Volume 27 June 2021
Indian Journal of Biological Sciences
(A Peer Reviewed Journal)
ISSN 0972-8503
http://dx.doi.org/10.62424/ IJBS.2021.27.01.08



# Management of Oviposition Attractant and Symbiotic Bacterial Flora of the Malarial Vector *Anopheles subpictus* (Grassi, 1899) by Plant Extracts

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## Abstract

*Anopheles subpictus* is one of the potent vectors of malarial diseases in different parts of the world. The present study is destined to control the bacterial strains which act as oviposition attractant as well as successful development of this vector species in its environment by an eco-friendly approach. Antibacterial efficacy of crude extracts of different plants were tested against eight bacterial isolates. Solvent extracts were prepared and antibacterial efficacy with MIC value was tested. Effect of most active solvent extract fraction on *An. subpictus* mosquito was determined. Among twenty-five different plant leaves extract tested, *Xanthium strumarium* gave highest antibacterial activity against all the tested bacterial isolates. Among different solvent extract fraction petroleum ether fraction gave highest ZDI value against all the tested bacterial isolates. Scanning electron microscopic analysis revealed several morphological deformities in bacteria. Application of active solvent fraction also reduced successful emergence of adult mosquitoes from eggs to a great extent. This study suggested that isolation and formulation of bio-active compounds from petroleum ether fraction of *X. strumarium* leaves will provide an alternative eco-friendly source of chemical insecticides to control malarial vector populations in Hooghly district.

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**Key words:** Bacteria, *Anopheles subpictus*, Plant extract, *Xanthium strumarium*

## Introduction

Mosquitoes are small dipteran arthropods serving as vector of numerous disease-causing pathogens such as malaria, filaria, dengue, chikungunya, japanese encephalitis throughout different parts of the world (Sahu et al., 2018; Chen et al., 2021). Among these diseases malaria is transmitted by different species of anopheline mosquitoes like *An. gambiae*, *An. stephensi*, *An. sundaicus*, *An. culicifacies*, *An. subpictus*, *An. dirus*, *An. fluviatilis* etc. and are responsible for a great number of mortality and morbidity (Bhattacharya and Sinha, 2019, Vythilingam et al, 2021). Previously *An. subpictus* mosquito was reported as primary malarial vector in India (Panicker et al., 1981) and as secondary vector in Sri Lanka (Hearth et al., 1983). There are five sibling species (A, B, C, D & E) of *Anopheles* among which *An. subpictus* Grassi (sibling sp. B), a fresh water breeder has been reported to act as a potent malarial vector in some rural areas of Hooghly district, West Bengal, India (Chatterjee and Chandra, 2000). The prevalence of malarial disease in any area depends upon the abundances of respective vector mosquitoes of that area. Furthermore, the abundance of mosquito vector depends on their rate of oviposition and successful survival in the environment. Several research studies showed that some bacterial strains present in the aquatic habitats attract the mosquito oviposition by releasing some volatile chemicals (Hasselschwert and Rockett, 1988; Arbaoui and Chua, 2014). At the same time bacterial strains inhabiting within mosquito mid-gut helps their host in healthy development and survival in aquatic bodies (Coon et al, 2017; Mukhopadhyay and Chatterjee, 2016; Seal and Chatterjee, 2022). Removal of these bacterial strains from aquatic habitats and mosquito mid-gut might have detrimental effect on their oviposition and successful survival in the aquatic environment, thereby reducing their prevalence.

Now a days, vector control strategy mostly based on the application of chemically synthesized insecticides including both adulticides and larvicides which have several

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harmful effects on the environment as well as on non-target organisms (Qi et al., 2020). Although some recent studies have used several compounds of botanical origin to control the mosquito vectors, but scanty literatures are available regarding the control of these beneficial bacterial flora from mosquito habitats as well as from their gut environment. So, the present study is aimed to control one of the malarial vector mosquitoes (*An. subpictus*) of Hooghly district, West Bengal by controlling their oviposition attractant as well as their beneficial gut bacterial flora. This would certainly create an alternative and effective vector management strategy in the study areas.

## Methods

### Collection of plant materials

Healthy fresh leaves of altogether twenty-five plants were collected from Golapbag campus of The University of Burdwan, Purba Bardhaman, West Bengal, India.

