Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(3): 803-809

Received: 21-03-2017
Accepted: 22-04-2017

Sudarshana Mahanta
Research scholar, Department of Zoology, Gauhati University, Guwahati-781014, Assam, India

Bulbuli Khanikor
Assistant Professor, Department of Zoology, Gauhati University, Guwahati-781014, Assam, India

Riju Sarma
Research scholar, Department of Zoology, Gauhati University, Guwahati-781014, Assam, India

Correspondence
Bulbuli Khanikor
Assistant Professor, Department of Zoology, Gauhati University, Guwahati-781014, Assam, India

Potentiality of essential oil from *Citrus grandis* (Sapindales: Rutaceae) against *Culex quinquefasciatus* Say (Diptera: Culicidae)

Sudarshana Mahanta, Bulbuli Khanikor and Riju Sarma

Abstract
The present investigation was made for assessing the larvicidal, ovicidal, adulticidal and repellent effect of essential oil from the peel and leaves of *Citrus grandis* against *Culex quinquefasciatus*. The results revealed that the oil was the most effective as ovicides having LC_{50} value14.02 ppm in case of essential oil of leaves and 17.06ppm in case of that of peel at 72 hour followed by larvicidal activity with LC_{50} value 18.53ppm and 40.59ppm at 72 hour respectively. But there was not much adulticidal affect recorded though it gave good protection time against *C. quinquefasciatus*. Thus, it is a potential repellent, ovicidal as well as larvicidal agent for the control of *C. quinquefasciatus*. One of the most important detoxifying enzyme i.e. glutathione-s-transferase level was found to decrease after applying the LC_{50} dose treated larvae and adults than the non-treated control groups

Keywords: *Citrus grandis*, *Culex quinquefasciatus*, essential oil, GST

1. Introduction
Mosquitoes are one of the most serious pests for humans and other animals as they are the vectors for many diseases such as dengue fever, malaria, filariasis and encephalitis of different types etc [1]. *Culex quinquefasciatus*, the Southern house mosquito is considered as "urban bridge vector" which bridges different hosts to humans because of its confrontation with different vertebrates [2]. It is a brown-coloured medium-sized mosquito found in tropical and sub-tropical regions of the world has been established as the vector of *Wuchereria bancrafti*, avian malaria and arboviruses including St. Louis encephalitis virus, western equine encephalitis virus, West Nile virus, various protozoans etc. Lymphatic filariasis which is caused by the parasitic infection (e.g. *Wuchereria bancrafti*) and transmitted by *C. quinquefasciatus* can affect the lymphatic system of human beings [3]. About 1.10 billion people are threatened by this disease in 58 countries worldwide [4]. In India, 19 million people suffer from filarial disease manifestations [5]. The adaptive fitness, host specificity, high reproductive capacity etc. has made *C. quinquefasciatus* a smart vector [2].
The approach to overcome the serious obstacles is based on the interruption of the disease transmission cycle by either targeting the early developmental stages of mosquitoes through spraying of stagnant water breeding sites or by killing or repelling the adult mosquitoes using insecticides [6]. Some drawbacks with the use of chemical insecticides are there that they could be more harmful to other non-target organisms and be a pollutant to environment. Due to the frequent use of those insecticides, mosquitoes develop resistance against the same [1]. Considering the disadvantageous aspects of the mosquito-repellent chemicals or insecticidal chemicals modern people seem to give more and more importance on the concept of plant-based products because of their eco-friendly nature and selective mode of action [7].
Numerous products of plant- origin, especially essential oils, have received considerable attention as because they consist of a rich source of bioactive compounds that are effective against developmental stages of mosquitoes and naturally biodegradable into non-toxic products [8]. In has already been reported that more than 1,005 plant species are found to possess insecticidal properties, 384 contain antifeedants, 297 contain repellents, and 27 contain attractants and possess growth inhibitors [9].
Citrus, a member of the Rutaceae family has recently attracted noticeable interest because of its insecticidal properties against different insects [10]. The essential oils from *Citrus* genus have monocyclic monoterpenoides comprising d-limonene as their major compound which is

mainly responsible for their insecticidal quality [11]. Peel or seeds extracts or essential oil from peel or seeds of some of the species like *Citrus reticulata*, *Citrus hystrix*, *Citrus sinensis* and *Citrus aurantifolia* had been studied by different researchers against some of the medically important mosquito species as well as the agricultural pests [11-14]. Again, the North-Eastern region of India holds a distinctive position in the world map of *Citrus* occurrence and its diversity [15].Though the genus *Citrus* had been extensively studied for their insecticidal properties, anti-microbial properties or antioxidant properties [16-18], but the efficacy of the essential oil from the fruit peel and leaves of *Citrus grandis* has not been explored yet against *C. quinquefasciatus* which is a medically important mosquito species. It had already been established that the popularity of the fruit of *C. grandis* lies in its higher tolerance to insects than the other citrus fruits [17]. Glutathione S-transferase (GST) is an enzyme that catalyzes the conjugation of electrophile molecules with reduced glutathione (GSH) to increase toxic substances metabolism. Thus GST plays an important role in insecticide resistance [19]. Therefore, the present study was aimed at evaluating the potentiality of essential oil from the peel and leaves of *C. grandis* against different developmental stages of *C. quinquefasciatus.* Again the activity of Glutathione-s-transferase enzyme was also studied to observe the detoxification of the applied essential oil by the target mosquitoes.

