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Efficacy of naringenin on ovicidal, repellent, insect growth regulating properties, and enzymological parameters against *Aedesa egypti* and *Culex quinquefasciatus*

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Abstract
In mosquito control programs, plants secondary metabolites may have the potential to be used as eggs, larvae, and adult, and also prevent insecticide resistance and pollution of the ecosystem. Therefore, the aim of the present study was to validate the effect of naringenin on ovicidal, repellent and insect growth regulating (IGR) properties, and enzymological parameters against *Aedesa egypti* and *Culex quinquefasciatus.* Naringenin was investigated for its ovicidal, repellent and IGR properties, and its effect on glutathione s-transferase (GSH), acetylcholinesterase (AChE) and cyclic AMP (cAMP) levels in third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus.* The treatment of naringenin at different concentrations (2, 4, 6, 8 and 10 ppm) produced egg hatchability of 49.2, 18.4, 10.6, 0 and 0 per cent in *Ae. Aegypti*, while 53.6, 24.22, 9.81, 1.67 and 0 per cent in *Cx. quinquefasciatus*. The biting protection was 89.1, 95.2 and 98.82, whereas 87.83, 93.20 and 97.23 at 0.5, 1 and 1.5 mg/cm^2 concentrations of naringenin for 2 hours in *Ae. Aegypti* and *Cx. quinquefasciatus*, respectively. The larvae emergence was notably declined in naringenin treated mosquitoes than control. Further, the GST activity was significantly declined in naringenine treated larva, pupa, male and female adult of selected mosquitoes when compared to control. The AChE activity was significantly declined while cAMP level was notably increased in naringenine treated *Ae. aegypti* and *Cx. quinquefasciatus* when compared to control. These findings collectively suggest that naringenin possess all the criteria of insecticides, making it a good candidate for controlling mosquito proliferation.

Keywords: ovicidal, repellent, insect growth regulatory activity and naringenin

Introduction
Mosquitoes play as chief vectors in the spread of various vector-borne diseases affecting both human beings as well as animals. Vector-borne diseases in India, i.e., dengue, chikungunya, malaria, filariasis, and Japanese encephalitis, cause thousands of deaths per year [43]. Among all the mosquitoes, the *Aedes aegypti* vector transmits several yellow fevers like dengue, chikungunya, viruses and other diseases (WHO, 2009). The *Culex quinquefasciatus* is the chief vector that is involved in the spread of lymphatic filariasis [8]. According to World health organization, the prevalence of dengue affected people had been estimated to be 3.9 billion, in 128 countries [45]. Based on [45] report, each year nearly 120 million people get infected by filariasis, over 40 million get severely disfigured and disabled, and nearly 76 million have hidden damage to the lymphatic and renal system while remaining are asymptomatic.
The control of mosquito population is a very crucial strategy towards the preventive measures for hindering the transmission of diseases and epidemic outbreaks. In the present time, over use of chemical insecticides leads to development of resistance in mosquitoes for the same. To overcome this problem, scientists have initiated the search for alternative control measures [37]. Thus, many researchers are focusing on finding newer insecticides of plant origin with high potency, safety and easy availability at low cost [19] and [28].
Naringenin, a polyphenol, belongs to flavonoids, is primarily present in citrus fruits, and is one of the common components in the human diet. Previously, [25] reported that *Citrus sinensis* orange peel extract had larvicidal, pupicidal, repellent and adulticidal activity against *Anopheles stephensi*, *Aedesa egypti* and *Culex quinquefasciatus.* Naringenin is major secondary metabolites in *Citrus sinensis* orange peel [25]. Recently, naringenin has received profound attention for its beneficial effects on health and disease prevention, leading to an increase interest in its pharmaceutical and nutritional effects [35]. It also has been analysed for various other pharmacological effects, such as antioxidant [47] anti-inflammatory [4] hepatoprotective [14], anti-cancer [22], anti-atherosclerotic [23] and anti-diabetic properties [36]. Additionally, in our previous study, we have reported naringenin to possess larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* by utilizing *in silico* and *in vitro* assays (add your 1st paper reference after publish). Thus, the present study was designed for evaluating the effect of naringenin on ovicidal, repellent, insect growth regulating properties, and enzymological parameters against *Aedesa egypti* and *Culex quinquefasciatus.*

Materials and Methods
Chemicals
Naringenin was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals as well as the reagents used were of analytical grade and were purchased from Merck, Himedia, Mumbai, India.

