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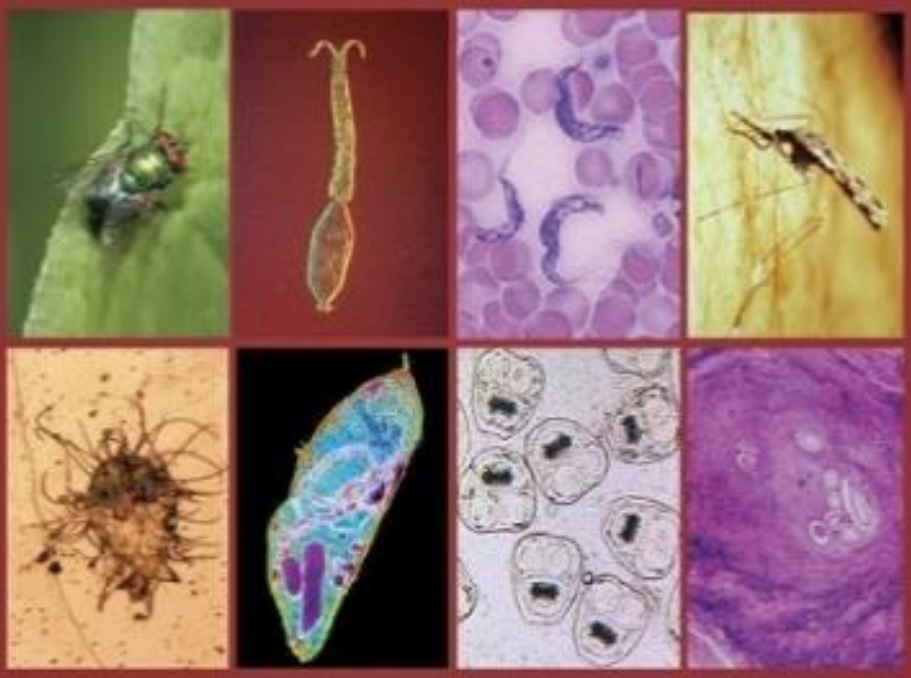

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Characterization of Some Medicinal Plants and Their Role in Mosquito Control

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ABSTRACT

Background & Objectives: *Culex quinquefasciatus* causes millions of deaths every year being the vector of many serious human diseases like Elephantiasis, and West Nile fever and may lead to serious problems to health. Chemical insecticides are quite effective to control mosquitoes but they pose certain major problems like the development of resistance and ecological imbalance. Therefore, plant products are now being screened all over the world for vector control. The present study evaluated the potential of *Datura stramonium and Morus alba* against *Culex quinquefasciatus* **Material andMethod:** The GCMS analysis was led to investigate the presence of major compounds in the extracts.Different bioassays against the IVth instar larvae of *Culex quinquefasciatus* were carried out. Seven different concentrations of the *Datura stramonium and Morus alba* extract were performed to get LC50. Mortality, data was calculated by the Log-probit method of Finney (Finney 1971) using SPSS.16 (SPSS 2010) following Abbott method. **Results:** GC-MS analysis indicated that *Datura stramonium* shows 23 While Morus alba showed 6 major chemical compounds. The petroleum ether extracts of *Datura stramonium* and *Morus alba*, showed LC50 at 54.18 ppm and 133.47 ppm respectively after 24 hours. Whereas LC50 after 48 hrs is recorded at 37.08 ppm in *Datura stramonium* and 122.46 ppm in *Morus alba* against *Culex quinquefasciatus*. At 55 ppm, *Datura stramonium* showed ovicidal activity at 59.24%, ovipositional activity at 89.70% and adulticidal activity at 49.6%. On the other hand, *Morus alba at* 135 ppm exhibited 39.01% ovicidal activity, 79.30% ovipositional activity and 52%. adulticidal activity. **Conclusion:** It is concluded that the petroleum extract of *Datura stramonium* is more effective against *Culex quinquefasciatus* as compared to the petroleum extract of *Morus alba* and can be used to control the population of *Culex quinquefasciatus.*

