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Mosquito Larvicidal Potential of Salicylic Acid and 3, 5-Di Nitro Salicylic Acid against Filarial Vector *Culex quinquefasciatus*

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Abstract

The present study was carried out to evaluate the larvicidal activity of an organic acid, namely Salicylic acid and its derivative, 3, 5-Di Nitro Salicylic Acid against larvae of *Culex quinquefasciatus*. Bioassay experiments with graded concentrations (50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm) of these organic acids were used against third instars larvae of *Cx. quinquefasciatus* at 24 hour, 48 hour and 72 hour of exposures to ensure their larvicidal potentiality. The results of bioassay experiments showed highest mortality rate at 72 h of exposure period for both the compounds. The toxicity of Salicylic Acid was higher than the 3, 5-Di Nitro Salicylic Acid. Regression analysis revealed that the mortality rate (Y) was positively correlated with the concentrations of the compounds (X). Respective Lethal toxicity values (LC_{50} values and LC_{90} values) gradually decreased with the exposure period. The effects of these organic acids were also tested on non target organisms such as *Anisops sardea*, larvae of *Toxorhynchites* sp. *Chironomus* sp. *and* Tadpole. There was no significant mortality occurred in non-target organism within 72h of exposure period. The results support that Salicylic acids and its Nitro derivative can effectively be used in mosquito breeding places to reduce the density of mosquito immature.

Keywords *Culex quinquefasciatus*; Larvicidal; Regression analysis; Lethal toxicity

Introduction

Mosquitoes act as a vector of many life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis etc., in almost all tropical and subtropical countries and cause morbidity associated with high mortality. To prevent the increased frequency of mosquito borne diseases and to increase the quality of public health, mosquito control is essential. According to World Health Organization (WHO) 1981, the most effective strategy for the reduction of mosquito borne diseases is to destroy the vectors or intermediate hosts in the immature condition because of their restricted habitat and minimum dispersal. The most effective method to control mosquito larvae is the application of chemical insecticides, such as Organophosphates, Organchlorides etc. The application of chemical larvicides are mostly found to be effective against immature forms of mosquito vectors; but the over and injudicious application of the chemical insecticide produce harmful effect on human health and non- target populations as they are non biodegradable in nature. Due to their repeated application and having higher rate of biological magnification through ecosystem, the insecticide resistant varieties of vectors are selected in nature. So it is an urgent need to develop new insecticides which are more environmentally safe and also biodegradable and target specific in nature. In view of this, some biodegradable organic products, synthetic chemicals and plant derived secondary metabolites are now recommended in the alternative vector control programme.

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Salicylic acid (SA) is a monohydroxybenzoic acid, a type of phenolic acid and a beta hydroxy acid which shows bacteriostatic, fungicidal and keratolytic actions (Dhaval et al., 2013; Srinivasa Babu et al., 2012). It is soluble in water, biodegradable in nature having low potential of bioaccumulation (Kilpi et al., 1980). This organic acid is a phytohormone and helps in plant growth and development (Thomas et al., 2008). SA has been recognized as a regulatory signal mediating plant response to abiotic stresses such as drought (Munne´-Bosch and Pen˜ uelas., 2003), chilling (Kang and Saltveit, 2002), heavy metal tolerance (Freeman et al., 2005), heat (Larkindale et al., 2005), and osmotic stress (Borsani et al., 2001). Salicylic acid and its derivatives are used in pharmaceutical and cosmetic industry also (Thomas et al., 2008).

The biocidal activity of Salicylic acid (SA) and its derivatives were earlier documented in the literature. Fumigant activity of a SA derivative, methyl salicylate derived from clove bud oil (*Eugenia caryophyllata*) was reported against eggs and females of *Pediculus humanus capitis* (Yang et al. 2003). Rathore and Swarup (1978) demonstrated the biocidal action of aspirin (acetylsalicylic acid) against mosquito larvae. Novelino et al., demonstrated the mild acaricidal activity of salicylic acid against *Boophilus microplus* (acari: ixodidae). Salicylic acid EC at 10 % concentration showed the nematicidal effect against second stage larvae of *Meloidogyne* sp. Respective EC_{50} values after 96 hours was 6.6 ppm and the ET_{50} value was 19 hours (Abd-alla et al., 2013). Salicylate is a naturally occurring plant secondary metabolite that also inhibits the growth of insect species such as the moth larvae *Operophtera brumata* (Ruuhola et al., 2001)] and the corn earworm *Helicoverpa zea* (Li et al., 2002). But no study was carried out to evaluate the larvicidal activity of nitro derivatives of SA against *Culex quinquefasciatus*. So, present study was designed to evaluate the larvicidal activity of Salicylic acid and its derivative, 3, 5-Di Nitro Salicylic Acid against larvae of *Culex quinquefasciatus*, a common vector of filariasis mainly in South East Asia.