Collection of eggs and maintenance of larvae
The eggs of *Ae. aegypti* and *Cu. quinquefasciatus* were

collected from VCRC (Vector Control Research Centre) Puducherry a unit of ICMR, using an "O"-type brush. These eggs were brought to the laboratory and transferred to 18×13×4-cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were pedigree dog biscuits and yeast at 3:1 ratio. The larvae were reared in plastic containers providing commercial fish food as a source of meal, till they are used. Water was changed on alternate days. The breeding medium was regularly checked for dead larvae and they were removed. During the present study, breeding cups were used for normal cultures and the experiment cups were kept closed with muslin cloth for preventing contamination through foreign mosquitoes. Once the larva attained the pupal stage, it was transferred to the cages. The egg, III instar larvae and pupae were sacrificed whenever required.

Culture of mosquitoes

The mosquitoes, *aegypti* and *Cx. quinquefasciatu*, were reared in the Department of Zoology, Annamalai University, Chidambaram, Tamil Nadu and India. The adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28 ± 2) ℃, 70%-80% relative humidity (RH), with a photoperiod of 14 h light, 10 h dark.

Ovicidal activity

The ovicidal activity was assessed by the method of Su and Mulla (1998) [40] with slight modifications. The naringenin was diluted in the appropriate water to achieve various concentrations ranging from 2, 4, 6, 8 and 10 ppm. The eggs of mosquito species (100 nos.) were exposed to each of the naringenin concentrations. After the treatment of the eggs with each concentration, they were individually transferred to the distilled water cups for hatching assessment. Then the eggs were counted under a microscope. Each of the experiment was replicated for six times along with the appropriate control. The hatch rates were then assessed after 48 hours of treatment and calculated by the following formula:

$$\% \text{ of egg mortality} = \frac{\text{No. of hatched larvae}}{\text{Total No. of eggs}} \times 100$$

Repellent test

The percentage of protection in relation to the dose method was used [46] and 3-4 days old blood of starved female *Ae. aegypti* and *Cx. quinquefasciatus* mosquito (100) were kept in a net cage (45 x 30 x 45 cm^2). All the arms of the volunteer were washed and cleaned with ethanol and ethanol served as control. After the arms were air dried, only 25cm^2 dorsal side of the skin on each arm was exposed, and rubber gloves covered the remaining area. The naringenin was applied at 0.5, 1 and 1.5 mg/cm2, separately. Both the control and the treated arms were introduced simultaneously into the cages. The number of bites was counted every 30 minutes from 18:00h to 06:00h. In the event of no bites, in the initial 5 minutes the test arm was exposed after every 30 min for the duration of 5 min until a confirmed bite was received. The test was over after the 3 confirmation of mosquito bites in the extract to be tested. The mosquito repellency of different naringenin concentration was measured on the basis of the protection time (min), i.e., the time until the first confirmed bite after application. The experiment was replicated six times in each concentration. It was observed that no skin irritation occurred from the naringenin tested. The percentage of repellence was calculated by the following formula:

$$\% \text{ Repellence} = [(Ta - Tb)/Ta] \times 100$$

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

Insect growth regulator activity (IGR)

The naringenin was tested for IGR activity against 25 numbers of early 2nd instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* by following standard procedure (Amalraj *et al*., 1988). The naringenin was volumetrically diluted to 250 ml dechlorinated water to obtain the test solution of 2, 4, 6, 8 and 10 ppm. All larvae were monitored till adult emergence and were provided with larval food and observations were made at 24 hours intervals and dead larvae and pupae were removed daily and counted. Symptoms of test larvae such as convulsion, unnatural position, tremor, rigor, sluggish movement, failure of the body to balance in water and feeding were recorded at the time. The percentage emergences at different concentration of naringenin were recorded. The LI_{50} (larval inhibition) was calculated by Finney (1971) [10].