INTRODUCTION

Vector-borne diseases are a serious threat to public health as it causes millions of deaths every year and account for causing more than 700,000 deaths annually which is more than 17% of all infectious diseases (WHO, 2020). They can be caused by parasites, bacteria, or viruses. Mosquitoes (Insecta: Diptera) are one such vector for most of the life-threatening diseases causing many deadly diseases like malaria, filariasis, Japanese encephalitis, West Nile fever, and yellow fever (Juliano, S.A.*et. al.*, 2019). Therefore, vectors and the diseases caused by vectors are a serious challenge to human beingsas it impedes national economic and social development. In order to minimize the spread of vector-borne diseases, the major strategies are to control the vector or immediate host (Iqbal, J. *et. al.*, 2018).

In India, approximately 25 million people contain microfilaria and around nineteen million people suffer from filarial diseases. *Culex quinquefasciatus* (Diptera: Culicidae), is an obligatory ectoparasitic vector of many deadly diseases, which is a widely distributed tropical disease (Deepalakshmi, S., & Jeyabalan, D. 2017). 863 million people in 47 countries worldwide remain threatened by lymphatic filariasis and 51 million people were infected as of 2018 (WHO 2022). The use of chemical insecticides is the most effective control strategy against mosquitoes but various ecological problems have been showing up to the negative effects of chemical insecticides like the development of resistance in insect strains, ecological imbalance, and harm to mammals, water contamination, toxicity to non-target organisms and residual effects (Rahuman, A. A. *et.al.,*2008). Due to such factors, botanicals were considered as an alternative for managing insects. Several secondary metabolites present in plants serve as a defence mechanism against insect attacks. A wide range of potential phytochemicals is present in a plant which shows potential mosquitocidal activity. They are more effective against these vectors because they are target specific, rapidly biodegradable, eco-friendly, and less toxic to human health. This attracts the attention of researchers toward plant-based chemicals for insect control, (Kumar, D *et.al.,* 2014). *Datura stramonium* (F.) is a plant that belongs to the Solanaceae family that growsin many parts of the world (Abbasipour, H., *et. al.,* 2011). It is well known for its toxicity and hallucinogenic to herbivores. It also has medicinal importance to treat epilepsy, burns and rheumatism (Chintem, D. G. *et. al.,* 2014). Whereas *Morus alba* Linn, commonly known as white mulberry, belongs to the Moraceae family. Phenolic acids, flavonoids, flavonols, anthocyanins, macronutrients, vitamins, minerals, and volatile aromatic compounds are some of the bioactive compounds which are abundant in *Morus alba* (Chen, C *et. al.,* 2021). Many pharmacological effects like antioxidative, diuretic, antiobesity, hypoglycemic, hypotensive, anticholesterol, antidiabetic, and antimicrobial were shown by these natural bioactive compounds. (Chang, B. Y *.et. al.,* 2021). Essential oils from the leaf of *Morus alba* show antibacterial and anticancer activity. The present study aimed to investigate the bioefficacy ofpetroleum ether extract of *Datura stramonium* and Morus alba against *Culex quinquefasciatus*.

MATERIALS AND METHODS

1.Collection and preparation of Plant Extract: Healthy leaves of *Datura stramonium* and *Morus alba* were collected from the local area and verified by the Botanical Survey of India, Pune. The selected leaves were kept for drying at room temperature (27□2□c) under shade till they dried completely. The dried leaves were powdered and subjected to Soxhlet extractions with petroleum ether solvent. The filtrate was concentrated to dryness by rotary evaporation at 50^0 c and kept in freeze for further use. (Rajendrabhoopathy, S.et al,2017)

2.Gas chromatography -mass spectrometry analysis:

Analysis of effective components: For the identification of constituent compounds of the selected plants, the samples were subjected to gas chromatography (Agilent 7890A) and mass spectrometry (Accu TOF GCv, Jeol). Helium was used as carrier gas at a constant column flow rate of 1ml/min. The GC programme was set for *Datura Stramonium* as 10;60-1M-8-200-1M-8-275-15M-5-280 ET and for*Morus alba* as 10;60-1M-8-200-1M-8-200-15M-5-280 ET.

