Effect of botanicals on Yellow Fever Mosquito Aedes aegypti(Diptera: Culicidae)

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ABSTRACT
The current studies were conducted during 2018-2019 to evaluate the efficacy of various botanicals against *Aedes aegypti*. Mosquitoes from ancient times are kind of the insect creatures which are known to transmit serious human diseases, causing millions of deaths every year. The number of problems prevailing such as resistance of synthetic insecticides, residue problems in the environment and bad effects on human population and non-target organism, researchers focus their whole area of research towards the formation of new plant based insecticides with less hazardous effects. Despite the huge operational costs, the application of synthetic chemical insecticides to control vector mosquitoes has resulted in physiological resistance and ruthless environmental consequences. Insecticides compound of botanical origin including phenolic, terpenoids and alkaloids have been reported as useful for control of mosquitoes and may result in jointly or independently contribution to the larvicidal activities of mosquitoes. The satisfactory results were exhibited by Pine oil extract used and established the efficiency when the results were drawn from the analysis. However, the mortality of the test species got increased with increase in plant extract concentrations and complete death was recorded at 8 to 10 percent concentration. The lower concentrations were also helpful as they showed restless movement for some times with abnormal wagging and then died. The LC_{50} and LC_{99} of plant extract were extremely toxic after 40-48 hours of exposure. The maximum efficacy was observed in the pine oil extract. The LC_{50} and LC_{99} values of pine oil extract against adults of *A. aegypti* after 20-24 hour and 40-48 hour were 0.28, 0.891 ml and 0.082, 0.678 ml, respectively though; no evidence of mortality was recorded in controls.The lethalkilling percentage of Neem oil and Til oil considered low adulticidal activity within 20-24 hours exposure period nevertheless, pine oil put forththe huge adulticidal activity while after 40-48 hours exposure high killing activity against mosquito population were shownwhen treated with plant bio-extracts. From the results, it was determined thatthe crude extraction of pine oil extract was apotent killer for controlling dengue vector mosquito, *Aedes aegypti.*
Keywords: Mosquitoes, Synthetic insecticides, Resistance, Adulticidal activity

INTRODUCTION

According to the World Health Organization (WHO), contagious diseases hold the sixth rank in causing number of deaths globally (WHO Coronavirus Disease). Among them mosquitoes are prime agents responsible for transmission of serious human diseases (Rachel and Anna-Bella 2021), causing millions of deaths every year (Masterson, 2021). Mosquito *Aedes aegypti* belongs to the family Culicidae and the genus Aedes is the major vector of dengue fever disease. Being a viral malady, dengue has levieda serious economic crises in both tropical and sub-tropical regions (WHO 2020). In addition to this more than 300 million cases were recorded annually with nearly 22,000 deaths (WHO, 2020). On a global level, dengue fever encompasses approximately 2.5 billion people with an estimated 50–100 million new cases annually. Dengue being most prominent cause of Haemorrhagic Fever (DHF) has been one of leading public health issues in South-East Asia, including Indonesia (Ranawaka *et al*. 2021). It is considered as one of the most important arthropod-borne viral diseases in Southeast Asia. The ailment due to dengue is commonly found in tropical and subtropical areas, commonly transmitted to humans by mosquito bites. The virus infection is transferred by its own mechanical vectors and the species *A. aegypti* and *A. albopictus*, will cause DHF to occur(Fortuna et al., 2021; Fernandes et al., 2020).