Enzymological studies

The activity of glutathione s-transferase was determined in larvae, pupa, adult male and female selected mosquitoes by using the model substrate 1-chloro-2,4-dinitrobenzene (CDNB) and reduced GSH as substrate, based on the method by [12]. The AChE level was analysed in entire body of third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* by [7] and [13] (1986). The level of cyclic AMP was analysed in whole body of third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* by [31].

Statistical analysis

Data were arranged in an Excel sheet; statistical analysis of the experimental data was performed using the computer software Stat Plus 2009 (Analyst Soft, Canada) to find the lethal concentration against larvae (LC_{50} and LC_{90}) out in 24 hours by probit analysis with a reliability interval of 95%. Additionally, to determine if there was a significant statistically difference among different doses of naringenin against mosquito larvae, student's t-test was used to analyse the difference of the percentage of mortality. The results with different superscripts (a, b, c) in each experimental groups are significantly different at $p<0.05$.

Results

Ovicidal activity of naringenin

The eggs of *Ae. aegypti* were treated with different concentrations of naringenin (2, 4, 6, 8 and 10 ppm). The rate of hatchability of 49.2, 18.4, 10.6, 0 and 0 per cent were observed at concentrations of 2, 4, 6, 8 and 10 ppm naringenin, respectively. The naringenin exerted zero hatchability (100% mortality) at 8 and 10 ppm for *Ae. aegypti*. While, hatchability of *Cx. quinquefasciatus* were 53.6, 24.22, 9.81, 1.67 and 0 at concentrations of 2, 4, 6, 8 and 10 ppm naringenin, respectively. When the concentration increased the egg hatchability also significantly ($p<0.05$) declined (Table 1).

Table 1: Ovicidal activity of naringenin against selected mosquitoes

Concentration	Control	2 (ppm)	4 (ppm)	6 (ppm)	8 (ppm)	10 (ppm)
	Ae. Aegypti					
	100	49.2 ± 3.75[a]	18.4 ± 1.40[b]	10.6 ± 0.81[c]	NH	NH
	Cx. quinquefasciatus					
	100	53.6 ± 4.08[a]	24.22 ± 1.84[b]	9.81 ± 0.75[c]	1.67 ± 0.26[d]	NH

NH: No hatchability; Significant at $p<0.05$

Repellent activity of naringenin

The repellent activities of naringenin against *Ae. aegypti* and *Cx. quinquefasciatus are* represented in Table 2. Three different concentrations (0.5, 1 and 1.5 mg/cm^2) of naringenin were utilized to evaluate the repellent activity at different intervals viz., 2, 4, 6, 8, and 10 hours. The biting protection of 89.1, 95.2 and 98.82 per cent were observed during 2 hours interval at 0.5, 1.0 and 1.5 mg/cm^2 concentrations, respectively for *Ae. aegypti*. While, the repellent activity of naringenin against *Cx. quinquefasciatus* was found to be 87.83, 93.20 and 97.23 at 0.5, 1 and 1.5 mg/cm^2 concentration of naringenin for 2 hours. All three concentrations of naringenin provided protection from mosquito bites up to 6 hours from both *Ae. aegypti* and *Cx. quinquefasciatus*, but the rate of protection varied based on naringenin concentration. Protection from mosquito bites significantly differed between each concentration and interval. Increase in the exposure period showed a reduction in repellent activity, and it directly depended upon the concentration of the naringenin.

Table 2: Repellent activity of naringenin against *Ae. aegypti* and *Cx. Quinquefasciatus*

Time period (h)	Percentage of protection* Concentration of Naringenin (mg/cm^2)		
	0.5	1.5	2.5
Ae. Aegypti			
2	89.1 ± 6.79[a]	95.2 ± 7.25[a]	98.82 ± 7.48[a]
4	78.7 ± 5.99[b]	88.9 ± 6.76[b]	94.81 ± 7.22[ab]
6	58.4 ± 4.45[c]	70.4 ± 5.51[bc]	82.2 ± 6.26[b]
8	47.9 ± 3.65[d]	52.8 ± 4.48[c]	63.83 ± 4.86[c]
10	0	0	56.93 ± 4.34[d]
Cx. Quinquefasciatus			
2	87.83 ± 6.69[a]	93.20 ± 7.10[a]	97.23 ± 7.40[a]
4	75.62 ± 5.76[b]	85.26 ± 6.50[b]	90.31 ± 6.86[ab]
6	56.85± 4.71[c]	67.43 ± 5.14[c]	82.43 ± 6.26[b]
8	39.74 ± 1.61[d]	49.84 ± 3.79[d]	65.24 ± 4.97[b]
10	0	0	35.29 ± 2.68[c]