3.Identification of major compounds of different plants: The final confirmations of compounds of each plant extract were made on the basis of their area percentage calculated from the GC- chromatogram and mass spectrometry results in reference to NIST standard database (NIST 2008).

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4.Rearing of Mosquito: The mosquito species were obtained from Ross life sciences, Pune. and were reared as per WHO guidelines, Larvae and eggs population were maintained according to the guidelines in different setups (Kauffman, E. *et. al.,* 2017). Temperature and relative humidity were maintained at 27°C ± 2°C and 75% ± 5% respectively under the 12:12 light and dark photoperiod cycle (Ham, H., *et. al.,* 2014), (Rajendrabhoopathy. S and Jayakumar. S, 2017).

5.Larvicidal assay: Larvicidal effects of petroleum ether extracts of *D. stramonium* and *M. alba* were carried out on laboratory-reared 4th instar larvae of *C. quinquefasciatus*. WHO (2005) bioassay protocol with slight modifications, is used to calculate percentage mortality and LC_{50} values. It was assured that the larvae under testing were free from any exposure to any insecticides or chemicals. A stock solution was prepared. A series of concentrations of 5 ppm, 10ppm, 20 ppm, 40 ppm, 80 ppm, 160 ppm, and 320 ppm was prepared for *Datura stramonium* as well as for Morus alba from the stock solutions. Twenty-five larvae were taken and mortality was checked after every 24 and 48 hours. Also, 25 IV^{th} instar larvae in 100 ml of water were taken as control. Three replicates were set to check the mortality for each concentration. The percentage of larval mortality was recorded after 24 and 48 h and corrected using Abbott's formula (Abbott, 1925, Iqbal, J.*et., al.* 2018).

Table 1: Mortality % of fourth instar larvae of *C. quinquefasciatus* exposed for 24 and 48 h to differentconcentrations of Petroleum Ether extracts of *D. stramonium* and *M. alba.*

Sr. No.	Plant extract	Exposure (h)	Control	5 ppm	10 ppm	20 ppm	40 ppm	80 ppm	160 ppm	320 ppm
1	*D. stramonium*	24 hrs	0.7	4.3	20.5	47.5	50.8	77.2	91.0	100.0
		48 hrs	1.7	8.2	24.3	51.3	57.8	84.2	100.0	100.0
2	*M. alba*		Control	60 ppm	80 ppm	100 ppm	120 ppm	160 ppm	180 ppm	200 ppm
		24 hrs	2.5	6.0	18.3	32.8	49.5	55.0	70.5	97.2
		48 hrs	2.0	11.2	23.5	38.0	54.7	60.2	80.8	100.0

6.Ovicidal Assay:

Five replicates of different concentrations (50 ppm, 70 ppm, 80ppm, 100ppm, 120ppm) of *D.stramonium* and *M. alba* extract were prepared and the eggs of *C. quinquefasciatus* were separately exposed to each concentration (Tu Su *et al.*, 1998). The eggs were exposed for five days. A separate set for the Control group was exposed to acetone. The percent ovicidal activity was calculated by the following formula (Abbasipour, H *et. al.,* 2011), (Reegan, A. D., *et al.,* 2014).

$$\text{Percent ovicidal activity} = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$$

7.Oviposition Deterrent Assay:

The deterrent efficacy of *D. stramonium* and *M alba* was tested against *C. quinquefasciatus* mosquitoes(Elango, G., et al 2009).10 day old fifteen gravid females were kept in the mosquito cage (45*38*38*cm) after 4 days of blood feeding. The cage was covered with a plastic screen, a glasstop, and a muslin sleeve for access. 10% sucrose solution was kept in the cage for feeding mosquitoes at all times. For testing the ovipositional deterrence, one bowl with the test material was kept in one corner of the cage, whereas, the other one with control solvent was kept in the opposite corner of the cage. The position of both the bowls was interchanged at different intervals to nullify the effect of position on oviposition. Serial dilutions of the extract were made in ethanol. The desired

concentration of test solutions was obtained by treating 100 ml of water with the extract. Three replications for each concentration were set up. The temperature was fixed at 27±2ºC and relative humidity at 70-80%. The number of eggs laid in treated and control bowls was counted and recorded after 24 hours of the experiment (Reegan, A. D.*et. al.,* 2014), (Karimzadeh, J., & Rabiei, A. A. 2020).

Following formula is used to get the ovipositional deterrence percentage.

ER%=NC-NT/NC*100

Where,

ER= Percent effective repellency NC= Number of eggs in control NT= Number of eggs in treatment

8.Adulticidal Assay:

The adulticidal activity of the plant extracts was evaluated following the WHO standard method. Briefly,plant extracts were dissolved in acetone to prepare a testing concentration of 10 mg/ml. Whatman paper was impregnated with testing concentration. The impregnated papers were air-dried for 5 minutes and then inserted into an exposure tube in the WHO testing kit. Negative control was set with acetone. Twenty, 2–5 day old, blood-starved female mosquitoes were used for the adulticidal experiment. These mosquitoes were introduced into the holding tube and held for 1 hour to acclimatize and later were transferred to the exposure tube by gentle blowing for 1 hour and then transferred back to the holding tube to recover. The recovering mosquitoes feed on a pad of cotton soaked with a 10% glucose solution. The number of dead mosquitoes was recorded and the percent mortality was calculated after the 24-hourrecovery period. Each extract was tested in duplicate and the assay was repeated three times. (Afolabi O.*et. al.,* 2018).

9.Gas chromatography -Mass Spectroscopy Analysis:

The GC-MS analysis of Petroleum ether extracts of *D. stramonium* and *M. alba* reveals the presence of various types of phytochemicals. *D. stramonium* shows the presence of 23 major phytochemicals out of which Phytol ($C_{20}H_{40}O$), Tetratetraacontane ($C_{44}H_{30}$), Pentanoic acid ($C_5H_{10}O_2$) are the major compound. While *M. alba* shows 6 phytochemicals, out of which Mibemycin b,13-chloro-5-o- demethyl-28-deoxy-6,28-epoxy-25-1{1-mwthyl propyl}-(6r-13s,25A(S), Acetic acid, 17-{4-chloro-5-methylhexyl}-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1- phenanthyl and Lup-20{29}-en-3-one are the major compounds.(Figs.1 and 2).