The disease itself have resulted inhuge negative impacts to humanbeings, espacially hospital expenses, lost of productivity and unbearable one that is even death as per. The dengue infection, a viral infection, has rapidly increased in humans through extensive transmission by the bites of infected female *A. aegypti* mosquitoes. About 10 years ago, high dengue alert was issued nationwide as well as in Malaysia, due to the total deaths management because larvae occur in specific areas and of more than 103 people between January and August 2010 (Cruez, 2010). The main strategy in controlling mosquito breeding and population dispersal is that the larva can be controlled by modification of habitat with insecticides. The insecticides directly attack the cuticle and ruptures it causing easy penetration of pathogenic organisms, thus reducing the mosquito population at a very promising rate. The four different closely linked virus serotypes responsible for dengue were identified as DENV-1, DENV-2, DENV-3 and DENV-4. It has been studied that these virus particles are predominantly transmitted when an infected female mosquito *A. aegypti* bites a healthy human being. According to the reports of (WHO, 2016) after the inception of infection in human body once, humans act as carriers and provide congenialatmosphere for proliferation of the virus bodies, which subsequently gets transferred to uninfected vectors during bites. The disease development in humans lead to an eminent high fever associated with some symptoms such as moderate to severe headache, joint pain and behind eyes, vomiting, rashes and inflammation of glands.If not diagnosed and treated well, dengue leads to severe complications and patient suffers due to respiratory distress, fluid accumulation, severe bleeding, organ damage etc. which becomes fatal in critical cases (WHO, 2016). In a present scenario, dengue is endemic in more than 100 countries across the globe including India; prior to 1970, only nine countries were reported to have epidemics of severe dengue (WHO, 2016). Bhatt et al. in the year 2013further studied that under-reporting of the actual number of dengue cases and estimated that annually about 390 million infections occur throughout the globe. Isolation of dengue virus inIndia was first done in the year 1944 in Kolkata from the serum samples of infected US soldiers (Sabin and Schlesinger, 1945).The first evidence of dengue was in a Chinese medical encyclopedia of 265–420 AD, in which the dengue has been described as a “poison water

withwith flying insects. The first major epidemics of Dengue Haemorrhagic Fever (DHF) and/or Dengue Shock Syndrome (DSS) was observed near Delhi and Lucknow in Uttar Pradesh and thereafter the virus started spreading rapidly across India killing thousands of people mercilessly(Kumar 2010). About 3400 species of mosquitoes belonging to 42 genera have been identified (Service Cambridge University Press, Cambridge, 2008). Universally 950 species of mosquitoes have been reported under genus Aedes, however in neighbouring country Pakistan 30 species of genus Aedes have been reported. Amongst all the species, two are majorly responsible and acts as a vector for viral diseases i. e. *A. aegypti* and *A. albopictus*. Primary vector of dengue is *A. aegypti*while as *A.albopictus* (Asian tiger mosquito) acts as a secondary vector (CDCP *Arboviral Encephalitides*, 2001). Dengue is a life-threatening arboviral disease, with high mortality worldwide (WHO, 2017). As per the survey conducted by World Health Organization,reportsaround 22,000 deaths occur due to dengue annually, especially in pediatric patients (WHO, 2019). The worst hit of the common denguenuisance fever outbreaks took place in 17th century in the Caribbean region. The first dengue epidemic in South Asia (Sri Lanka)happened in the period from 1965 to 1968, resulting in 51 dengue hemorrhagic fever cases and 15 deaths, while the greatest of all time epidemic took place in 2017, infecting 186,101 individuals (Epidemiology Unit, 2019). In India, until 2015, Delhi has experienced eight dengue outbreaks since 1967, with the last epidemic reported in 2006 (Ahmed and Broor, 2015).
Dengue is a feverishinfection that attacks infants, young children and adults with symptoms appearing 3-14 days after the infective bite. Dengue fever infects 50 million people annually, and approximately two fifth population of the world is at risk of infection. According to World Health Organization, about 2-3 per cent deaths globally occur every year and large proportion of which are children. The dengue outbreak has been documented in Eastern Mediterranean Region possibly at the end of 17th century in Egypt and its frequencies of repeated uprising continue to increase as parallel studies done by WHO (2005) over a course of years. Recent outbreaks have been recorded in Pakistan, Saudi Arabia and Yemen.

Surveillance Report

InAndhra Perdashreport of the denguerecordedthousands of suspected cases seasonally.The govt. data exhibited the cases of seasonal diseases like dengue, typhoid, diarrhea and malaria fell with the seasonal percentage of 97.4%, 96%, 81.7% and 50.4%, respectively. Dengue cases fell 97.4 per cent in current year and overall only 24 positive cases were found in between June and August. This % were higher compared to 944 cases during previous year. Whileas, the total number of typhoid cases came down to 355 with 96.05 drop compared to previous year. In case of diarrhoea there was 81.7 per cent drop. Further, the malaria showed a lowest drop of only 81.7 per cent with 601 cases only. The reports says thatthe rapid urbanization at an intense pace has made technology driven human evolution to evolve multiple breeding places of Aedes mosquitoes many folds in tropical and sub-tropical climatic zones of the world which culminate in significant expansion in incidence of dengue cases. Despite the technical and operational anomalies with the use of synthetic insecticides and their implications on human health, there is a dire need to searched out an alternate sources of mosquito population control which are more target-specific, easily biodegradable and effective against mosquitoes. The present investigation was conducted to scientifically asses the adulticidal efficiency of three plant extracts including*Azadirachta indica* (Neem tree), *Sesamum indicum* (Til) and *Pinus*