Significant at $p<0.05$

Insect growth regulatory activity of naringenin

The naringenin was studied for IGR activity against *Ae. aegypti* and *Cx. quinquefasciatus* and is represented in table 3 and 4, respectively. The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to different concentrations of naringenin *viz*., 2, 4, 6, 8 and 10 ppm. The larvae emergence Inhibition was 20, 34, 56, 74 and 96 per cent at 2, 4, 6, 8 and 10 ppm naringenin concentrations for *Ae. aegypti*, respectively. The EI_{50} and EI_{90} values of naringenin against *Ae. aegypti* were 4.266 and 10.420 ppm, respectively. The chi-square value was 0.065, which showed that the IGR activity was significant at 0.05 % level.

IGR activity of naringenin against *Cx. quinquefasciatus* were 17, 31, 52, 74, 91 at concentration viz. 2, 4, 6, 8 and 10 ppm, respectively. The value of EI_{50} and EI_{90} naringenin against *Cx. quinquefasciatus* were 4.952 and 10.20 ppm, respectively. The value of chi-square was 0.212, which proved that the IGR activity was significant at 0.05 % level.

Table 3: Insect growth regulator activity of naringenin against *Ae. Aegypti*

concentration (ppm)	Emergence Inhibition (%)	EI_{50} (ppm)	EI_{90}(ppm)	Regression Equation	95% confidence limit		x^2
					LCL (ppm) EI_{50} (EI_{90})	UCL (ppm) EI_{50}(EI_{90})	
2	20 ± 1.52	4.266	10.420	Y= 3.5749x+2.7541	3.289(8.035)	5.532(13.513)	0.065
4	34 ± 2.59						
6	56 ± 4.26						
8	74 ± 5.64						
10	96 ± 7.31						

Control: No mortality; EI: Emergence inhibition; LFL: lower fiducidal limit; UFL: upper fiducidal limit; x^2: Chi-square value; df: degrees of freedom.

Table 4: Insect growth regulator activity of naringenin against *Cx. Quinquefasciatus*

Compound concentration ppm)	Emergence Inhibition (%)	EI_{50} (ppm)	EI_{90} (ppm)	Regression Equation	95% confidence limit		x^2
					LCL (ppm) EI_{50} (EI_{90})	UCL (ppm) EI_{50} (EI_{90})	
2	17 ± 1.29[a]	4.952	10.20	Y=3.1915x+2.8733	3.516(9.251)	6.156(12.997)	0.212
4	31 ± 2.36[b]						
6	52 ± 3,97[c]						
8	74 ± 5.64[d]						
10	91 ± 6.93[e]						

Control: No mortality; EI: Emergence inhibition; LFL: lower fiducidal limit; UFL: upper fiducidal limit; x^2: Chi-square value; df: degrees of freedom.

Table 5: Effect of naringenin on GST in the different life stages of *Ae. aegypti* and *Cx. Quinquefasciatus*

S. No	Larvae (µg protein/mg weight)		Pupae (µg protein/mg weight)		Adult male (µg protein/mg weight)		Adult female (µg protein/mg weight)	
	Control	NG	Control	NG	Control	NG	Control	NG
	Ae. Aegypti							
1	78.56 ± 5.83	59.45 ± 4.53	84.63 ± 6.44	62.83 ± 4.74	87.43 ± 6.66	65.87 ± 5.02	89.96 ± 6.85	68.98 ±5.25
	Cx. Quinquefasciatus							
2	82.67 ± 6.30	61.52 ± 4.69	85.98 ± 6.55	64.21 ± 4.89	91.78 ± 6.99	69.76 ± 5.31	94.87 ± 7.23	72.56 ± 5.52