2. Materials and Methodology

The whole study was conducted in the Entomology Laboratory of the Department of Zoology, Gauhati University, Assam. The study period was from the month of May to June, 2016.

2.1 Collection of plant materials

The fruits and leaves of *Citrus grandis* were collected from Pathsala (26.5119^0 N, 91.1809^0 E) of Barpeta district, Assam, India. The essential oil from peels and leaves of *C. grandis* was extracted by hydrodistillation method using Clevenger apparatus.

2.2 Rearing of *C. quinquefasciatus*

The culture of *C. quinquefasciatus* was maintained at 28±2ºC and 65-70% RH in the Entomology Laboratory, Department of Zoology, Gauhati University. The reared larvae were fed on dog biscuits and yeast powder in the ratio of 3:1 in plastic tray (24x35x5cm). Adults were fed with 10% sucrose solution and the female mosquitoes were also fed with live rat blood every alternate day to lay satisfactory number of eggs [20].

2.2 Bioassay Procedure

2.3.1 Ovicidal Activity

The ovicidal bioassay was performed according the method described by Tennyson *et al*. [21] and Puspanathan *et al*. [22] with little modifications. Seven different concentrations like 1000 ppm, 750 ppm, 500 ppm, 250 ppm, 100 ppm, 50 ppm and 10 ppm of the essential oil were prepared for the testing. 50 numbers of eggs of the mosquito species were exposed to each concentrations of essential oil. Each experiment was replicated thrice along with appropriate control (DMSO as positive control and water as the negative control). The number of hatching was assessed 72 hour post treatment.

Percent ovicidal activity= (% of eggs hatched in control-% eggs hatched in treated/ %of eggs hatched in control) ×100

2.3.2 Larvicidal activity

Larvicidal activity was determined by following the method described by Govindaranjan *et al*. [23] with little modification. Twenty numbers of third instar larvae of *C. quinquefasciatus* were kept in 100 ml of water of the plastic cup with the desired concentration. Seven different concentrations like 1000 ppm, 750 ppm, 500 ppm, 250 ppm, 100 ppm, 50 ppm and 10 ppm with three replicates for each concentration were maintained. Dimethyl sulphoxide (DMSO) was used for the control. Mortality of larvae was recorded after 24 h of treatment. The median lethal concentration LC_{50} values were calculated.

2.3.3 Pupicidal activity

The pupicidal activity was tested following the method described by Aruna *et al.* [3]. 20 numbers of mosquito pupae were kept in a plastic cup containing 100 ml of water with the particular concentration. Three replicates were made for each concentration and the mortality was observed after 24 hours of the treatment and the LC_{50} value were calculated. DMSO was used as the control.

2.3.4 Adulticidal bioassay

The adulticidal activity of essential oil was assessed following the technique described by Kovendan *et al*.[24].Sugar-fed adult female mosquitoes (5 to 6 days old) were used for the bioassay. Different concentrations of essential oils were impregnated on filter papers (140×120 mm). The papers were left to dry. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in culture tube (10 cm depth) which was wrapped from inside by essential oil treated filter papers. Sucrose-fed and blood-starved mosquitoes (20) were then released into the tube, and the mortality effects of the essential oil were observed. LC_{50} value was calculated for 24 hour, 48 hour and 72 hours. The above procedure was carried out in triplicate for each concentration of the oil. Acetone was used as the control.

2.3.5 Repellent bioassay

Protection time was observed following the method described by Barnard *et al*. [25]. For the study of the protection time of this oil, three-four days old blood-starved female *C. quinquefasciatus* mosquitoes (50) were kept in a net cage (47 x 35 x 31 cm^2). The dorsal side of the arms of the test person was covered with the rubber gloves except an area of $5cm^2$.This area was covered with a muslin cloth. On the cloth 0.5ml of tested solution was applied where olive oil was used as control. After air drying the arms of the test person, the control and treated arms were introduced simultaneously into the cage. The landing of mosquitoes was observed by exposing the treated area inside the cage for 5 minutes after every 30 minutes from 5:00h to 7:00 h. The experiment was conducted three times for each concentration. It was observed that there was no skin irritation from the essential oil tested. The olive oil was used as the positive control against *C. quinquefasciatus*. The protection time was calculated by the following formula-

Protection time=Time of first landing of mosquito in treated - Time of first landing of mosquito in control

2.3 GC-MS Analysis

GC-MS analysis was carried out to identify the constituents of the essential oil extracted from the fresh leaves and peels of *C. grandis*. Sample of the essential oil was analysed using Gas Chromatography(Programme 2M-10-200-3M-10-270-5M-10-280-1M-HP5).The identity, retention time, area and

percentage composition of the essential oil extracted from the peels and leaves of *Citrus grandis* is presented in the Table-4 and Taable-5.