sabinaena (Pine tree) against the adults dengue vector (*A. aegypti*). Besides that it was studied that leaves and seed of neem tree may contain an active plant botanical used as natural insecticides, azadirachtin. In studies done by various authors it was believed that leaves or seed of Neem can be simply processed to obtain neem oil, which is commonly used as plant pesticides and insect repellents in African countries (Asogwa et al., 2009). Due to the high larvicidal properties of Neem oil it was considered as potent larvicide of *A. aegypti* and *Culex pipiens* (Alouani et al., 2009), as well as adult *A. aegypti* mosquito repellent in cream formulation (Kiplang'at and Mwangi, 2013). Biologically 99 active compounds are present in neem seeds out of which azadirachtin, nimbin, nimbidin and nimbolides are major ones. Azadirachtin, which is a biologically active compound in the Neem plant *A. indica*has been put forward as environment friendly insecticide than synthetic insecticides which contribute in high cost and health effects. Nevertheless, many byproductspossesantifeedancy efficacy, ovicidal activity, fecundity suppression properties besides insect growth regulation and repellency against insects.To examine adulticidal efficiency of Til, Pine and Neem oil most effective phytochemical among them that can be used against the management of adult of primary disease causing mosquito*A. aegypti*.

MATERIALS AND METHODS

During the current experiment the stock solution of different concentrations of plant based natural extracts i.e. 2%, 4%, 6%, 8% and 10% was purchased and prepared. The efficiency of crude Neem oil extract, Pine oil extract and Till oil extract in adult lethality with five different concentrations i.e. 2%, 4%, 6%, 8% and 10% of each plant oil extracts in acetone was tested against five to six days old sugar fed female of *A. aegypti*. The mortality percentage of adult was observed after 24 hours and 48 hours under laboratory conditions. *A. aegypti* mosquitoes were reared in the insectary of the Department of Medical Entomology and Disease Vector control at Health Services Academy, Haryana. The liver food were fed to larvae while as adults were given with 10% sucrose solution and albino rats for blood meal. Mosquitoes were reared at 28^0 C and 70-85 per cent relative humidity, with a photo period of 12 light and dark hours.

Adulticidal bioassay

The adulticidal efficiency of each plant extract was checked by using standard World Health Organization (WHO) procedure. Sugar-fed Adult female mosquitoes five to six days old were used and at least 15 female mosquitoes were used in three replicate. The multiple concentration of plant botanicals were impregnated on filter papers by dipping them in stock solution for 5 minutes. A blank paper impregnated with only acetone (solvent) was taken as control. The papers were left to dry at room temperature to ward off the acetone overnight.

Filter paper drenched with number of concentrationsas aforementioned of plant extracts from stock solution was prepared freshly prior to testing. The bioassay was done in an experimental kit which contains two cylindrical plastic tubes following the guiding of WHO. One tube acts to expose the mosquitoes to the plant extracts and another tube was used to hold the mosquitoes before and after the exposure periods. The soaked filter paper used for impregnation were rolled and placed in the exposure tube. The tube was closed at one end from both the sides with a 16 mesh size wire screen. Five to six days old 15 mosquitoes which were sucrose-fed released into the tube.After the exposure periods 3hr the

mosquitoes were put in holding tube. The tube was kept in appropriate conditions (20-25 ±3 °C and 70% ± 5% relative humidity) for 20-24 hours and 40-48 hours. A Cotton pad soaked in 20% sugar solution was placed in the tube during the holding period. Three checks were taken to define the activity of extracts. Mortality in the range of 1-49 per centdepicts low activity; Mortality in the range of 55-75 per centshows moderate activity and Mortality in the range of 55-73 per cent indicates high activity.The exercise mentioned above was done in three replicates for each concentration of every plant oil extract and mortality in terms of percentage. The observations were recorded after one day and two days of the experiment.