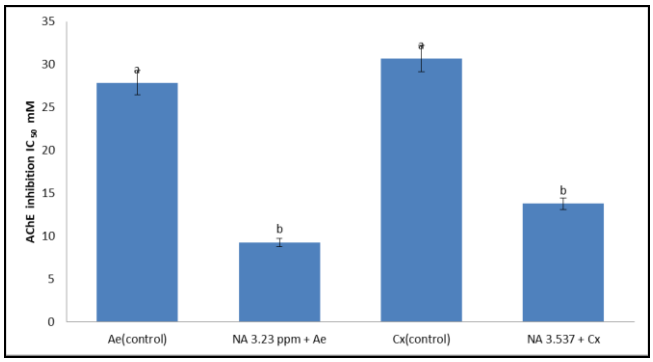
The values are expressed as mean ± SD

Effect of naringenin on GST

Table 5 shows the activity of GST in the different life stages of *Ae. aegypti and Cx. Quinquefasciatus*. The GST activity was 78.56, 84.83, 87.43 and 89.986 in control larvae, pupa, adult male and adult female *Ae. Aegypti*, respectively. In naringenin treated larvae, pupa, adult male and adult female *Ae. Aegypti* showed GST activities of 59.45, 62.83, 65.87 and 68.98 respectively. In *Cx. Quinquefasciatus*, the GST activity was 82.67, 85.98, 91.78 and 94.87 in control larvae, pupa, male adult and female adult while naringenin treated larvae, pupa, male adult and female adult were 61.52, 64.21, 69.75 and 72.56, respectively.

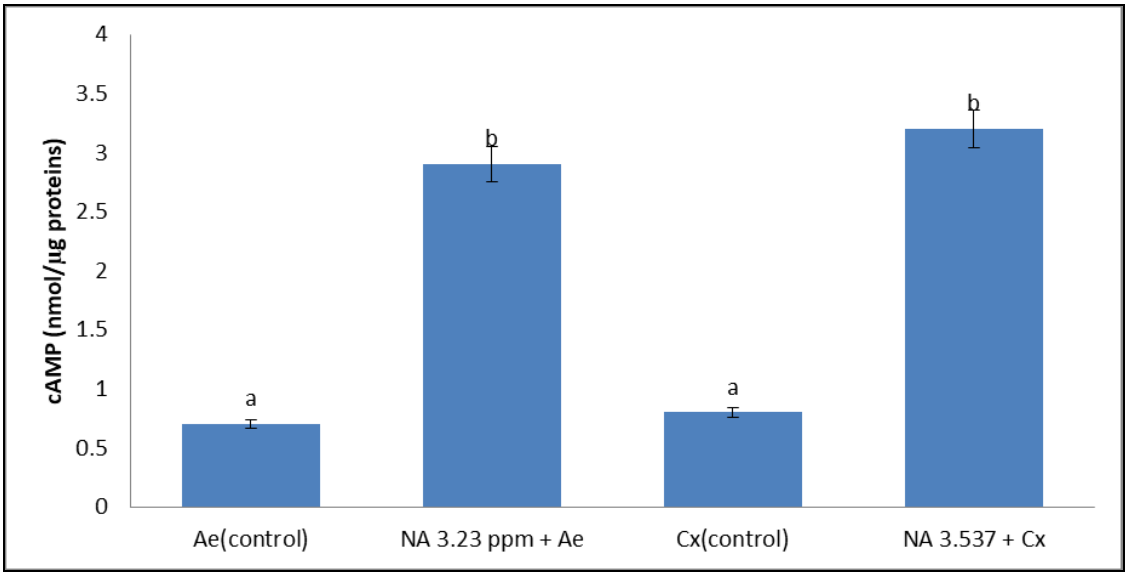
Effect of naringenin on AChE and cAMP

The activity of AChE and cAMP in the third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* represents in Figure 1 & 2. At the concentration of 3.23 and 3.537 ppm of naringenine were applied on 100 third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, respectively for 24 hours. The activity of AChE was 27.87 and 30.67 ug/ml, in control, and 9.23 and 13.76 ug/ml in 3.23 and 3.537 ppm of naringenine in *Ae. Aegypti* and *Cx. Quinquefasciatus* third instar larvae, respectively. The level of cAMP was 0.7 and 0.8 ug/ml in control, and 2.9, and 3.2 ug/ml in *Ae. Aegypti and Cx. Quinquefasciatus* instar larvae, respectively. The AChE activity was significantly declined while cAMP level was notably increased in naringenine treated *Ae. aegypti* and *Cx. quinquefasciatus* when compared to control.