Materials and Methods

Collection of mosquito larvae

Present study was conducted at Bankura (23.14 ℃N and 87.4 ℃E), West Bengal, India during January to March 2013. *Cx. quinquefasciatus* larvae were collected from cemented drains surrounding the Bankura Sammilani College campus. The collected larvae were taken to the Department of Zoology, Bankura Sammilani College and kept in enamel tray containing tap water. The larvae were kept free from exposure to pathogens, insecticides or repellents. The larvae were reared in the laboratory with dried Yeast powder and dog biscuits (1:3) as food up to the third instars larval forms that were used in bioassay experiments.

Test materials

Salicylic Acid was purchased from E. Merck (India) Limited, Mumbai and 3,5- Di Nitro salicylic Acid was purchased from Nice Chemicals Pvt. Ltd, Cochin. 500 ppm of stock solution was prepared by adding 0.05g of both acid in 100 ml distilled water in separate glass beakers, and kept in room temperature for further use. For bioassay experiment graded concentrations (50, 100, 150, 200 and 250 ppm) were prepared through mixing of stock solution with variable amount of distilled water.

Larvicidal bioassay

The bioassay experiments were conducted according to standard WHO procedure (1981) with slight modifications. During experiment only third instars larvae of *Cx. quinquefasciatus* were used. At first five concentrations (50,100,150,200 and 250 ppm) of Salicylic Acid and 3, 5-Di Nitro Salicylic Acid were applied into separate glass Petri-dishes (9 cm diameter/150 ml capacity) containing twenty five third instars larvae and 100 ml of water to study the rate of larval mortalities. A control experiment was also carried out with experimental set up but without applying any test material in 100 ml of water. All bioassay experiments were replicated three times on three different dates both for treated and untreated controls. During each bioassay experiments all the concentrations were prepared fresh from stock solution. The larval mortality was recorded after 24, 48 and 72 hours of the exposure period. The data of mortality in 48 and 72 hours were expressed by the addition of the mortality at 24 and 48 hours, respectively. Dead larvae were identified when they failed to move after probing with a needle in the

siphon. The experiments were conducted in a BOD Incubator maintained in laboratory condition having temperature at 25–30 C and 80%~90% relative humidity.

Effect on non target organisms

Four non target species such as *Anisops sardea*, larvae of *Toxorhynchites sp.*, *Chironomus* sp. *and* Tadpole were collected from mosquito breeding places near college campus and maintained for few days in laboratory. Ten individuals of each non target species were exposed to drainage water (control set) as well as treated set where concentrations of both the acids were maintained at their respective LC_{50} values at 24 h reported during earlier bioassay experiments with mosquito larvae. The experiments were carried out for 24 h, 48 h and 72 h of exposure periods to observe their mortality and other abnormalities, such as sluggishness and reduced swimming activity, if any. The experiments were replicated three times on three different days both for treated and untreated controls.

Statistical analysis

The percentage mortality of *Cx. quinquefasciatus* larvae were corrected using Abbott's formula. Statistical analysis of the experimental data was performed using the computer software STATPLUS 2007 and MS EXCEL 2003 to find the LC_{50}, regression equations (*Y*= mortality; *X* = concentrations) and regression coefficient values.

Results and Discussion

The efficacies of different concentrations of Salicylic acid and it's derivative 3, 5-Di Nitro Salicylic Acid against third instars of *Cx. quinquefasciatus* larvae are shown in Table 1. The corresponding LC_{50}, LC_{90}, value regression equation, and R^2 values at 24, 48 and 72 hours of bioassay experiments are shown in Table 2. The results of the present study indicated that the mortality rate of *Cx. quinquefasciatus* larvae at 250 ppm concentration was higher ($p<0.05$)) than the mortality rates at 50, 100, 150, 200 ppm concentrations of both the acids. Highest mortality rate was recorded at 72h of exposure and the value was higher ($p<0.05$)) than those at 24 and 48 hour of exposures. The results of regression analysis revealed that the mortality rate (Y) was positively correlated with the period of exposure (X). The results of log probit analysis (95% confidence level) revealed that LC_{50} values and LC_{50} values gradually decreased with the exposure period. In the larvicidal bioassay good LC_{50} projections were noticed with SA at 24h of exposure (107.12 ppm, R^2 =0.89) and 3,5-Di Nitro Salicylic acid at 72h of exposure(65.92 ppm, R^2=0.67).

The activity of Salicylic acid or its derivatives against insects have been well documented in literature. Salicylic acid (SA) is known to regulate both basal and R.gene-mediated defense response against pathogens and insects (Kim et al., 2008). Salicylic acid was known as inducer of plant resistance to some pathogens (Kessman et al., 1994). Also it has been

Table 1 Efficacy of different concentrations of Salicylic acid and Di Nitro Salicylic acid on third instars larvae of *Culex quinquefasciatus* mosquito