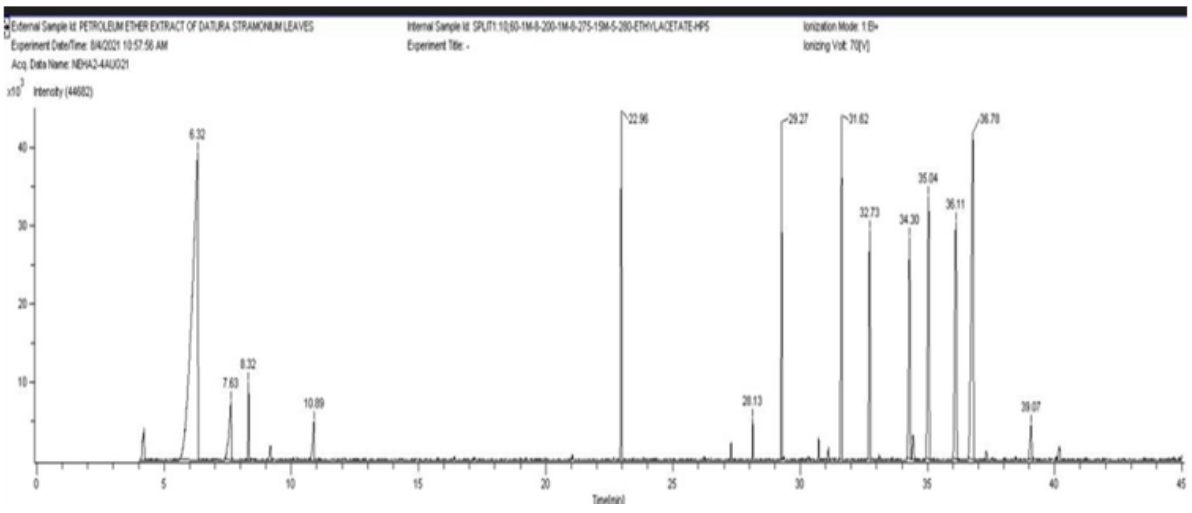

Fig. 1: GC-MS chromatogram of Petroleum ether extract of *D. stramonium*.

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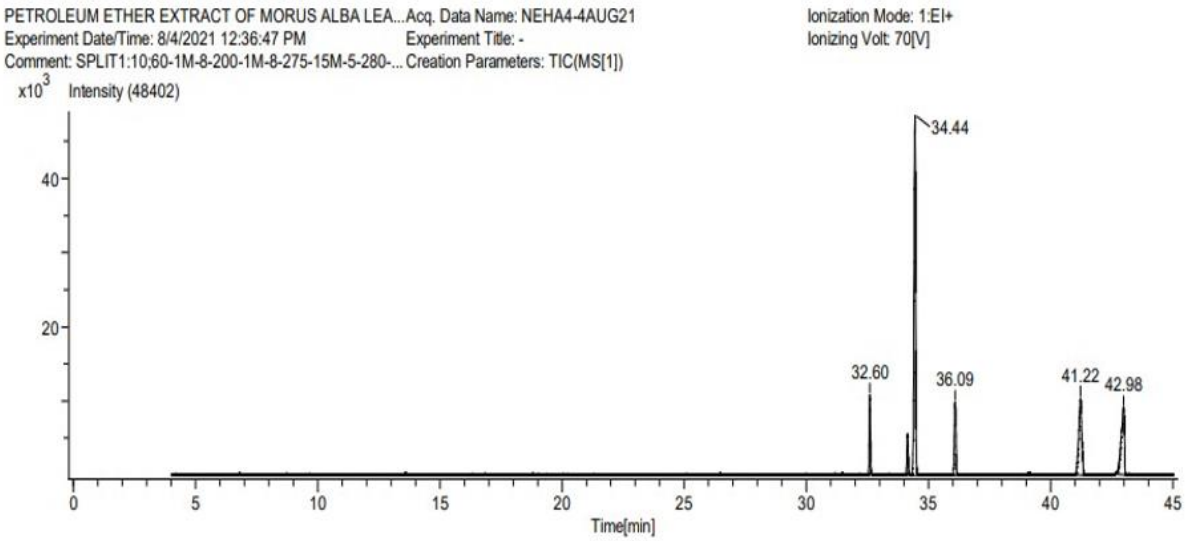

Fig. 2: GC-MS chromatogram of Petroleum ether extract of *Morus alba.*

RESULTS

1.Larvicidal Activity of Petroleum Ether extracts:

The results of larvicidal activity obtained after exposing IVth instar larvae of *C. quinquefasiatus* to various concentrations of *D. stramonium* and *M. alba* exhibits potent lethality against the mosquito species tested. Extract of *Datura stramonium* shows 20% - 40% mortality against IV instar larvae of *C. quinquefasiatus* at 10- 20 ppm when exposed for 24 hours, while *Morus alba* kills 20%-40% IV instar larvae *C. quinquefasiatus* at 80- 100 ppm when exposed for 24 hours. The data obtained from probit analysis of plant extract against IVth instar larvae of *C. quinquefasiatus* at different concentrations are represented in table 2. The LC50 and LC90 of *Datura stramonium* after 24 h post-treatment was 54.18 ppm and 123.67 ppm respectively whereas LC50 and LC90 after 48 hours of exposure were 37.08 ppm and 79.33 ppm respectively. The LC50 and LC90 of *Morus alba* after 24 h post-treatment was 133.47 ppm and 209.88 ppm respectively whereas LC50 and LC90 after 48 hours of exposure were 122.46 ppm and 195.38 ppm (Table 2).

Table 2: Lethal concentration of *D. stramonium* and *M. alba* against fourth instar larvae of *C. quinquefasciatus.*

Sr. No.	Plant extract	Exposure(h) against *C. quinquefasciatus*	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% Confidence limits			
						UCL		LCL	
						LC 50	LC90	LC50	LC90
1	*D. stramonium*	24 hrs	54.18	123.67	y= .99+0.018x	61.02	139.65	48.09	111.4
		48 hrs	37.08	79.33	y= -1.125+0.030x	41.575	89.81	33.121	71.4
2	*M. alba*	24 hrs	133.47	209.88	y=-2.239+0.017x	139.85	223.62	127.32	198.85
		48 hrs	122.46	195.38	y=-2.153+0.018x	128.45	207.58	116.53	185.47

2.Ovicidal Assay:

Datura stramonium recorded its highest ovicidal activity of 59.24% against *C. quinquefasciatus* at 55ppm and (Table 3) whereas *Morus alba* recorded 39.01% ovicidal activity against *C. quinquefasciatus* at 135 ppm. In contrast, the ovicidal activity of *C. quinquefasciatus* in control was 5.18% and 8.88% respectively in a set I and set II.

Table 3: Effect of plant extracts on fecundity and egg hatchability of *C. quinquefasciatus*

Sr no	Plant extract	Dose	Insect Species	Parameters	R-1	R-2	R-3	R-4	R-5	Avg	% Ovicidal Activity
1	*D. stramonium*	55 ppm	*C. quinquefasciatus*	Total Number Eggs exposed	85	119	108	145	171	125.60	59.24
				Number of Unhatched eggs	70	81	62	93	66	74.40	
2	*M. alba*	135 ppm	*C. quinquefasciatus*	Total Number Eggs exposed	135	154	129	160	186	152.80	39.01
				Numberof Unhatched eggs	44	84	56	65	49	59.60	

Where R= replication set

3.Oviposition Deterrent Assay:

The ovipositional deterrent activity of petroleum ether extract of *D. stramonium* against *C. quinquefasciatus* was higher at a very low concentration in contrast to *Morus alba. Datura stramonium* showed its highest ovipositional repellency activity of 89.70 % at 55 ppm whereas *Morus alba* shows 79.30% ovipositional repellency activity at 135 ppm against *C. quinquefasciatus.* On the other hand, the control exhibits minimal activity as shown in Table 4.

Table 4: Oviposition deterrence of *D. stramonium* and *M. alba* against *C. quinquefasciatus*

Sr No.	Plant extract	Dose	Insect Species	Parameters	R-1	R-2	R-3	R-4	R-5	Average	% Repellency	Ovipositional Activity Index
1	*D. stramonium*	55 ppm	*C. quinquefasciatus*	Control	407	361	506	334	382	398.00	89.70	-0.81
				Treated	43	69	34	42	17	41.00		
2	*M. alba*	135 ppm	*C quinquefasciatus*	Control	785	598	629	571	760	668.60	79.30	-0.66
				Treated	169	136	135	109	143	138.40		

Where R= replication set

4.Adulticidal Activity:

The duration (days) of adult development was recorded after exposure to extracts of *Datura stramonium* and *Morus alba* (table 5). Analysis of petroleum ether extracts revealed the fact that bothextracts show promising adulticidal activity against *C. quinquefasciatus. Morus alba* shows 52.00% *w*hereas *D.stramonium shows* 49.60% adulticidal activity against *C. quinquefasciatus.* On the other hand, adulticidal activity is negligible in control.