Ae: *Ae. aegypti*; Cx:*Cx. quinquefasciatus*; NA: Naringenin. Each bar represents the mean ± standard error of triplicate samples of three independent experiments. Description a and b indicates significant ($p<0.05$) between control and naringenin treated mosquitoes.

Fig 1: Effect of naringenin on AChE in third instar larvae of *Ae. aegypti* and *Cx. Quinquefasciatus*



Ae: *Ae. aegypti*; Cx:*Cx. quinquefasciatus*; NA: Naringenin. Each bar represents the mean ± standard error of triplicate samples of three independent experiments. Description A and B indicates significant ($p<0.05$) between control and naringenin treated mosquitoes.

Fig 2: Effect of naringenin on cAMP in third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*

Discussion

Development of insecticide resistance has frequently been reported for various commercially utilized synthetic insecticides [2] and [20]. In order to avoid the resistance, alternative methods in vector control are necessary. Plant secondary metabolites are an excellent source for controlling mosquitoes due to their efficiency, easy biodegradability, development of less to non-toxic products, and may be applied to mosquito breeding places [34] and [27]. The present study demonstrated a high potential for

naringeninas mosquito as repellent against *Ae. aegypti*and *Cx. Quinquefasciatus*, without causing any allergic reaction to the test person. The findings in the present study may help to produce new and more effective strategies to prevent and control mosquitoes.

The egg is the least studied life stage and understanding it can contribute with novel strategies for mosquito control [9]. Ovicidal compounds can interrupt embryonic development and impair the survival of the larvae inside the egg. Previously, [35] and [11] revealed that flavonoids are able to interrupt embryo development, impair the survival of larva inside the egg or block egg hatching. In this investigation, naringenin exerted zero hatchability (100% mortality) at 8 and 10 ppm for *Ae. aegypti* whereas zero hatchability of *Cx. quinquefasciatus*at concentrations 10 ppm naringenin. Naringenin may have retarded the growth of eggs by inhibiting the embryogenesis. These results were similar with [32] reported that flavonoids like poncirin, rhoifolin and naringin showed 100% zero hatchability at 0.6 (mg/l) against *Ae. aegypti*.

Currently, mosquito control, as well as protection from the mosquito bites are presently accepted as the key measures used to control mosquito bites. The use of repellents is one of the most efficient methods to protect oneself from mosquito-borne diseases [3]. Mosquito repellents could be one of the most effective tools to safeguard humans from mosquito attack and mosquito-borne diseases, such as dengue hemorrhagic fever, malaria, encephalitis, and filariasis [18] and [29]. Identification and evaluation of effective repellent compounds are essential to combat increasing concerns for the environmental safety and the unacceptability of synthetic counterparts [21]. Plants could be an easy and natural alternative source for mosquito repellents due to the presence of a wide range of potentially bioactive compounds [43] and are mostly free from harmful side effects [41] and [21] In the present study, the naringenin at 1.0, 1.5 and 2.0 mg/cm^2 concentrations provided 83.16, 89.11 and 93.77 per cent protection from all the mosquitoes bites to test volunteers. The naringenin provides mosquito repellent activity that may be due to their ability to block mosquito sensory organs. Moreover, during this study period, we observed no noticeable adverse effects on the human skin. This result was similar to [39], who reported that tetradecanoic acid showed prominent repellent activity at a concentration of 5.0 mg/cm^2 that provided 100% protection of up to 60 and 90 min against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively.

The insect growth regulators are a very unique class of insecticides with selective effects on the various life stages of some types of insects. Insect Growth Regulators disrupt as well as impede the life cycle of insects in the egg and larvae stage of development. IGR treated egg and larvae stage cannot reach adulthood, and thus cannot reproduce. IGR is also known as a "birth control" for pests that aids to keep the populations of unwanted pests under check by preventing the current as well as future infestations [26]. The present experiment, plant-derived Naringenin showed prominent larvae emergence inhibition of 96 and 91 per cent at 10 ppm concentration in *Ae. aegypti* and *Cx.Quinquefasciatus*, respectively. Thus, the present experiment provided evidence that naringenin have IGR activity against mosquitoes. Naringenin could have delayed the development by acting as a growth hormone suppressant (tanning hormone) or could have disrupted the feeding activities of mosquito species. This finding similar with [5] reported that flavonoids such as chlorogenic acid, quercetin and rutin delayed in development of *Helicoverpaarmigera* and *Spodopteralitura* larvae.