### Primary screening of antibacterial activity of collected plant leaves

### Preparation of crude extract

Freshly collected leaves were rinsed with water to remove all debris from the leaves. After that by using mortar and pestle the leaves were crushed to fine paste. Then the aqueous portion of the leaves were filtered and collected within test tubes.

### Antibacterial assay

For antibacterial assay total eight bacterial isolates were selected. Four of them viz., *Bacillus cereus* HABW1 (MN153450), *Bacillus megaterium* HABW4 (MN173350), *Bacillus subtilis* HABW10 (MN166905) and *Bacillus tequilensis* HABW14 (MZ363639) were oviposition attractant bacterial isolates of gravid *An. subpictus* of Hooghly (Seal and Chatterjee, 2023); and rest four of them viz., *Bacillus subtilis* HALG2 (MN894011), *Bacillus pumilus* HALG4 (MZ363627), *Bacillus cereus* HALG6 (MZ363632) and *Proteus vulgaris* HALG7 (MZ363637) were symbiotic gut bacterial isolates of larval forms of *An. subpictus* of Hooghly (Seal and Chatterjee, 2022). All of these eight bacterial isolates were inoculated separately within 20 mL of sterilized nutrient broth media and incubated in a B.O.D. shaker incubator at $32\pm1^{0}$C temperature

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for 24-48h until until it reaches desired optical density (0.257) ( ~ $10^8$ cells/mL). Antibacterial activity test was done over sterilized Mueller-Hinton agar plates. Ten µL of each of the homogenous bacterial cultures were spread on separate petri plates and wells of 6 mm diameter were prepared with the aid of a cork borer and 100 µL of each of the crude plant extracts were poured on these wells. The plates were incubated in a B.O.D. incubator for 24h at $32\pm1^0$C. After the incubation period antibacterial activity of plant crude extracts were recorded by observing clear halo zone surrounding the well on the culture plates.

**Detailed screening of antibacterial activity of collected plant leaves**

Plant leaves that gave good result (*Xanthium strumarium*) in primary screening was further chosen for detailed assay.

**Preparation of solvent extract (successive method)**

Leaves of *Xanthium strumarium* was collected, dried in shaded condition for 10-15 days, then ground into fine powder using grinder machine and stored in airtight container for further use. Solvent extracts of plant leaves were prepared by using soxhlet apparatus. Five different solvents (1000 mL) with increasing polarity from non-polar to polar gradient (petroleum ether, ethyl acetate, chloroform, methanol and distilled water respectively) were applied successively through 100gms of grounded leaves (extraction time 96 hours in each solvent). Solvent extracts were filtered and collected in pre-weighted petri dishes. All the extracts were evaporated to dryness in a hot air oven at $50\pm2^0$C and extractive yields (%) were calculated. Then stock solution of 10 mg/mL concentration was prepared in dimethyl sulphoxide (DMSO) solution.

**Antibacterial assay of successive solvent fraction of *X. strumarium* leaves**

Antibacterial efficacy of successive solvent fraction of *X. strumarium* leaves against all the eight bacterial isolates were performed by agar well diffusion technique over mullar-hinton agar plates following Brown, 2004. In this assay DMSO (100 µL) was used as a negative control and two standard antibiotics levofloxacin (LE; 5 µg/disc) and doxycycline (DO; 30 µg/disc) were used as positive control. Then minimum inhibitory

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concentration (MIC) of most active solvent fraction of *X. strumarium* leaves (XPEF) was determined over mullar-hinton agar plates. For this assay graded concentrations of 500 µg/mL, 250 µg/mL, 200 µg/mL, 150 µg/mL, 100 µg/mL, 50 µg/mL were used. MIC value was determined by observing no visible growth surrounding the well having the lowest concentration of solvent fraction (XPEF).

**Scanning electron microscopy of plant extract treated bacteria**

Scanning electron microscopy was done to observe any morphological abnormalities in the bacterial cells after treatment with plant extract. For that purpose, 100 µL standardized culture of each of the bacterial isolates ($10^8$ cfu/mL) were inoculated separately in sterilized 2 mL nutrient broth media within eppendorf tubes and 10 µL plant extract was added to it. At the same time negative control (treated only with 10 µL DMSO) and untreated control (without any plant extract or solvent) of bacterial cultures were prepared. All the tubes were incubated for 24h in a B.O.D. incubator at $32\pm1^0$C. Thin smear of plant extract treated bacterial cells as well as untreated and only DMSO treated bacterial cells were made over the cover glasses and were prepared for the scanning electron microscopic study (Seal and Chatterjee, 2022). Images of the bacterial cells were captured through scanning electron microscope (Sigma 300, ZEISS).