Organic acids	Concentrations	Mean mortality(%) ± SE		
		24 h	48 h	72 h
Salicylic acid	50ppm	16.67 ±1.67	27.78 ±2.22	51.11 ±2.60
	100ppm	24.44 ±1.76	43.33 ±3.33	70.00 ±4.08
	150ppm	64.44 ±5.03	93.33 ±2.36	100 ±0
	200ppm	98.89 ±1.11	100 ±0	100 ±0
	250ppm	100 ±0	100 ±0	100 ±0
3,5-Di Nitro Salicylic acid	50ppm	08.89 ±2.00	22.22 ±3.24	44.44 ±4.12
	100ppm	12.22 ±2.22	28.89 ±4.84	58.89 ±4.55
	150ppm	18.89 ±2.60	40 ±3.33	68.89 ±2.60
	200ppm	23.33 ±2.36	43.33 ±3.33	83.33 ±4.08
	250ppm	33.33 ±2.36	46.67 ±2.89	88.89 ±3.88

Note: SE: Standard error

Table 2 Log probit analysis and regression analysis of larvicidal activity of Salicylic acid and Di Nitro Salicylic acid against third instars of *Cx. quinquefasciatus*

Organic acid	Period of bioassay(h)	LC_{50} value	LC_{90} value	Regression equations	R^2 value
Salicylic acid	24	107.12	203.78	Y=0.4822 X+11.44	0.89
	48	80.25	154.56	Y=0.4022 X+12.56	0.80
	72	55.24	119.06	Y=0.2556 X+45.89	0.73
3,5-Di Nitro Salicylic acid	24	636.30	5916.14	Y=0.1200 X+1.33	0.61
	48	295.49	5239.42	Y=0.1267 X+17.22	0.42
	72	65.92	324.11	Y=0.2267 X+34.89	0.67

thought to play an important role in systemic acquired resistance because exogenous salicylic acid induces this resistance and accumulated in pathogen infected tissue (Malamy et al., 1990). Topical application with 100 ng/ml, 200 ng/ml and 300 ng/ml phytohormone salicyclic acid on commercial traits was analysed by Kochi and Kaliwal in 2005 in bivoltine CSR2, CSR4 and CSR2XCSR4 crossbreed races of the silkworm, *Bombyx mori* L. The results showed that there was significant increase in larval weight female cocoon weight, male cocoon shell weight, and hatching percentage, silkgland weight, female cocoon shell ratio, male cocoon shell ratio, filament length, in all the treated groups of the silkworm, *B. mori* when compared over the respective controls.

Today many species of mosquitoes were reported to develop resistance against various chemical pesticides because of the repeated applications (Su and Mulla, 2004, Tia et al., 2006; Arunpandiyan, 2011). It also creates aquatic pollution as well as effects non target aquatic animals. Mosquitoes can effectively be controlled at their larval stage due to their low mobility in aquatic body in respect to time (Wiesman et al., 2005). Various phytochemicals (Chowdhury et al., 2008; Rawani et al., 2010; Ghosh et al. 2008; Haldar et al., 2011; Singha et al., 2011) and plant essential oil (Amer and Mehlhorn, 2006) are reported to be environmentally safe mosquito larvicides. Various organic acid also play an important role in mosquito control. Organic acid from plant origin namely Orthophosphoric acid, and lactic acid (Chakraborty et al., 2010), tartaric acid, thiourea, glacial acetic acid (Adhikari et al., 2012) and common salt (Mukhopadhyay et al., 2010) also showed good mosquito control potentiality.

In the present bioassay experiments 100% mortality was observed at 200 ppm concentrations in 48 hours exposure and 250 ppm in 24 hours of exposure of Salicylic Acid against 3rd instar larvae of *Cx quinquefasciatus*. Di nitro Salicylic Acid also showed 88.89% percent mortality in 250 ppm at 72 hours exposure. Salicylic Acid showed higher mortality in all concentrations in longer exposure. LC_{50} values of these compounds did not produce any adverse effect on the non target organisms as non significant mortality was recorded in case of *Toxorhynchites* larvae and *Chironomus* larvae (Table 3).

Table 3 Effect of particular concentrations (LC_{50} at 24h) of Salicylic acid and Di Nitro Salicylic acid on non target organisms

Organic acids	Concentrations	Non Target organism	Mean mortality(%) ±SE		
			24 h	48 h	72 h
Salicylic acid	107 ppm	*Anisops sardea*	0 ±0	0 ±0	0 ±0
		Tadpole	0 ±0	0 ±0	0 ±0
		Toxorhynchites	0 ±0	0 ±0	0.33 ±0.33
		Chironomus larvae	0 ±0	0 ±0	0.67 ±0.33
3,5-Di Nitro Salicylic acid	636 ppm	*Anisops sardea*	0 ±0	0 ±0	0 ±0
		Tadpole	0 ±0	0 ±0	0 ±0
		Toxorhynchites larvae	0 ±0	0 ±0	0.33 ±0.33
		Chironomus larvae	0 ±0	0 ±0	0.33 ±0.33

Note: SE: Standard error

From the present set of experiments, it can be concluded that, a biodegradable organic acid namely Salicylic Acid, and the Nitro derivatives of Salicylic acid (3, 5-Di Nitro Salicylic Acid) may be used as safe mosquito larvicide in the breeding places of mosquitoes.

Author's Contributions

RPM performed the Larvicidal bioassay experiment and systematized the data. AG performed the statistical analyses and drafting of the manuscript. GC supervised the whole work and approved the final manuscript.

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