Analysis plan

To calculate the average adult mortality probit analysis of lethal concentration 50 (LC_{50}) and lethal concentration 99 (LC_{99}) using computer software Mini tab was accomplished. Chi-square was also employed to check the homogeneity of tested population.

RESULTS

As per the mortality, the data gained from adults of *Aedes aegypti* under laboratory condition on the use of different concentration of plant extracts were recorded. The activity of adulticidal Neem oil extract against adult *A. aegypti* after 20-24 hours of exposure was recordedand it was revealed that the adult mosquito mortality ranges from 20.06 to 55.0 per cent approx.which washighest accolade in number. The mortality per cent of *A. aegypti*after 40-48 hours of exposure was much higher than 20-24 hours exposure and lie in between 75 to cent per cent. Maximum mortality (100%) was recorded at 8 per cent concentrations. The lethal activity of Pine oil extract used to kill adult *A. aegypti* after 20-24 hours of exposure was witnessed at all concentrations with mosquito mortality ranging from percentage of cent to 80-86 per cent. However, the highest mortality of cent per cent was observed at 8 and 10 per cent concentrations. The percentage mortality of *A. aegypti* after 40-48 hours of exposure ranged between 91 to 100 per cent. The huge mortality was again recorded at higher concentrations of 6, 8 and 10 per cent but til oil extract revealed significantly lower mortality in the mosquitoes exposed for 20-24 hours (ranging between 20-40 per cent) as compared to 40-48 hours exposure (ranging from 75.0-99.0 per cent).After one day (20-24hrs) of exposure the LC50 value of Neem oil extract was 1.21 ml however it was recorded as 0.09 ml subsequently after 40-48 hours of exposure. The graphic representation of mean mortality of *A.agypti* under different botanicals pesticides were presented in Fig (s)-1,2,3 and 4 below.

Table 1: Descriptive statistics of different doses of various botanical pesticides applied at varied concentrations during 2018-2019

Neem Oil		Time	Lethal dose	LFL	ULF	Slope±SE	χ2	P value	Regression equation
	LC_{50}	20-24 h	1.21	0.91	6.81	0.704±0.514	3.01	0.300	Y=-0.81+ 0.25X
		40-48 h	0.081	-0.29	0.281	2.51±0.609	6.49	0.110	Y=-0.19 + 2.10X
	LC_{99}	20-24 h	4.53	2.50	48.50	0.711±0.300	3.40	0.310	Y= -0.79+ 0.31X
		40-48 h	1.19	0.98	1.68	2.30±0.619	6.31	0.121	Y=-0.19+2.105X

Pine Oil	LC_{50}	20-24 h	0.28	-1.81	0.088	2.39±0.620	2.45	0.401	Y=0.502+ 0.13X
		40-48 h	0.082	-1.51	0.160	3.41±1.281	0.39	0.891	Y=0.233+ 3.10X
	LC_{99}	20-24 h	0.891	0.670	1.81	2.10±0.707	2.51	0.401	Y=0.592+0.243X
		40-48 h	0.678	0.510	1.41	3.10±1.610	0.42	0.881	Y= 0.210+ 3.11X
Til Oil	LC_{50}	20-24 h	1.21	1.101	6.10	-1.19±0.078	2.81	0.501	Y=-1.29+0.81X
		40-48 h	0.050	-0.803	0.210	1.149±0.517	0.91	0.798	Y=0.0810+1.41X
	LC_{99}	20-24 h	4.24	2.501	27.19	-1.31±0.758	2.45	0.480	Y=-1.21+0.70X
		40-48 h	1.30	1.101	2.30	1.601±0.525	0.89	0.799	Y=0.0851+1.05X