**Effect of plant extract on *An. subpictus* mosquitoes**

Effect of plant extract on different life stages of *An. subpictus* mosquito viz egg, larvae, pupae and adults were recorded in laboratory condition. Different stages of *An. subpictus* mosquito were maintained in natural habitat water and tap water at 1:1 ratio and were kept in an environmental chamber at $28\pm2^0$C temperature and 75±5% relative humidity. Treatment groups were given plant extract at 1mg/mL concentration. Percentage of egg hatching, pupa formation and adult emergence were calculated in both control and treatment groups and were compared for significant difference between these two groups by paired t-test using GraphPad prism 9.0.0.

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## Results

Sensitivity of bacterial isolates towards crude leaves extracts of all total twenty-five plants have been depicted in Table I. Among twenty-five different plants crude leave extracts only one plant i.e., *Xanthium strumarium* (L) showed good antibacterial activity against all the eight tested bacterial isolates. During solvent extraction of *X. strumarium* leaves highest yield was obtained in distilled water extract (40.6%) followed by methanol (23.58%), petroleum ether (10.56%) and chloroform (5.34%), whereas minimum yield was obtained with ethyl acetate (5.34%) (Table II). Physical characteristics also varied among different solvent extracts (Table III).

All the bacteria showed sensitivity towards petroleum ether, chloroform and ethyl acetate fraction of *X. strumarium* leaves but methanol and aqueous extracts did not show any inhibitory activity against all the eight isolates (Table III). Among the three active solvent fraction, petroleum ether showed highest antibacterial activity against all the four isolates, followed by chloroform and ethyl acetate fraction (Table III & Figure I).

Minimum inhibitory concentration assay indicated that MIC value of petroleum ether fraction of *X. strumarium* leaves was 25 µg/mL against *B. cereus* HABW1, *B. megaterium* HABW4, *B. tequilensis* HABW14, *B. cereus* HALG6 and *P. vulgaris* HALG7. This value was 20 µg/mL against *B. subtilis* HABW10, *B. subtilis* HALG2 and 15 µg/mL against *B. pumilus* HALG4 (Table IV & Figure II).

Scanning electron microscopic analysis of plant extract treated bacterial cells exhibited deformed cell shape in case of *B. subtilis* HABW10 (Fig IIIA), damage in the cell wall of *B. pumilus* HALG4 (Fig IIIB) and puncture in the cells of *B.cereus* HALG6 (Fig IIIC). Rest of the bacterial isolates did not exhibit any significant morphological abnormalities when compared with untreated bacterial cells.

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## Table I. Sensitivity of bacterial isolates to crude extracts of selected plant leaves.

| Name of plants | *B. cereus* HABW 1 | *B. megaterim* HABW 4 | *B. subtilis* HABW 10 | *B. tequilensis* HABW 14 | *B. subtills* HALG 2 | *B. pumilus* HALG 4 | *B. cereus* HALG 6 | *P. vulgaris* HALG 7 |
|---|---|---|---|---|---|---|---|---|
| ***Tecoma stans*** | - | - | - | - | - | - | - | - |
| ***Saraca asoca*** | + | - | + | - | + | - | + | - |
| ***Antigonon leptopus*** | - | - | - | - | - | - | - | - |
| ***Solanum nigrum*** | - | - | + | - | + | - | - | + |
| ***Ruellia tuberosa*** | + | - | - | - | + | - | - | - |
| ***Nyctanthes arbor-tristis*** | - | - | - | - | - | - | - | - |
| ***Synedrella nodiflora*** | - | - | + | + | + | - | - | - |
| ***Syzygium cumini*** | - | + | - | - | + | + | - | - |
| ***Trema orientalis*** | - | - | + | - | + | - | - | + |
| ***Markhamia stipulata*** | - | - | - | - | - | - | - | - |
| ***Polyalthia longifolia*** | - | - | + | - | + | - | - | + |
| ***Tamarindus indica*** | + | - | + | - | + | + | - | - |
| ***Grevillea robusta*** | + | + | + | + | + | - | + | - |
| ***Lagerstroemia lanceolata*** | - | - | - | - | - | - | - | - |
| ***Calotropis gigantea*** | + | - | + | + | - | - | + | - |
| ***Murraya paniculata*** | - | - | - | - | - | - | - | - |
| ***Ocimum tenuiflorum*** | - | - | + | + | + | - | - | + |
| ***Bauhinia racemosa*** | + | - | - | - | - | - | + | - |
| ***Ziziphus mauritiana*** | - | - | - | - | - | - | - | - |
| ***Murraya koenigii*** | - | + | - | + | + | - | - | - |
| ***Tabernaemontana divaricata*** | - | - | - | - | - | - | - | - |
| ***Neolamarckia cadamba*** | + | - | - | - | - | - | + | - |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Psidium guajava*** | - | - | - | - | - | - | - | - |
| ***Acacia nilotica*** | + | - | + | - | + | - | + | - |
| ***Xanthium strumarium*** | + | + | + | + | + | + | + | + |

