benzaldehyde killed 100% *Ae. aegypti* larvae in 48 h. Propionic acid also showed good activity but its effect was not comparable with benzaldehyde.

Benzaldehyde is the simplest aromatic aldehyde; it is an important component of almond oil and also found in many nuts, seeds and leaves. The larvicidal activity of 14 different individual compounds including benzaldehyde which were identified as the main constituents of *Cinnamomum osmophloeum* leaf essential oil, were tested against *Aedes albopictus* larvae and it was found that benzaldehyde was one of the most toxic compounds against the larvae and recorded a LC_{50} of 47.0 and 45.4 μg mL^{-1} in 24 and 48 h of treatment , respectively (Cheng *et al*., 2009); the ppm equivalents of the above median lethal concentrations were high compared to the LC_{50} values of benzaldehyde against *Ae. aegypti* and *Cx. quinquefasciatus*, calculated in the present study. Benzaldehyde was a component of the essential oil of *Pogostemon parviflorus* and the isolated benzaldehyde exhibited repellent activity against *Sitophilus oryzae* and *Bruchus chinensis* (Saxena and Koul, 1982). Benzaldehyde is present in the essential oil of *Goniothalamus macrophyllus* (Humeirah *et al*., 2010). Benzaldehyde has also been detected as a component of the essential oil of *Suregada zanzibariensis* leaves which showed mosquito repellent activity against *Anopheles gambiae* (Innocent *et al*., 2010).

Plenty of essential oils have been screened as alternatives against *Ae. aegypti* and *Cx. quinquefasciatus* life stages. The mosquito larvicidal, pupicidal, repellent or knockdown activities of essential oils were due to the presence of one or more volatile compounds in the essential oils. The larvicidal activity of *Cymbopogan citratus* essential oil was tested against *Cx. quinquefasciatus* larvae (Pushpanathan *et al*., 2006) and the results showed that the LC_{50} of the oil against third instar larvae was 165.7 ppm in 24 h which is higher than the LC_{50} of benzaldehyde in the present study.

An interesting observation in this study was that *Cx. quinquefasciatus* larvae were less susceptible than *Ae. aegypti* to all the three treatments. The LC_{99} of benzaldehyde against *Cx. quinquefasciatus* was nearly two folds higher than that of *Ae. aegypti* and the LC_{99} of propionic acid against *Cx. quinquefasciatus* larvae was nearly three folds higher than that of *Ae. aegypti* larvae. This was in accordance with the findings of some earlier reports. Dharmagadda *et al*. (2005) reported that *Ae. aegypti* larvae were more susceptible than *Cx. quinquefasciatus* to *Tagetes patula* essential oil. Manilal *et al*. (2011) reported that *Ae. aegypti* larvae were more prone to death by extracts of marine algae at low LD_{50} concentrations than *Cx. quinquefasciatus*. Similarly Raghavendra *et al*. (2011) have stated that *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* to *Eugenia jambolana* solvent extracts. On the contrary the pupa stage of *Cx. quinquefasciatus* was more susceptible than *Ae. aegypti* to all the treatments.

Besides larvicidal and pupicidal activities, the compounds tested in the present study also exhibited knockdown activity. Benzaldehyde was found to be the potentially effective treatment. Benzaldehyde registered a KT_{50} of 9.44 and 12.08 min against *Ae. aegypti* and *Cx. quinquefasciatus* adults , respectively. Knockdown activity of many volatile oils and their components has been recorded against *Ae. aegypti* and *Cx. quinquefasciatus* adults. *Cx. quinquefasciatus* adult was slightly resistant to benzaldehyde compared to *Ae. aegypti*. This result coincides with the findings of Adanan *et al*. (2005) who evaluated the knockdown effect of mosquito mats which had the active ingredients d-allethrin (36 mg mat^{-1}) and prallethrin (15 mg mat^{-1}) against *Ae. aegypti* and *Cx. quinquefasciatus* adults. They found that *Cx. quinquefasciatus* adults were less susceptible to d-allethrin (8.36 min) and prallethrin (12.68 min) than *Ae. aegypti*. However, an opposite trend was found in propionic acid and benzaldehyde+propionic acid treatments. Jahangir *et al*. (2008) studied the knockdown effect of acetone vapour on *Ae. aegypti, Cx. quinquefasciatus* and *Toxorhynchites splendens* adults. They recorded 11.9 and 15.7 and 15.2 min as KT_{50} against *Cx. quinquefasciatus, Ae. aegypti* and *T. splendes* adults , respectively and *Cx. quinquefasciatus* was found to be the most susceptible species to acetone vapour among the three species. Further, studies are needed to understand these contrasting findings.

Benzaldehyde is a volatile compound and works as a fumigant against the adult mosquitoes. Benzaldehyde is used in cosmetics as a denaturant, a flavoring agent (almond flavouring) and as a fragrance and is a Generally Regarded As Safe (GRAS) food additive in the United States and European Union (Andersen, 2006). Both benzaldehyde and propionic acid are organic compounds and easily biodegradable. These two compounds can be used in mosquito control programmes.

CONCLUSION

The results of the present investigation clearly indicate that benzaldehyde can be used as a potent mosquito control agent since this organic compound has the capacity to kill mosquito larvae and adults at very low doses. An easy method of application of benzaldehyde for adult mosquito control should be investigated and the non-target effects against common aquatic organisms should also be checked before it can be considered for natural aquatic ecosystems.

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