2.4 GST activity

GST assay was performed by the method described by Farahnak *et al*. [26]. GST activity was assayed spectrophotometrically at 30°C with 1-chloro-2, 4-dinitrobenzene (CDNB) as the standard second substrate and reduced glutathione (GSH). For this essay, insect was first homogenized in 5 ml of 200 mM EDTA and 20 mM potassium phosphate solution (1:1). Then the homogenate was centrifuged in 4^0C at 10000rpm for 10 minutes. For each assay, one ml of cocktail (980 µl PBS pH 6.5, 10 µl of 100 mM CDNB and 10 µl of 100 mM GSH) was prepared, and then removed 100 µl of cocktail and its remaining placed into 1.5 ml plastic cuvet. Distilled water was used as zero and 100 µl PBS will be added to 900 µl of cocktail in the blank cuvet and absorbance was measured at 340 nm for 30 min. 100µl supernatant of sample was added to 900 µl cocktail in the test cuvet and mixed and absorbance measured at 340nm.

2.5 Statistical Analysis

The LC_{50} values were calculated by probit analysis using SPSS and MINITAB software.

3. Results

3.1 Ovicidal activity

During the study of the ovicidal activity of the essential oil against the target species, hatching of larva from the eggs were observed from 24 hours to 72 hours. No hatching was recorded after 72 hours of treatment. Therefore LC_{50} value of ovicidal activity was recorded at 72 hour of exposure period (Table-1). The essential oil from the peel of *C. grandis* had shown higher efficacy against the eggs of the target mosquitoes than that of leaves. At a high dose of 1000 ppm concentration of essential oil of peel, 7.64% and 8.2% hatching was observed at 48 hour and 72 hour respectively. At 24 hour, no larvae were found to hatch from the treated eggs. Again, 15.39%, 56.8% and 56.8% hatching were noticed at 24 hour, 48 hour and 72 hour of treatment at a lowest dose of 10 ppm concentration of the said essential oil respectively (Table-2). The potentiality of the essential oil from leaves as ovicides was relatively lower than that of peels. At 1000 ppm and 10 ppm concentrations of the oil, 0%, 0%, 0% and 29.8%. 26.7% and 24.6% hatching was noticed after 24 hour,48 hour and 72 hour of the treatment respectively. The LC_{50} value along with the regression equation and 95% confidence level is listed in Table-1. At 72 hour of treatment, the LC_{50} value was recorded as 17.06 ppm in case of essential oil of peel and 11.58 ppm in case of essential oil from leaves of *C. grandis*.

Table 1: Ovicidal, Larvicidal, Pupicidal and Adulticidal effects of essential oil from the peel and leaves of *C. grandis* against the respective developmental stages of *C. quinquefasciatus*

Serial no.	Effect	Essential oil	Time	LC_{50} value	Regression Equation	Lower Bound	Upper Bound	Chi-square value
1	Ovicidal	Peel	72 Hour	17.06 ppm	Y=3.85+.94x	.911	1.029	41.960
		Leaves	72 Hour	11.58 ppm	Y=3.02+1.87x	1.209	1.423	59.21
2	Larvicidal	Peel	24 Hour	61.046 ppm	Y=2.31+1.5x	1.226	1.782	26.720
			48 Hour	60.55 ppm	Y=2.17+1.59x	1.322	1.852	25.935
			72 Hour	40.59 ppm	Y=2.61+1.48x	1.223	1.744	16.193
		Leaves	24 Hour	64.89 ppm	Y=-1.40+3.53x	2.252	4.834	6.007
			48 Hour	26.65 ppm	Y=2.58+1.69x	1.244	2.173	13.136
			72 Hour	18.53 ppm	Y=1.87+2.47x	1.600	3.039	3.073
3	Pupicidal	Peel	24 Hour	441.42 ppm	Y=-1.80+2.57x	4.339	7.715	15.792
		Leaves	24 Hour	247.27 ppm	Y=1.17+2.79x	2.043	3.298	15.10
4	Adulticidal	Peel	24 Hour	-	-	-	-	-
			48 Hour	-	-	-	-	-
			72 Hour	-	-	-	-	-
		Leaves	24 Hour	-	-	-	-	-
			48 Hour	-	-	-	-	-
			72 Hour	-	-	-	-	-

"-"Implies no results

Table 2: Hatching percentage of eggs and the survivability percentage of adults of *C. quinquefasciatus* after the treatment of essential oil from the peel and leaves of *C. grandis.*