**Table II. Percentage yield and physical characteristics of different solvent extracts of *Xanthium strumarium* leaves.**

| **Extracts** | **Quantity used for extraction** | | **Nature of extract** | | **Yield (%)** |
|---|---|---|---|---|---|
| | **Leaves (gm)** | **Solvent (mL)** | **Colour** | **Consistency** | |
| **Petroleum ether** | 100 | 1000 | Yellowish green | Sticky | 10.56 |
| **Chloroform** | 100 | 1000 | Black | Semisolid | 5.34 |
| **Ethyl acetate** | 100 | 1000 | Green | Sticky | 2.1 |
| **Methanol** | 100 | 1000 | Dark brown | Sticky | 23.58 |
| **Distilled water** | 100 | 1000 | Dark brown | Solid | 40.6 |

**Table III. Sensitivity of bacterial isolates to different solvent extract of *Xanthium strumarium* leaves.**

| **Bacterial isolates** | **XPEF** | **XCHF** | **XEA** | **XMET** | **XAQU** |
|---|---|---|---|---|---|
| ***Bacillus cereus* HABW1** | 31.2±0.58 | 28.2±0.37 | 17.4±0.50 | - | - |
| ***Bacillus megaterium* HABW4** | 26.6±0.50 | 19.8±0.37 | 12±0.44 | - | - |
| ***Bacillus subtilis* HABW10** | 30±0.44 | 23.2±0.58 | 15.6±0.50 | - | - |
| ***Bacillus tequilensis* HABW14** | 24.8±0.37 | 16.4±0.50 | 10.8±0.37 | - | - |
| ***Bacillus subtilis* HALG2** | 30.2±0.37 | 27±0.31 | 20.6±0.50 | - | - |
| ***Bacillus pumilus* HALG4** | 28.2±0.37 | 21.6±0.24 | 12.4±0.50 | - | - |

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| | | | | | |
|---|---|---|---|---|---|
| ***Bacillus cereus* HALG6** | 29.4±0.40 | 22.8±0.86 | 18.8±0.37 | - | - |
| ***Proteus vulgaris* HALG7** | 27.2±0.48 | 18.2±0.37 | 10.8±0.37 | - | - |

**Table IV. Minimum inhibitory concentration of *Xanthium strumarium* extract (XPEF) and two standard antibiotics against the bacterial isolates.**

| Name of isolates | MIC value of XPEF (µg/mL) | MIC value of standard antibiotics | |
|---|---|---|---|
| | | Doxycycline (µg/mL) | Levofloxacin (µg/mL) |
| ***Bacillus cereus* HABW1** | 250 | 0.50 | 0.19 |
| ***Bacillus megaterium* HABW4** | 250 | 0.50 | 0.75 |
| ***Bacillus subtilis* HABW10** | 200 | 0.094 | 0.47 |
| ***Bacillus tequilensis* HABW14** | 250 | 0.25 | 0.50 |
| ***Bacillus subtilis* HALG2** | 200 | 0.125 | 0.38 |
| ***Bacillus pumilus* HALG4** | 150 | 0.16 | 0.125 |
| ***Bacillus cereus* HALG6** | 250 | 0.50 | 0.25 |
| ***Proteus vulgaris* HALG7** | 250 | 0.75 | 0.30 |