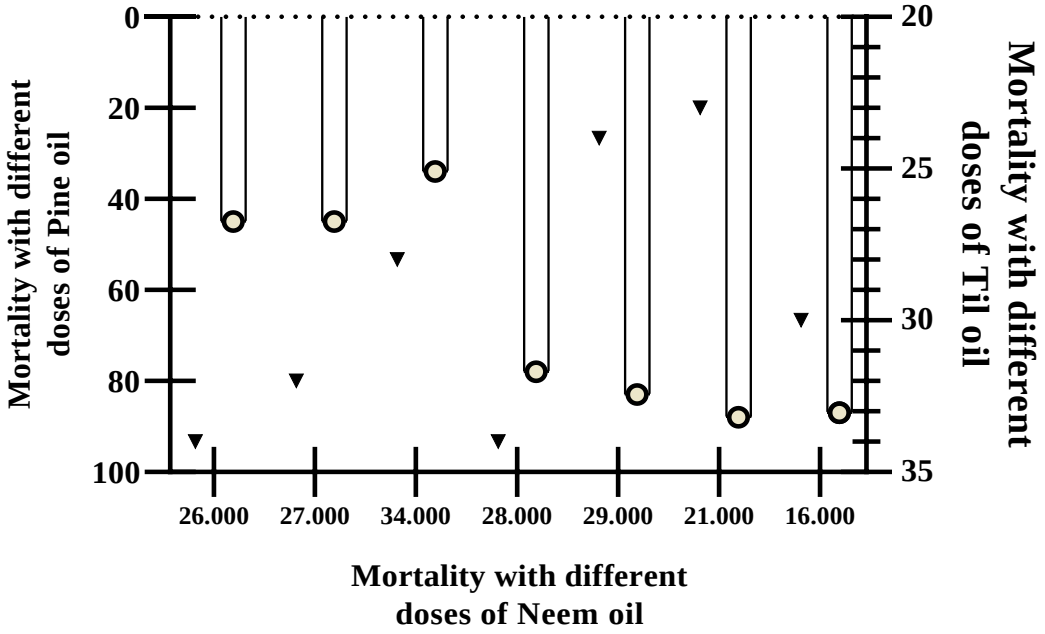

Fig 1: Graphic representation of mean mortality of *A.agypti* under different botanicals pesticides

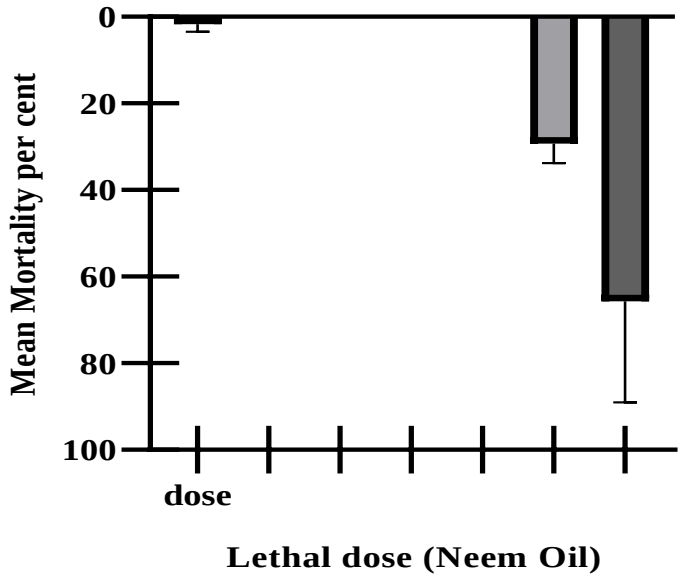

Fig-2: Mean mortality per cent by different doses of Neem oil.

