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Bio-larvicidal activity of *Prunus persica* (L) crude leaf extracts against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*) (Diptera: Culicidae)

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Abstract
Mosquitoes are the major causative agents of vector borne diseases namely as malaria, lymphatic filariasis, dengue fever etc. worldwide. Mosquito control programs are used to reduce this problem but, it is completely rely on the use of synthetic insecticide which creates lots of risk on human health, aquatic animals and environment. Natural insecticides discovered in plants that are environmentally friendly and biodegradable provide an alternative method of mosquito control. Therefore, in the current study we investigate on the larvicidal activity of *Prunus persica* leave extract against the 4th instar larvae of three different species of mosquitoes such as *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. The *Prunus persica* leaf extract with various solvent caused moderate mortality at concentration of 25, 50, 100, 200, 300 ppm against the mosquito larvae. However, *Prunus persica* methanol leaf extact (PPMLE) showed the highest larvicidal efficacy with LC_{50} and LC_{90} value 55.432, 221.027 ppm for *Anopheles stephensi*; 70.961, 321.561 ppm for *Aedes aegypti*; 104.104, 483.622 ppm for *Culex quinquefasciatus*. These results indicate the potential larvicidal activity of methanol extract of *Prunus persica* leaf against malarial, filarial and dengue vectors.

Keywords: *Prunus persica*, crude extract larvicidal, *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus*

Introduction
Mosquitoes belong to the Diptera order and the Culicidae family of insects. They are classified into 41 genera and 3500 species and about 404 of them are found in India [1, 2]. Among the 41 genera, *Anopheles*, *Culex*, and *Aedes*, are the most important as it transmit a several deadly diseases [3, 4]. Mosquitoes are the primary vectors of some protozoan and viral diseases, including malaria, lymphatic filariasis, Japanese encephalitis, Yellow fever, Zika fever, and Dengue fever. These diseases continue to be a major public health issue in developing countries [5]. *An. stephensi* (L) is the major causative agent of malaria in India and other West Asian countries [6]. The World Health Organization estimates that 435,000 people died as a result of malaria in 2017. Malaria affects children under the age of five the most, accounting for 61% of all malaria deaths worldwide [7]. The primary causative agent of dengue, dengue hemorrhagic fever, and chikungunya is *Aedes aegypti*. Dengue fever is prevalent in South East Asia, including India, Bangladesh, and Pakistan [8]. Every year the infection of dengue are nearly 390 million of which 96 million manifest clinically, with varying degree of severity [9]. *Culex quinquefasciatus* is a causative agent of lymphatic filariasis in many countries [10]. Lymphatic filariasis affects approximately 120 million people globally, with 44 million people suffering from the common chronic manifestation [11]. Furthermore, most of these diseases, such as West Nile fever, St Louis encephalitis, and lymphatic filariasis, are not preventable in humans through vaccination [12].
Synthetic chemical insecticides are the most effective in the mosquito control strategy. However, the use of synthetic insecticides cause numerous adverse effects on human well-being and non-target organisms, as well as contributing to environmental pollution, has resulted in ecological disturbance without addressing the issue of resistance development to insecticides, the emergence of new species [13, 14]. As a result, alternative sources to synthetic chemical insecticides are required in mosquito control strategies to reduce these issues. Mosquitocidal activity was discovered in a large number of botanical derivatives. Plants that contain a high concentration of bioactive chemicals, are active against a limited number of species, including specific target insects, and are biodegradable, could be a source of alternative mosquito control agents. They could be beneficial in integrated pest management programmes [15, 16]. *Prunus persica* belongs to the family R*osaceae* and the subfamily *Amygdyloideae* and it is up to 10m in height [17]. It is commonly cultivated in West Asia, Europe, Himalayas and India up to an altitude of 1000 ft [18]. It is commonly known as “Aaru” and in English popularly called “Peach” has been extensively consumed worldwide [19]. Green leaves are very useful as astringent, expectorant, demulcent, diuretic, laxative, febrifugal, and parasiticide. Expectorant (used in cough, chronic bronchitis and whooping cough), sedative, demulcent, anti-scorbutic, stomachic, diuretic are effective bark aid [20]. Nitin kumar, Anurag Chaudhary (2015) [21] reported on different extracts of *P. persica* leaves as anthelmintic activity. The bark is used for the treatment of leprosy, and jaundice. Leaves of *P. persica* have been investigated for their antioxidant [22], anti-inflammatory activities [23]. Fruits of *P. persica* have been reported to have a hypoglycemic effect in the prevention of Type 2 diabetes [24]. Rakesh Raturi, P. P. Badoni and Radha Ballabha, 2016 investigated the insecticidal and fungicidal activities of the *Prunus persica* (L.) methanolic extract of stem bark [25]. In overview of all the past studied activities of *Prunus persica* the present study is undertaken to investigate the mosquitocidal activities on different species of mosquitoes.