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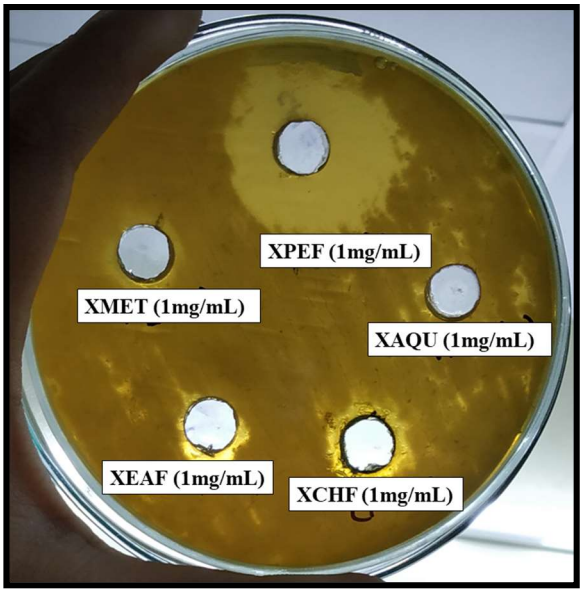


**Fig I. Antibacterial activity of different solvent extract fraction of *Xanthium strumarium* leaves. XPEF-petroleum ether fraction, XCHF- chloroform fraction, XEAF- ethyl acetate fraction, XMET- methanol fraction, XAQU- aqueous fraction.**

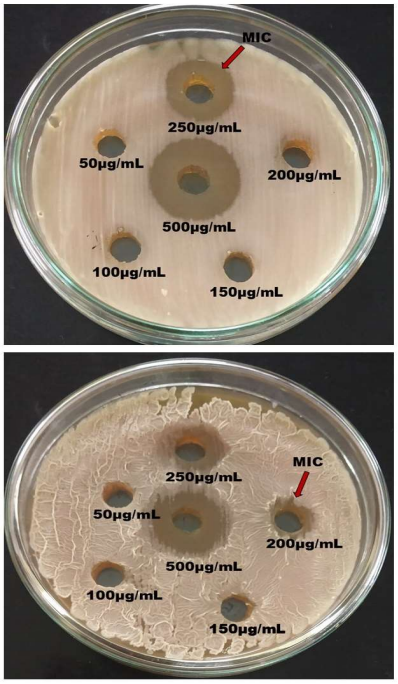


**Fig II. Minimum inhibitory concentration (MIC) of petroleum ether extract of Xanthium strumarium leaves against bacterial isolates.**

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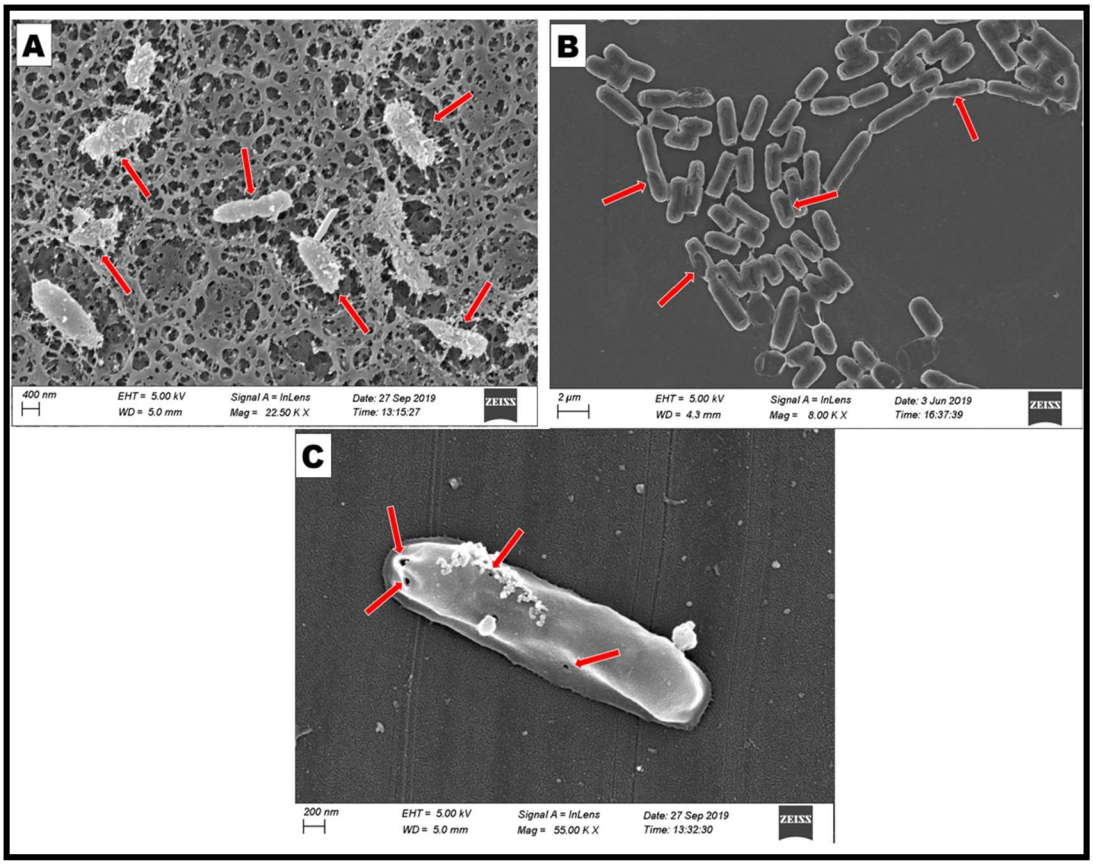