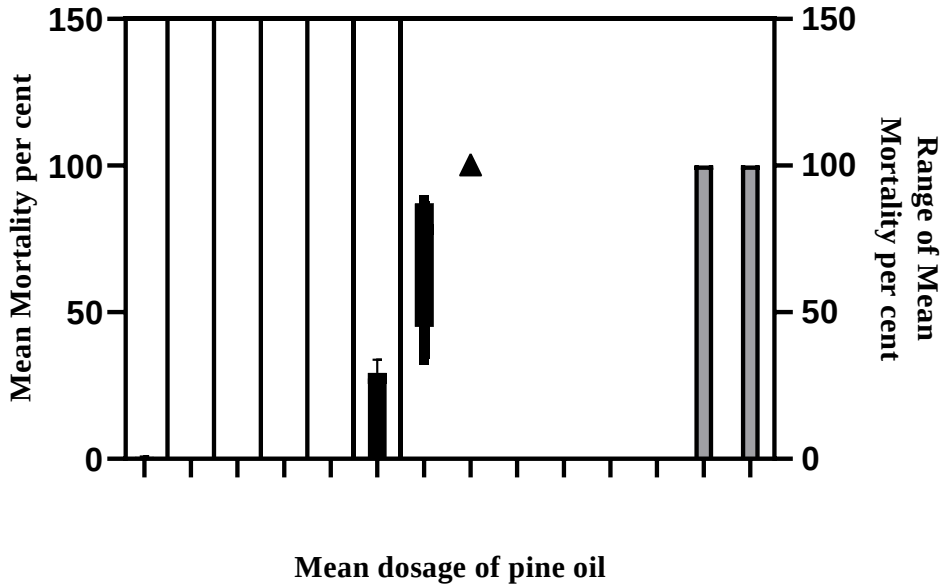

Fig-3: Mean mortality per cent by different doses of Pine oil.

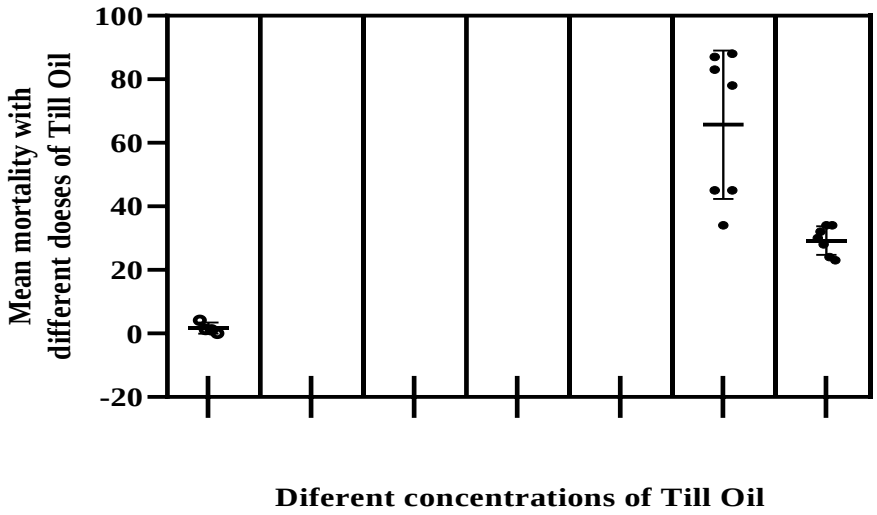

Fig-4: Mean mortality per cent by different doses of Til oil.

However, the LC99 value was 4.3 ml after 20-24 hours and 1.21ml after 40-48 hours of exposure but in case of Til oil extract LC50 value was 1.21 ml after 20-24 hours of exposure and 0.050ml after 40-48 hours of exposure. The LC99 value was recorded as 4.23 ml after 20-24 hours and after 40-48 hours of exposure it was observed as 1.30ml.Keeping in view the LC50 and LC 99 it is very clear that the plant extract was extremely toxic after 40-48 hours of exposure. In the tested plant oil extracts concentration, the maximum efficacy was observed in the pine oil extract. The LC50 and LC99values of pine oil extract against adults of *A. aegypti* after 20-24 hour and 40-48 hour were 0.28, 0.891 ml and 0.082, 0.678 ml, respectively though; no evidence of mortality was recorded in controls.Though, results were recorded to be statistically significant. The data generated of killing efficiency of acetone extract of Neem oil, Pine oil and Til oil are presented in Table 1**.**

DISCUSSION

The increasing resistance of the mosquitos *A. aegypti*populations to the current commercial pesticides has put a great barriers to the efforts for managementof dengue vector efficiently (Djiappi-Tchamen et al. 2021). Besides, other major problems such as high environmental and toxicity to non-target organism have been established by the continuous use of synthetic chemical pesticides. Therefore, there has been an increasing interest in the development and creation of alternative tools of mosquito control which are less hazardous to humans and other living things. Furthermore, plant based compounds have emerged as potential candidates, not only as new effective and precise tools in vector management but also as environmentally safer tools. The currentinvestigation was undertaken to put forward the adulticidal properties of pine oil extract, Neem oil extract and till oil extract against the dengue vector mosquito, *A. aegypti*. The results drawnfrom this investigationrevealed that Pine oil extract possessed marked adulticidal activity against adult *A. aegypti* after 20-24 hours of exposure with low LC_{50}value, indicating its role as promising and efficient adulticides, same were reported by Singh *et al*. (2021). The LC_{50} values of 1.21, 1.50as against the Neem and till oil was observed respectively, which