Materials and Method

Plant sample and Preparation of crude extract

Collection of the fresh matured leaves of *Prunus persica (L)* has been done from Bajengdoba (25°51'25.9"N 90°28'35.6"E) North Garo Hills, Meghalaya, India during January, 2020 and were washed with water to clean the dust particle. Then shade dried at room temperature and ground into fine powder using mechanical grinder. Fine powder were extracted with various solvents namely petroleum ether, chloroform, ethyl-acetate and methanol (10 g plants powder for each solvent) using soxhlet apparatus. Then the extract were filtered through whatman No.1 filter paper and concentrated at rotatory evaporator. The crude extract was then kept at -4°C until bioassay was carried out.

Fig 1: *Prunus persica* plant.

Collection and rearing of mosquito

Larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex. quinquefasciatus* were collected from in and around the agriculture department rice field at Annamalai University. The larvae were kept in plastic trays filled with tap water in the laboratory, and all experiments were conducted at 27±2 °C and 75–85 % relative humidity under 14:10 light and dark photoperiod cycles. The mixture of dog biscuit and yeast powder in the ratio of 3:1 were use to fed the larvae. They were kept and raised in the laboratory [26].

Larvicidal test

Larvicidal activities were carried out against three different species of mosquitoes (*Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus*) using WHO, 2005 protocol [27]. Different crude extract such as petroleum ether, chloroform, ethyl acetate and methanol leaf extract (PPPLE, PPCLE, PPEALE, and PPMLE) were dissolved separately in DMSO. 25th fourth instar larvae were introduced into each disposable cup filled with 100 ml of water, to this appropriate volume of dilution was added to get desired concentration like 25,100,200,300 ppm respectively, and each concentration have five replicates. For control also the same number of replicates were maintain containing DMSO and water.

The larvae were not given any food during this experiment. After 24 h post-treatment the mortality of larvae were calculated.

When larvae did not move to the solution's surface, they were considered as dead.

Statistical analysis

Percentage mortality and standard error were calculated from five replicates. All the data were subjected to probit analysis to calculate the $LC_{50,}$ LC_{90} at 95% fiducial limit of upper confidence and lower confidence limit and chi-square values were calculated using IBM- SPSS Statistic version 22.0.