Serial no	Observations	Essential oil	No of individual/replica	Concentration	24 hour (%±SE)	48 hour (%±SE)	72 hour (%±SE)
1	Hatching percentage of eggs	Peel	50	1000 ppm	0±0	7.64±1.14	8.2±0.89
			50	500 ppm	3.4±.32	8.4±0.89	8.4±0.89
			50	100 ppm	5.2±0.89	25.5±0.33	25.6±0.59
			50	10 ppm	15.39±.33	56.8±1.20	56.8±1.20
		Leaves	50	1000 ppm	0±0	0±0	0±0
			50	500 ppm	0±0	0±0	2.14±.88
			50	100 ppm	23.3±1.15	10.2±1.73	10.4±1.73
			50	10 ppm	29.8±1.20	26.7±1.15	24.6±1.15
2	Survivability percentage of adults	Peel	20	1000 ppm	100±0	67±0.33	44±0.33
			20	500 ppm	100±0	87±.33	77±.33
			20	100 ppm	100±0	100±0	86.7±0.89
			20	10 ppm	100±0	100±0	100±0
		Leaves	20	1000 ppm	96.7±.33	46.7±.33	26.7±.33
			20	500 ppm	100±0	76.6±.33	60±.33
			20	100 ppm	100±0	96.7±0	84±.33
			20	10 ppm	100±0	100±0	93.3±0.66

3.4 Larvicidal activity

During the study of larvicidal activity of essential oil of peel and leaves of *Citrus grandis*, seven different concentrations of the oil were prepared and tested against 3rd instar larval stages of *C. quinquefasciatus*. In the study, it was observed that the larval mortality was directly related to the exposure time and concentration of the oil. Larval mortality was recorded upto 72 hours. The statistical analysis revealed the LC_{50} values of essential oil extracted from the peels of *C. grandis* as 61.04 ppm, 60.55 ppm and 40.59 ppm and 64.89 ppm, 26.65 ppm and 18.53 ppm for the essential oil from the leaves of *C. grandis* at 24 hour, 48 hour and 72 hour respectively. As a larvicide, essential oil from the leaves of *C. grandis* was more efficient than that of peels against *C. quinquefasciatus*. The values of sub-lethal concentrations are presented along with regression equations, 95% confidence level in the Table-1.

3.5 Pupicidal activity

The toxicity of the essential oil was also studied against the pupal stages of *C. quinquefasciatus* exposing them in seven various concentrations and LC_{50} value was found to be 441.42 ppm in case of essential oil from the peels of *C. grandis* and 247.27 ppm in case of that of leaves of *C. grandis* after 24 hours. The LC_{50} value after 48 hour and 72 hour could not be calculated as the living individuals were transformed to adult stages after 24 hours (Table-1)

3.6 Adulticidal activity

No effective adulticidal activity was observed of the essential oil from *Citrus grandis*(peel) against the adult stage of *Culex quinquefasciatus*. Mortality was absent till 24 hour after the treatment with 100 to 1000ppm concentration in case of essential oil from the peel of *C. grandis*. But after 48 hour and 72 hour of treatment, the survivability of adults were found to be decreased 53.3% to 73.3% respectively at the concentration of 1000ppm and 3.3%, 13.3% at 100 ppm which was not a very good result of adulticidal activity of the essential oil. The concentration of 1000 ppm of the essential oil from the leaves of *C. grandis* showed little effect against the adult stages as the survivability rate was 96.7%, 67% and 44% at 24 hour, 48 hour and 72 hours respectively. The survivability percentage of adult *C. quinquefasciatus* at two concentrations is listed in the Table-2.

3.7 Repellent activity

The result of the repellent effect of the essential oil was assessed in terms of determining the protection time. The result showed protection time of 30 minutes by the two essential oil against *C. quinquefasciatus.* (Table-3)

Table 3: The protection time of the essential oil from *C grandis* (peel & leaves) as repellent.

Serial no.	Treatment	Protection time
1	*C. grandis*(peel)	30 minutes
2	*C. grandis* (leaves)	30 minutes
3	Olive oil (Control)	5 Minutes

3.8 GC-MS Analysis

The GC-MS report showed nootkatone, eudesmol as probable major constituents of the essential oil extracted from the peels of *C. grandis* and ß citronellol, 5- hepten-1-ol-2,6- dimethyl as probable major compounds of the essential oil of leaves of *C. grandis*(Table-4 and 5, Fig-1 and 2)