reveled lower adulticidal activity after 20-24 hours of exposure. The LC_{99} value of Pine Oil was much lower as compared to that of the extracts from Neem and til oil when compared with LC_{99} values of the three plant extracts after 20-24 hours of exposure. After 24h of exposure under lab conditions the percent mortality of adult mosquito *A. aegypti* revealed that Pine oil had the highest percent mortalities as 96 per cent when compared to Til and Neem oil respectively. The adult showed restless movement for some times at higher concentrations with abnormal wagging and then died. The mortality rate was directly proportional to concentration in case of 40-48 hrs exposure for Neem and Til oil. The chi-square value are significant at $\alpha<0.05$ level. In control no mortality was observed after both the exposure periods. In the present investigation Neem and Til Oil extracts did not show effective adulticidal affect after 24 hr exposure period however, after 48 hrs of exposure period both plant extracts showed high *A. aegypti* mortality as did the Pine oil extract. The values of LC_{50} and $LC_{99}$of Neem and Til Oil extract are provided in the tables presented above. Govindarajan, 2010 revealed that the chi-square value is significant at *αC. flexuous>Eucalyptus citriodora>C. deodara>P. roxburghii>T. minuta*.For the testing against adult *Anopheles gambiae* mosquitoes and the findings showed that the usage of traditional insecticide such as *N. mitis* has been effective when incorporated against Anopheles gambiae and can be further used for the control of adult mosquitoes species particularlyat the breeding sites the bio-formulations and compounds from tubers of Neorautaneniamitis were employed as studies done. It was studied that methanolic extract of leaves Pinus roxburghii were tested against the adult of *Anopheles squamosa* on *Culex quinquefasciatus*. The bio-extract based formulation conferred dose dependent activity, exhibited significantly shorter knock down KD50 and KD_{90} values and produced huge mortality. The results showed the efficientmosquitocidalefficiency of the plant extract against *Anopheles squamosa* and *Culex quinquefasciatus*. The methanol extracts of seven species of Malaysian tunicates, the mortality values of the extracts on the adult mosquitoes were dosedependent and increased with exposure period.The present era drives the population towards the world to the door where indiscriminate use of synthetic insecticides is employedthe plant-based insecticides can prove as the promising alternative to chemical supplements. The results of the present study would be very helpful in promoting research aiming at the development of new agent for mosquito control based on plant source.

CONCLUSION

Due to the un-availability of target specific drugs against dengue disease it must have creating ambiguity in the minds of people suffering due to this sickness. This situation not only creates panic in the patients but also puts pressure on clinicians who are in management line of the disease. This also helped our society to find out alternative options for treatment and prevention of dengue. For combating dengue numerous plants and their extracts have been employed traditionally in various states of India. Owing to the developmentation of resistance property, response to synthetic insecticides, residue problems in the environment and ill effects on human beings and non-target organism can be easily witnessed.The researchers now change their focus towards the development of new bio-insecticides with minimumhazardous effects. Some of the compounds are known to exist in plants such as phenolic, terpenoids and alkaloids which jointly or independently confer to the generation of activities against the immature stages (larvae) of mosquitoes. The newly studies identified that with the help of pine oil extract adult mortality increases upto 8-10 percent with elevation in plant extract concentrations. Nevertheless,the less

concentrated extract was still beneficical as they showed restless movement for some times with abnormal wagging and dying of the insect. Therefore the study put forth that Neem oil and Til oil with low adulticidal activity at 24 hours exposition time period while as Pine oil was recorded with high properties of adultscumbness. The killing efficiency of all the oils after 48 hrs changed and showed cent per cent mortality of adults. There is a dire need to search more such herbal formulations which are being practiced at local level, document them properly and ratify them in a scientific manner to confirm efficiency, understand mechanistic action and safety so that they can be steadfastly used for their anti-dengue prospectus.

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