**Fig III. Morphological abnormalities of plant extract treated bacterial cells. A: deformed shape of bacteria, B: damage in bacterial cell wall, C: puncture in the bacterial cell.**

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**Fig IV. Larva and pupa of *Anopheles subpictus*. A: Control live larva, B: Plant extract treated dead larva, C: Control live pupa, D: Plant extract treated larva started pupation but died before completion of pupa formation.**

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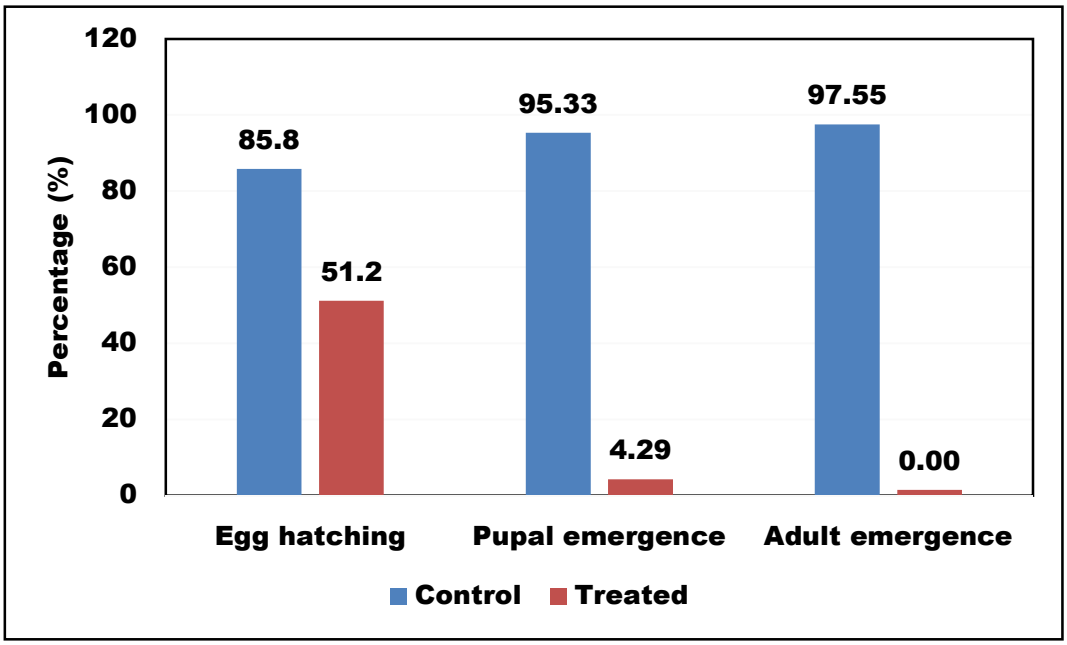