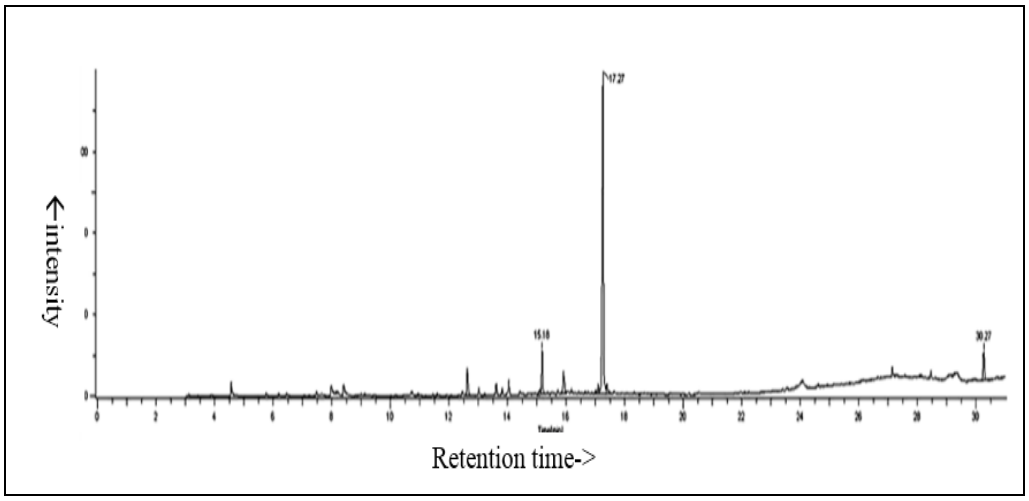

Fig 1: Chromatogram of GC MS analysis of the essential oil from *Citrus grandis* (peel)

Table 4: Four probable major constituents of the essential oil from *C. grandis* (peel)

Serial no.	Component	Molecular weight	Retention index	Chemical formula	Area	Retention time
1	1-Naphthalenol, deca-hydro-1,4 a-dimethyl 7-[(1-methyllthylidone)(1-R(1α, 4αβ, 8aα)]	222	1681	$C_{15}H_{26}O$	7.65	15.19
2	2(3H)-N aphthalenone 4, 4a, 5, 6, 7, 8 hexahydro 4, 4a- dimethyl 6(1-methylethyenyl, 4R(4α, 4aα, 6β)	218	1781	$C_{15}H_{22}O$	69.14	17.25
3	6.10,14, 18,22, Tetra cosapentaen-2-ol, 3 bromo,2,6,10,15,19,23hexamethyall E	506	3253	$C_{30}H_{51}BrO$	5.98	30.27
4	Geranylgeraniol	290	2192	$C_{20}H_{34}O$	5.26	15.92

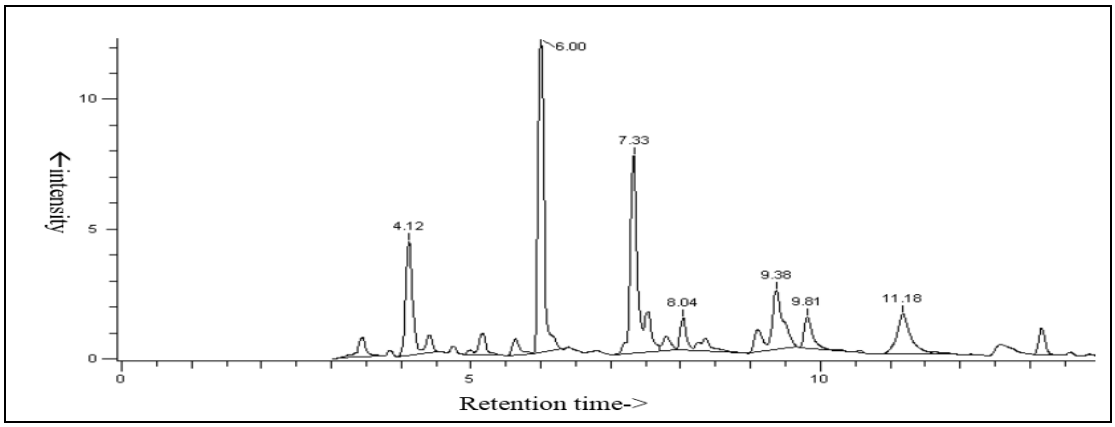

Fig 2: Chromatogram of GC MS analysis of the essential oil from *Citrus grandis* (leaves)

Table 5: Four probable major constituents of the essential oil from *C. grandis* (leaves)

Serial no.	Component	Molecular weight	Retention index	Chemical formula	Area	Retention time
1	(R) –(+)- β- Citronellol	156	1179	$C_{10}H_{20}O$	22.87	7.33
2	Cyclohexanol,2-(2-hydroxy-2-propyl)-5- methyl	172	1320	$C_{10}H_{20}O_2$	11.37	9.37
3	Citronellal hydrate	172	1239.7	$C_{10}H_{20}O_2$	7.75	11.19
4	α-citral	152	1249	$C_{10}H_{16}O$	4.87	8.04

a. Effect on GST activity

In the present study, regarding the effect of essential oil of leaves of *Citrus grandis* against the 3rd instar larvae and adult of *C. quinquefasciatus*, the GST activity was observed. Since the essential oil extracted from the peels of *C. grandis* was less effective than that of leaves, therefore the essential oil from the leaves was tested to highlight the effects on GST-activity In case of essential oil treated individuals, the GST activity was seen lower as compared to the positive control (Fig. 3 and Fig. 4).