Results

Bio-larvicidal activity of petroleum, chloroform, ethyl-acetate and methanol extract of *Prunus persica* leaf has been studied here against the fourth instar larvae of three most important mosquito species namely *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. The results revealed that the different solvent extract after 24th h of post treatment have moderate larvicidal activity however, *Prunus persica* methanol leaf extract(PPMLE) showed the highest larvicidal activity with LC_{50} and LC_{90} value 55.432, 221.027 ppm for *Anopheles stephensi*; 70.961, 321.561 ppm for *Aedes aegypti*; 104.104, 483.622 ppm for *Culex quinquefasciatus*. The larvicidal efficacy of *Prunus persica* methanol leaf extract (PPMLE) was followed by *Prunus persica* petroleum ether leaf extract (PPPLE) (LC_{50} and LC_{90}=78.699, 417.117; 101.133, 505.924; 116.802, 524.469 ppm) > *Prunus persica* chloroform leaf extract(PPCLE) (LC_{50} and LC_{90}=109.288, 742.771; 154.336, 897.593; 235.287, 1866.154 ppm) > *Prunus persica* ethyl-acetate leaf extract(PPEALE) (LC_{50} and LC_{90}=131.999, 1069.749; 177.737, 1069.749; 274.186, 1949.576 ppm) against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. All the larvicidal activities and their LC_{50} and LC_{90} values were shown separately for each species of mosquitoes.

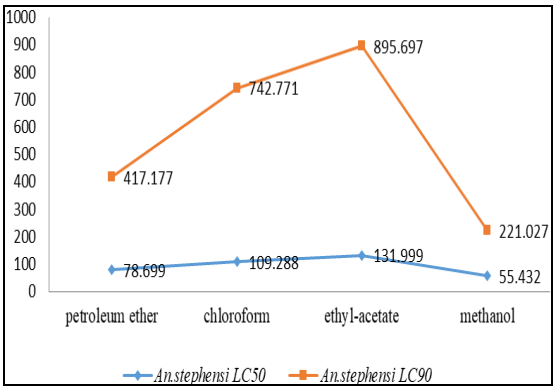

Fig 2: Larvicidal activity of *Prunus persica* leaf extacts against *Anopheles stephensi* after 24 hour of post treatment.

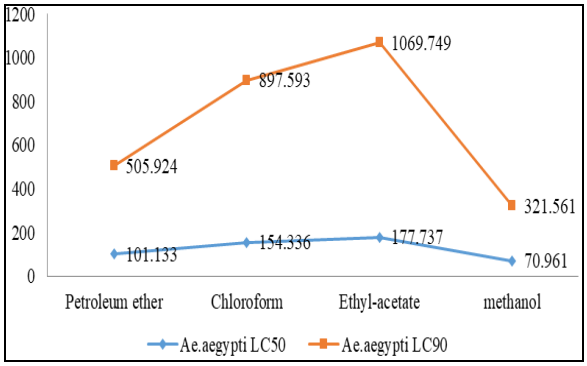

Fig 3: Larvicidal activity of *Prunus persica* leaf extacts against *Aedes aegypti* after 24 hour of post treatment.

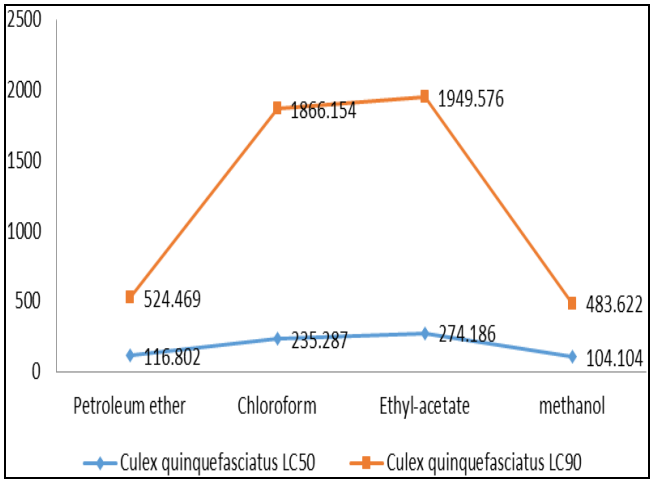

Fig 4: Larvicidal activity of *Prunus persica* leaf extacts against *Culex quinquefasciatus* after 24 hour of treatment.