**Fig V. Effect of plant extract (XPEF) at different life stages of *Anopheles subpictus* mosquitoes.**

Effect of most potent solvent extract fraction of *X. strumarium* leaves (XPEF) on different life stages of *An. subpictus* mosquitoes showed that rate of egg hatching in plant extract treated eggs were much reduced (51.2%) than untreated control eggs (85.8%) and showed significant differences between these two groups when compared by paired t-test ($t_{(8.233, 4)}$, p= 0.0012). Most of the plant extract treated larvae were recorded to die during 2nd or 3rd instar stages and majority of the larvae that were survived even up to 4th instar stage were failed to pupate and became died at the start of pupation (Fig IV). Rate of pupation in plant extract treated larvae was 4.29 % and showed significant difference ($t_{(49.75, 4)}$, p<0.0001) with pupation rate of normal larvae (95.33%). Very fewer number of pupae (1-3) that were formed in treated groups, failed to emerged as adult. Rate of

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adult emergence from pupae in untreated control group was 97.55% (Fig V).

## Discussion

Adaptation to survive in diverse types of aquatic environments and insecticidal resistance to commonly used insecticides in anophelines impart a great challenge for vector controlling programmes (Surendran et al., 2020). Further, the enormous use of chemical insecticides to control the mosquito population has posed negative effects on the environment. Therefore, the application of eco-friendly products for mosquito control is becoming utmost necessary (Theochari et al., 2020). Several studies had indicated that *An. subpictus* mosquitoes prevalent in different geographic regions of the world becoming resistant to various synthetic insecticides used for controlling them (Elango et al., 2009; Tikar et al., 2011; Raghavendra et al., 2017).

The present study was conducted to explore eco-friendly plant extract that have good antibacterial property against some selected bacterial isolates, which modulates oviposition of gravid female *An. subpictus* mosquitoes as well as their succful survival in rural areas of Hooghly. Antibacterial activity study of different botanical extracts revealed that sensitivity of these bacterial isolates to the tested plant extracts were varied to a great extent, some of them were sensitive, whereas some of them were resistant in most of the botanical extract tested. Among the tested plant extracts, only the extract of *X. strumarium* leaves was able to inhibit all of the bacterial isolates. Minimum inhibitory concentration of the plant extract was also recorded to vary among the tested bacterial isolates from 150-250 µg/mL.

*Xanthium strumarium* is an annual herb that is distributed throughout the world (Scherer et al., 2013). This plant commonly grows along the road sides, waste places, river banks in warmer regions (Kamboj and Saluja, 2010). Several previous studies had indicated that botanical extracts from different parts like leaves, bark, fruits, roots etc. of *X. strumarium* have antibacterial (Wahab et al., 2017; Ingawale et al., 2018; Khan et al., 2020; Jawalkar et al., 2020), antifungal (Kim et al., 2002; Ma et al., 2007; Wahab et al., 2017), antioxidant (Lee et al., 2001; Scherer et al., 2013; Malpani et al., 2019), anti-

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inflammatory (Kim et al., 2005; Han et al., 2007;Xia et al., 2020), antidiabetic (Ingawale et al., 2018; Guemmaz et al., 2018; Shelke and SM, 2021) anti-cancerous (Piloto-Ferrer et al., 2019; Malekzadeh et al., 2020; Ly et al., 2021) properties. Leaves and fruit extracts of *X. strumarium* was found to repel the potato beetle *Leptinotrasa decemlineata* (Cetinsoy et al., 1998). Studies by Chandel et al. (2012) indicated that leaves extract of *X. strumarium* have anti-plasmodial activity and so they suggested that *X. strumarium* might be used as remedy of malarial diseases. Further study by Sahoo et al. (2020) clearly indicated that there is a scope to develop antimalarial phyto-pharmaceutical from active compounds of *X. strumarium* extracts that had inhibitory activity against *Plasmodium falciparum*. In the present study crude leaf extracts of *X. strumarium* was found to be effective as antibacterial agent against both tested gram-positive (*B. cereus* HABW1, *B. megaterium* HABW4, *B. subtilis* HABW10, *B. tequilensis* HABW14, *B. subtilis* HALG2, *B. pumilus* HALG4, *B. cereus* HALG6) and gram-negative bacteria (*P. vulgaris* HALG7) and among the successive solvent extracts of *X. strumarium* leaves, petroleum ether extract was recorded to have highest antibacterial activity against all the tested bacterial isolates.

Several previous studies reported antibacterial activity of *X. strumarium* against *Bacillus cereus* (Hassan et al., 2014) and *Bacillus subtilis* (Devkota and Das, 2015; Sharifi-Rad et al., 2016; Kumar et al., 2019). Besides these bacterial species, antibacterial activity of extracts from different parts of *X. strumarium* was reported in wide number of both gram-positive and gram-negative bacteria (Scherer et al., 2009; Ullah et al., 2015; Khan et al., 2021).