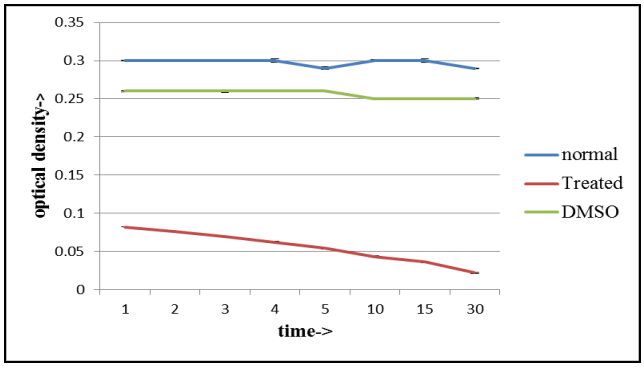

Fig 3: Graph showing the GST activity in the larval stages of *C. quinquefasciatus* after the application of essential oil of *C. grandis* (leaves).

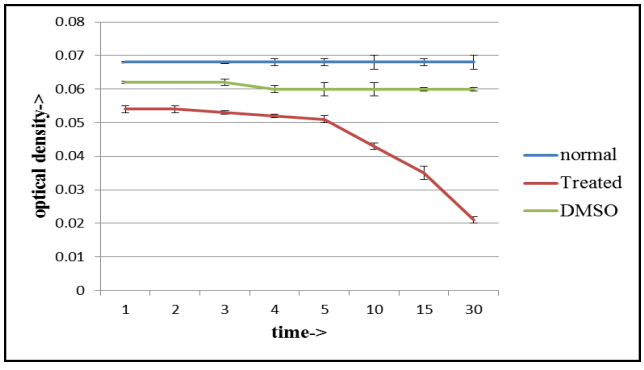

Fig 4: Graph showing the GST activity in the adult stages of *C. quinquefasciatus* after the application of essential oil of *C. grandis* (leaves).

4. Discussion

According to the results of the current study, it can be summarised that the essential oil from the leaves and peels of *C. grandis* showed promising insecticidal activity against the *C. quinquefasciatus* mosquitoes. The one of the important factors which is responsible for this effect is the major compounds of the essential oil including its quality and quantity [8] found in the said essential oil. The compounds including alcohols, aldehydes, fatty acid derivatives, terpenoids, and phenolics may jointly or independently contribute to insecticidal, ovicidal, repellent as well as antifeeding activities [27]. Mansour *et al.* [28] already found that Limonene was the dominant compound with an occurrence ranging from 51.97 % to 95.32 % in different *Citrus* species. In the present study, different compounds of the essential oil from the peel of *C. grandis* had been analysed using the GC-MS technique. This finding showed nootkatone, eudesmol as the probable major compounds of the oil which was not same as the previous findings found by Manorenjitha *et al.*[29] as they found limonene as the major compound of the essential oil from fruit peel of *C. grandis*. Geographic location, method of extraction, time of harvesting are some of the factors which influence those variations of the profile of constituents of an essential oil [13]. The present study also revealed that the essential oil from the leaves of *C. grandis* was more effective against the developmental stages of the target mosquitoes than that of peels. It was due to the presence of some other probable major compounds like citronellol, 5-hepten-1-ol-2,6-dimethyl etc. as found in the GC-MS analysis.

The oil demonstrated the efficacy in eggs, larval and pupal stages, but no effect was observed in case of the adults as an adulticidal agent. Larvicides or ovicides or pupicides kill the respective developmental stages in their breeding habitat before they can mature into adults, hence they can minimize the application of other adulticiding chemicals [30]. The eggs of *C. quinquefasciatus* were more susceptible than other developmental stages as the encapsulation of the eggs actually increased exposure to stresses by holding embryos in stressful condition that larvae or other stages could easily avoid through passive dispersal or vertical migration [31].Exposure time also has a crucial role in toxicity of the oil against the

eggs of the target species. The differences in susceptibility to the two different essential oil as ovicides are due to the differential rate of intake, penetration through the egg-membrane, detoxication etc [32]. Mosquitoes vary in their response to essential oil and it has already been established that the sensitivity of different developmental stages of the same species of mosquito could be quite different for the same compound. Again essential oils can either be inhaled, ingested or absorbed by the skin of insects. Therefore, the different toxicity levels in different developmental stages were due to those physiological as well as morphological variations [33].

Though the efficacy of essential oil from the peel and leaves of *C. grandis* has not been studied against *C. quinquefasciatus* yet, many researchers had found the essential oil of *Citrus sinensis*, *Citrus hystrix* and *Citrus reticulata* as potent larvicides against different mosquito species [8, 12]. Again, Hafeez *et.al* [14] reported the essential oil of *Citrus sinensis* (LC_{50}=53.61 ppm) as adulticidal agent against *Aedes albopictus* which is not same as the present findings. This difference in toxicity may be due to the presence of different constituent compounds of the essential oil as mentioned above.