The investigations on the modes of action of the naturally occurring phyto-compounds may help in providing useful information for the development of bio-insecticides with novel target sites and for future resistance management [30] and [17]. The main modes of insecticidal action of naturally occurring compounds are mainly due to AChE inhibition and also for the interference with the octopaminergic system [16]. Inhibiting the AChE generates the accumulation of the neurotransmitter acetylcholine in neuronal synapses, which creates a state of permanent stimulation and results in a general lack of coordination in the neuromuscular system and subsequent death. Earlier Perumalsamy *et al*. (2015), stated that AChE is the main target site of mosquito larvicidal action of flavonoids. The AChE activity was significantly declined in naringenine treated *Ae. aegypti* and *Cx. Quinquefasciatus* larvae when compared to control. This finding clearly showed that naringenine can inhibit AChE in larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. This result similar with [30], who confirmed that flavonoids such as karanjachromene, pongamol and pongatorene strongly inhibited mosquito larval cholinesterase.

Insect populations survive the effect of toxic insecticidal compounds by different physiological mechanisms, including reduced target site sensitivity and elevated detoxifying enzyme production [42]. GST is one of the important detoxifying enzymes that catalyzes the conjugation of reduced glutathione and plays a crucial role in detoxification of xenobiotic compounds including insecticides. Plant- origin natural products such as essential oils and extracts were also reported as an activator of GST activity in insects [6]. In present investigation, Treatment of naringenin was notably decreased the activity of GST in larvae, pupa, male adult and female adult of *Ae. aegypti and Cx. Quinquefasciatus* when compared to control. The activity of GST enzyme may vary in different stages of insects probably due to different detoxicative capacity, developmental stage, and with their dwelling nature. Variation in this activity may be responsible, at least in part, for the selective toxicity of insecticides and their development of resistance to insecticides [36]. The current observation indicates that the naringenin reduces activity of GST enzyme thus potentially rendering them more susceptible to toxins. This study was supported by [22], who reported that essential oil from *Citrus grandis* treated *Culex quinquefasciatus* showed declined activity of GST at larva and adult stages.

The second messenger cAMP has also been also implicated in modulation of signaling associated with the cell death in a wide variety of cells [48] and [15]. The increases in the level of cAMP stimulate protein kinase A (PKA). The activation of the cAMP/PKA signalling pathway initiates a series of cellular events that affect homeostasis and fundamental biological processes like the destabilization of the cytoskeleton and ion channels in the cell membrane. These cellular events, in turn, hampers the cellular processes and leads to the loss of structural and functional integrity of the cell and cause cell death by membrane blebbing and cellular swelling [48]. In the present study, the level of the cAMP was 0.7, 0.8, 2.9 and. 9 and 3.2 ug/ml in control *Ae. aegypti, Cx. quinquefasciatus,* naringenine treated *Ae. aegypti* and *Cx.*

quinquefasciatus third instar larvae, respectively. The increasing level of cAMP indicate that the mechanism of insecticidal action of naringenine might be due to interference with the octopaminergic system. It is the first hand information that naringenine can increase cAMP levels in larvae, which leads to cell death. This study was supported by [30], who reported that elaidic acid, arachidic acid and behenic acid were kill the larvae by increasing cAMP. [43] stated that honokiol isolated from *Magnolia denudate* was killing the larva of *C. pipiens pallens, A. aegypti, A. albopictus* and *A. sinensis* by increasing cAMP.

Conclusion

The present analysis demonstrates substantial evidence that naringenin has ovicidal, repellent and IGR activities. Moreover, it also induced a decline in the GST level, AChE activity and increase in cAMP levels in both *Ae. aegypti* and *Cx. quinquefasciatus*. However, further examination is warranted to understand naringenin's long term effects in the field conditions and on the ecosystem.

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