Table5: Effect of plant extracts for adulticidal activity against *C.quinquefasciatus*

Sr. No.	Plant Extract	Insect Species	Replication Reading after 1hour of Exposure						Average in %	Replication Reading after 24 hours of Exposure						Average In %
1	*D. stramonium*	*C. quinquefasciatus*	17	14	22	22	16	18.20	72.80	13	10	9	18	2	12.40	49.60
2	*M. alba*	*C. quinquefasciatus*	20	17	16	15	17	17.00	68.00	16	13	12	11	13	13.00	52.00

DISCUSSION

Mosquitoes are the vector of the world's most deadly diseases (WHO 2020). All three main species (Anopheles, Culex and Aedes) are well known for causing diseases like malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis all over the world (Zuharah, Wan Fatma *et. al.,* 2021). Due to the emergence of resistance in mosquito species, studies are conducted to find alternative effective control of mosquitoes like phytochemicals as they are eco-friendly, biodegradable, and diverse in nature. These phytochemicals are capable of causing a range of acute and chronic toxic effects (Shaalan, E. A. S *et.al.* (2005). The same plant extract is found effective for joint action to control not only one stage of mosquito development but most of them like larval, Ovicidal, ovipositional deterrence and adult stages and therefore such extract can be applied to control mosquito species irrespective of their developmental stage (Mahanta, S. & Khanikor, B. 2021), The mosquitocidal activity of crude plant extracts is often attributed to the complex mixture of active compounds (Kamaraj C. *et al.*,2011).It is evident from the results that chemical compounds analysed from *Datura stramonium* has promising larvicidal activity with an LC_{50} value of 54.18 ppm as compared to the LC_{50} value of *Morus alba* i.e. 133.47 ppm. The results are in accordance with earlier studies that have shown the larvicidal activity of aqueous extracts of *Datura stramonium leaves* showing 100% mortality against Culex (Olofintoye, L. K., *et al.,* 2011). Other studies revealed encouraging larvicidal and repellent activities of ethanolic leaf extract of Datura stramonium against the vectors (Swathi *et.al.,* 2011). *Datura stramonium* proves to be a better ovicidal agent against *C. quinquefasciatus* with an ovicidal activity of 59.24% than *Morus alba* with 39.01% ovicidal activity. Similar earlier studies revealed that progeny development of *Callosobruchus maculatus* was significantly reduced by acetone extracts of aerial parts of *Datura stramonium* (Abbasipour, H *et. al.,* 2011). *Datura stramonium* also shows higher ovipositional activity with 89.7 % at 55 ppm against *C. quinquefasciatus* over *M. alba* with 79.3 % ovipositional activity at 135 ppm. Earlier studies where ethanolic extracts of *Datura* show repellent activity against ladybird adults. (Kumral, N. A.et. al. 2013). On the other hand, the adulticidal activityof *Morus alba* is 52% at 135 ppm in comparison to *Datura stramonium* with 49.6 % at 55 ppm. Findings showed that the essential oils from the *Datura stramonium* plant extracts also have adulticidal and repellent effects on the *An. gambiae* mosquitoes (Afolabi, O. J.*et al.,* 2018). It is concluded that the active chemical compounds such as which Phytol ($C_{20}H_{40}O$),Tetratetraacontane($C_{44}H_{30}$), Pentanoic acid from *Datura stramonium* can be use in pure form to control the population of C. *quinquefasciatus* as compaired to *Morus alba*.

Conflict of interest: The authorities declare no conflict of interest.

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