Essential oil(s) possess some of the drawbacks like they are highly volatile due to which their repellent activity and protection time are lesser than that of synthetic products. This problem can be nullified by using appropriate fixatives to the formulation. Generally, repellent properties of some of the essential oils appear to be associated with the presence of lower isoprenoides [34].

Since essential oil(s) are the mixtures of major as well as minor constituents, they act synergistically in the target individuals in contrast to synthetic insecticides. Moreover, the essential oil has been found potent against those species of pests which are resistant to synthetic products [35].*Culex quinquefasciatus* had been considered as one of the three" world's resistant mosquitoes" and it had been reported that the involvement of glutathione-s-transferase in resistance to organophosphate, organochlorines and pyrethroides [36]. 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. Plant- origin natural products such as essential oils and extracts were also reported as an activator of GST activity in insects [37]. In present investigation, effects of the essential oil from *C. grandis* (leaves) on the GST activity of the 3rd instar larvae, and adult have been studied. The pupal stage was avoided due to its short duration of the life span. The results showed distinctive effect on GST activity of each stage. In the larva, very low GST activity is observed in comparison to the normal and positive control. GST level was found to be decreased in both the larval and adult stages of the target species (Fig-3 and 4).

Change in GST activities in insects implies different chemical susceptibilities [19]. Insects can metabolize or degrade toxic substances or endogenous compounds. 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 [38]. The current observation indicates that the essential oil from *C. grandis* (leaves) reduces levels GST enzyme thus potentially rendering them more susceptible to toxins.

5. Conclusion

Essential oil from the peels or leaves of *C. grandis* could be an alternative measure for the control of *C. quinquefasciatus* as they contain efficient source of bioactive compounds which are generally free from health hazards, environmental pollution. Further studies are needed to identify the active compounds responsible for their effectiveness to observe the mode of action of the natural products on the target species and last but not the least; to highlight the field trials.

6. Acknowledgement

The authors are very much thankful to Dr.Gajen Sarma, curator of the Department of Botany, Gauhati University for helping in the identification of the plant species. We are also grateful to the SAIF, IIT Bombay for their valuable assistance and UGC,DST for their financial support in this investigation.

7. References

1. Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. Malaria Journal. 2009; 8:124.
2. Bhattacharya S, Basu P. The Southern House Mosquito, *Culex quinquefasciatus*: profile of a smart vector. Journal of Entomology and Zoology Studies. 2016; 4(2):73-81.
3. Aruna P, Murugan K, Priys A, Ramesh S. Larvicidal, pupicidal and repellent activities of *Gaultheria* oil (Plantae: Ericaceae) against the filarial vector, *Culex quinquefasciatus*(Insecta: Diptera: Culicidae). Journal of Entomology and Zoology Studies. 2014; 2(4):290-294.
4. World Health Organization. 2016.
5. Kovendan K, Murugan K, Thiyagarajan P, Kumar N, Abirami D, Asaikkutti A. Impact of climate change on the filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). In: International Congress of Global Warming on Biodiversity of Insects: Management and Conservation. 2009; 62.
6. Tiwary M, Naika SN, Tiwary K, Mittal PK, Yadav. Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. Journal of Vector Borne Disease. 2007; 44:198-204.
7. Khanikor B, Parida P, Yadav RNS, Bora D. Comparative mode of action of some terpene compounds against octopamine receptor and acetyl cholinesterase of mosquito and human system by the help of homology modelling and Docking studies. Journal of Applied Pharmaceutical Science. 2013; 3(02):006-012.
8. Suttthanont N, Choochote W, Tuetun B, Junkum A, Jitpakdi A, Chaithong U *et al*. Chemical composition and larvicidal activity of edible plant-derived essential oils against pyrethroid-susceptible and-resistant strains of *Aedes aegypti* (Diptera: Culicidae). Journal of Vector Ecology. 2010; 35(1):106-115.
9. Jayaraj S. Neem in pest control. International World neem conference. 1993, 37-43.
10. Khan HAA, Akram W. Citrus-based essential oils could be used for dengue vector mosquitoes control. 2013, 504-504.
11. Zia S, Sagheer M, Razaq A, Mahboob A, Mehmood K, Haider Z. Comparative bioefficacy of different citrus peel extracts as grain protectatnt against *Callosobruchus chinensis*, *Trogoderma granarium* and *Tribolium castaneum*. World Applied Sciences Journal. 2013; 21(12):1760-176.