Some earlier studies reported a number of extracts from different botanical origin to control vector mosquito populations including population of *An. subpictus* mosquitoes. These studies include eco-friendly control of *An. subpictus* and *Cx. Tritaeniorhynchus* by application of flower methanol extract and leaf petroleum ether extract of *Cassia auriculata*, seed and leaf methanol extracts of *Solanum torvum* and leaf hexane extract of *Vitex negundo* (Kamaraj et al., 2009); leaf ethyl acetate extract of *Andrographis*

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*paniculata* and leaf hexane extract of *Eclipta prostrata* (Elango et al., 2009); control of *An. subpictus* and *Cx. vishnui* by application of chloroform: methanol (1:1) leaf extract of *Solanum nigrum* (Rawani et al., 2017).

Through scanning electron microscopic analysis, the present study recorded several morphological abnormalities in plant extract treated bacterial cells when compared with untreated bacterial cells. The bacterial cells showed deformed shape, damages in bacterial cell wall, puncture in cell etc. Previous studies by several workers reported different types of morphological anomalies in bacterial cells after treating with plant extracts, such as treatment with *Ocimum basilucum* leaf methanolic extracts resulted in damage in the cell surface of *Pseudomonas aeruginosa* and *Staphylococcus aureus* as observed by Kaya et al. (2008); treatment by *Aquilaria crassna* leaf aqueous extract resulted in swollen cells in *Staphylococcus epidermidis*as recorded by Kamonwannasit et al. (2013); study by Gupta et al. (2015) recorded that treatment with *Curcuma longa* rhizome extract resulted in damage in the cell membrane, spilling of cytoplasm out of the cells of *Staphylococcus aureus*.

Primary metabolites of plants includes proteins, lipids and carbohydrates, which are essential compounds for growth and metabolism of plants. In contrast, secondary metabolites are those compounds, which are produced by the plants as products of primary metabolism and they serve in defence mechanisms of plants against herbivores, insects and microorganisms (Vaghasiya et al., 2011; Gajger and Dar, 2021). Phenolic compounds present in plant, such as tannins and flavonoids reported to have antimicrobial activities (Shan et al., 2007; Omogbai and Eza, 2010; Kamonwannasit et al., 2013). Studies by earlier workers have reported presence of a good sources of phytochemicals including alkaloids, steroids, flavonoids, terpenoids, glycosides in different extracts from *X. strumarium* (Ullah et al., 2015; Sahoo et al., 2020). The antibacterial activity of *X. strumarium* is due to the presence of toxic compound xanthol, xanthanolide sesquiterpenoids, xanthinin (Sato et al., 1997; Pandey and Rather, 2012) etc. There are several reports which suggested that the presence of specific metabolites

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in plants depends on a number of factors, such as, surrounding environmental conditions of plant (Radusiene et al., 2012; Wen et al., 2020); season of collection (Chaves et al., 2013; Soni et al., 2015; Prinsloo and Nogemane, 2018), extraction procedure (Starmans and Nijhuis, 1996), extraction time (Falleh et al., 2012) and types of solvents used during extraction (Falleh et al., 2012; Dirar et al., 2018). So, the antibacterial efficacy of a plant extracts can be improved by testing all these factors which is able to provide maximum concentration of desired compounds with activity.

So far, there are many reports which suggested that phyto compounds of *X. strumarium* could be used as an alternative of antibiotics to control diseases caused by infectious bacteria (Hassan et al., 2014; Ullah et al., 2015; Fan et al., 2019). But there are no reports of using this plant source for controlling the mosquito populations. This is the first-time report which suggested that phyto extracts from *X. strumarium* leaves might be used as an alternative of chemical insecticides for controlling oviposition attractant and larval symbiotic bacteria of *An. subpictus* mosquitoes in order to control the vector populations in rural areas of Hooghly.

**Acknowledgement**

The authors are thankful to UGC, DST-PURSE and DST-BT, Govt. of West Bengal for providing support to carry-out this work.

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(A Peer Reviewed Journal)
ISSN 0972-8503
http://dx.doi.org/10.62424/ IJBS.2021.27.01.08



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