Discussion

Mosquitoes are the most annoying and dangerous insects because they transmit pathogens. Synthetic pyrethroids are extremely effective against mosquito vectors and have been used to control mosquito vectors in the field. However, these insecticides had a number of unintended consequences for humans and the environment. Because of the consistent use of insecticides for mosquito control, multiple insecticide resistance has developed [28]. Many studies have found that plant extracts have the potential to control mosquito-borne diseases [29, 30] due to active ingredient synergisms, plant crude extracts may be more effective than individual bioactive compounds in managing resistant mosquito populations (Maurya *et al*., 2012) [31]. To date, over 2,000 potential species of plants have been assessed for insecticidal properties around the world [31, 32].

In the present study, *Prunus persica* leaf was extracted with various solvents *viz*., petroleum ether, chloroform, ethyl-acetate, and methanol against three vector mosquito species, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Among the extract PPMLE after 24h of post treatment showed the highest larvicidal activity with LC_{50} and LC_{90} values of value 55.432, 221.027 ppm for *Anopheles stephensi*; 70.961, 321.561 ppm for *Ae. aegypti*; 104.104, 483.622 ppm for *Cx. quinquefasciatus*.

Many researchers have reported that *An. stephensi* and *Ae. aegypti* larvae were more susceptible than *Cx. quinquefasciatus* larvae, as obtained in our result. *Cichorium intybus* methanol extract after 12h and 24h of treatment have been reported to have more effective against *Anopheles stephensi* than *Aedes aegypti* and *Culex quinquefasciatus* with LC_{50} and LC_{90} values of 66.16, 18.88 and 197.56, 107.16 ppm; 78.51, 40.15 and 277.31, 231.28 ppm; 103.99, 64.56 and 314.04, 247.54 ppm[33]. *Terminalia chebula* methanol extract was found to be more efficient against *An. stephensi* than *Ae. aegypti* and *Cx. quinquefasciatus*, with LC_{50} values of 87.13, 93.24, and 111.98 ppm, respectively [34]. In support of our findings, A. B. Umar, A. H. Dankaka, and M. Manjur Shah (2020) [35] demonstrated that the bark methanol extract of *G. triacanthos* has the highest larvicidal activity against *Cx. quinquefasciatus* larvae, with an LC_{50} value of 20.36 mg/l.

As observed in the current study, an increase in the concentration of plant extracts was the primary cause of mortality in the tested mosquito species. Many researchers have noticed a similar trend [36, 33]. S.I. Ali *et al*., (2018) [37] reported the similar results of petroleum ether, chloroform, ethyl acetate, and methanol extracts of the root and leaves of *Senecio laetus Edgew* yielded the highest larvidal activity with LC_{50} values of 24.68 and 16.70 ppm and LC_{90} = 200.42 and 109.19 ppm for *An. stephensi*;; LC_{50} = 44.25 and 22.30 ppm and LC_{90} = 266.19 and 144.67 ppm for *Ae. aegypti* and LC_{50} = 84.14 and 30.68 ppm and LC_{90} = 291.17 and 183.84 ppm for *Cx. quinquefasciatus* at 200 to 12.5 ppm respectively.

This result is also consistent with previous reports by Thangapandi Veni *et al*.,(2017) [34], who found that all crude extracts had moderate larvicidal activity, with the highest efficacy being in the methanol extracts of *T. chebula* against the larvae of *An. stephensi*, *Ae. aegypti*, and *Cx quinquefasciatus*, with LC_{50} and LC_{90} values of 87.13. Death of the larvae may be due to effects of the crude extract on midgut columnar cell vacuolization, microvilli damages reported by R.M. Al-Mehmadi, A.A. Al-Khala (2010) [40].

Conclusion

The current study showed that the *Prunus persica* leaf extracts have remarkable larvicidal activities against the mosquito larvae and therefore these extracts could be used as mosquitocide alternative to synthetic insecticide. The current study findings indicate that further research on the biological properties of the single constituent is needed to conduct.

Conflict of Interest

Authors declare to have no conflict of interest.

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