12. Vera SS, Zambrano DF, Sanchez SCM, Sanabria FR, Stashenko EE, Luna JED. Essential oils with insecticidal activity against larvae of *Aedes aegypti*. Parasitology Research. 2014; 113:2647-2654.
13. Din SU, Akram W, Khan HAA, Hussain A, Hafeez F. Citrus waste-derived essential oils: alternative larvicides for dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera).Pakistan Journal Zoology. 2011; 43(2):367-372.
14. Hafeez F, Aktam W, Suhail A, Khan MA. Adulticidal action of ten citrus oils against *Aedes albopictus* (Diptera: culicidae).Pakistan Journal of Agricultural Science. 2010; 47(3)241-244.
15. Hore DK, Barua U. Status of citriculture in north eastern region of India- a review. Agriculture Review. 2004; 25(1):1-15.
16. Singh SK, Singh IP, Singh A, Parthasarathy VA, Vinoth S. Pummelo[*Citrus grandis* (L.) Obseck] Diversity in India, Indian Journal of Plant Genetic Resources. 2015; 28(1):44-49.
17. Rehman ZU. Citrus peel extract – A natural source of antioxidant. Food Chemistry. 2006; 99:450-45.
18. Herman A, Tambor K, Herman A. Linalool affects the antimicrobial efficacy of essential oils. Current Microbiology. 2016; 72:165-172.
19. Mendoza AC, Penilla RP, Rodriguez DA. Insecticide resistance and glutathione S-transferases in mosquitoes: A review. African journal of Biotechnology. 2009; 8(8):1386-1397.
20. Ramar M, Ignacimuthu, Paulraj GM. Biological activity of nine plant essential oils on the filarial vector mosquito, *Culex quinquefasciatus* Say.(insect: Diptera: Culicidae). International journal of research in biological science. 2013; 4(1):1-5.
21. Tennyson S, Ravindran KJ, Arivoli S. Screening of plant extracts for ovicidal activity against *Culex quinquefasciatus* Say(Diptera: Culicidae). Applied Botany. 2011; 40:5456-5460.
22. Pushpanathan T, Jebanesan A, Govindarajan M. Larvicidal, ovicidal and repellent activities of *Cymbopogon citratus* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus*(Say) (Diptera : Culicidae).Tropical Biomedicine. 2006; 23(2):208–212.
23. Govindaranjan M. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f.ex Benth (Rutaceae) against three mosquito species. Asian Pacific Journal of Tropical Medicine. 2010, 874-877.
24. Kovendan K, Murugan K, Kumar PM, Thiyagarajan P, William SJ, Ovicidal, repellent, adulticidal and field evaluations of plant extract against dengue, malaria and filarial vectors. Parasitology Research. 2013; 112:1205-1219.
25. Barnard D, Posey KH, Smith D, Schreck CE. Mosquito density, biting rate and cage size effects on repellent tests. Medical and Veterinary Entomology. 1998; 12:39-45.
26. Farannak A, Seyyedi MA, Jalali M. Study on Glutathione-S-Transferase inhibition assay by Triclabendazole. III: nematodirus parasite and sheep liver tissue. Acta Medica Iranica. 2007; 45(6):437-442.
27. Park IK, Shin SC. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). Journal of Agriculture and Food Chemistry. 2005; 53:4388-4392.
28. Mansour SA, Sharkawy EAZ, Ali AR. Mosquitocidal activity of citrus peel oils with respect to their limonene content. Egyptian Journal of Natural Toxins. 2004; 1:111-134.
29. Manorenjitha MS, Zairi J, Ling SK, Mailina J, Nuziah H. Chemical constituents of the fruit peel from white flesh *Citrus grandis* (L.) Osbeck. International journal of Pharmacy and Biological Science. 2016; 7(1):267-278.
30. Raquel M, Gleiser JA, Zyygadlo. Insecticidal properties of essential oils from *Lippia turbinata* and *Lippia polystachya* (Verbenaceae) against *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research. 2007; 101:1349-1354.
31. Fox CW, Roff DA, Fairbairn DJ. Evolutionary ecology: Concepts and Case Studies, Oxford University Press. 2001.
32. Valarmathy D, Govindaraju M, Elumalai K. Studies on ovicidal activity of plant essential oil formulation against the eggs of important vector mosquitoes, *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L.) at laboratory condition. International journal of current research. 2011; 3(6):378-381.
33. Roger CR. The potential of botanical essential oils for insect pest control, Integrated Pest Management Reviews. 1997; 2:25-34.
34. Rehman JU, Ali A, Khan IA. Plant based products: Use and development as repellents against mosquitoes: A review. Fitoterapia. 2014; 95:65-74.
35. Batish DR, Singh HP, Kohli RK, Kaur S. Eucalyptus essential oil as a natural pesticide. Forest ecology and management. 2008; 256:2166-2174.
36. Low VL, Chen CD, Lee HL, Tan TK, Chen CF, Leong CS *et al*. Enzymatic characterization of insecticide resistance mechanisms in field population of Malaysian *Culex quinquefasciatus* Say (Diptera: Culicidae). PLOS ONE. 2013; 8(11).
37. Ebadollahi A. Plant essential oil from Apiaceae family as alternatives to conventional insecticides. Ecologia Bolkanica.2013; 5(1):149-172.
38. Sivori JL, Casabe N, Zerba N, Wood EJ. Induction of Glutathione S-transferase activity in *Triatoma infestans*. SciELO Analytics.1997; 92(6):797-802.