



## Original article

# Entomofaunal survey and larvicidal activity of greener silver nanoparticles: A perspective for novel eco-friendly mosquito control



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## ABSTRACT

The entomofaunal survey and its toxicity of *Blumea mollis* (Asteraceae) leaf aqueous extract-mediated (*Bm*-LAE) silver nanoparticles (AgNPs) were assessed against selected human vector mosquitoes (HVMs). A total of 1800 individuals of 29 species belongs to 7 genera were identified. Month-wise and Genus-wise abundance of HVMs larval diversity were calculated and one-way ANOVA statistically analyzed the average physico-chemical characteristics. The relationship between physicochemical characteristics and HVMs larvae in KWS was interpreted. The total larval density and container index were 23530.18 and 1961.85 examined against 10 different containers. Various spectroscopic and microscopic investigation characterized *Bm*-AgNPs. The *Bm*- AgNPs tested against HVMs larvae, the predominant LC<sub>50</sub>/LC<sub>90</sub> values of 18.17/39.56, 23.45/42.49 and 21.82/40.43 µg/mL were observed on *An. subpictus* Cx. *vishnui* and *Ae. vittatus*, respectively. The findings of this investigation, improperly maintained drainages, containers and unused things in study sites, are engaged to HVMs development. This will be essential for designing and implementing HVMs control. The larval toxic potentiality of *Bm*- AgNPs had a prompt, inexpensive and compelling synthesis of multi-disperse action against HVMs.

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## 1. Introduction

Mosquitoes are well adapting to breed and survive in various extreme habitats (Senthamarai Selvan et al., 2015; Senthamarai Selvan and Jebanesan, 2016; Franklin and Whelan, 2009). World-wide, mosquitoes are great blood-sucking ectoparasites which provide major health issues to the public. Due to the climate change, urbanization, artificial containers, availability of food sources are

significant factors for utilization and rapid multiplication of human vector mosquitoes (HVMs) (Gokulakrishnan et al., 2012; Krishnappa and Elumalai, 2012a; Thomson, 2014; Rafael et al., 2019). They are transmitting many dreadful vector-borne diseases (VBDs) to public as the results causing millions of mortality and morbidity has been occurring every year (Govindarajan et al., 2005, 2013; WHO Zika virus report, 2016a; WHO malaria report, 2016b; WHO malaria report, 2018; WHO filariasis report, 2019; Govindarajan et al., 2016a; Benelli et al., 2017a). The VBDs are mainly transmitted by four genera of *Aedes*, *Anopheles*, *Culex*, and *Mansonia* of mosquitoes (Sivakumar et al., 2011; Benelli, 2015; Benelli and Govindarajan, 2017; Benelli et al., 2018). Recently, VBDs are very challenging problems and gives social-economic crisis in worldwide (Krishnappa et al., 2012a; Govindarajan and Rajeswary, 2015; Govindarajan et al., 2016b; Benelli et al., 2017b; Rekha et al., 2019).

*Aedes* is a cosmopolitan major HVM they breeds and proliferates in different household water containers even in a tree hole, flower pot, etc., (Govindarajan and Sivakumar, 2012; Govindarajan et al., 2013; Albaba et al., 2015; Sudeep and Shil, 2017). It considerably

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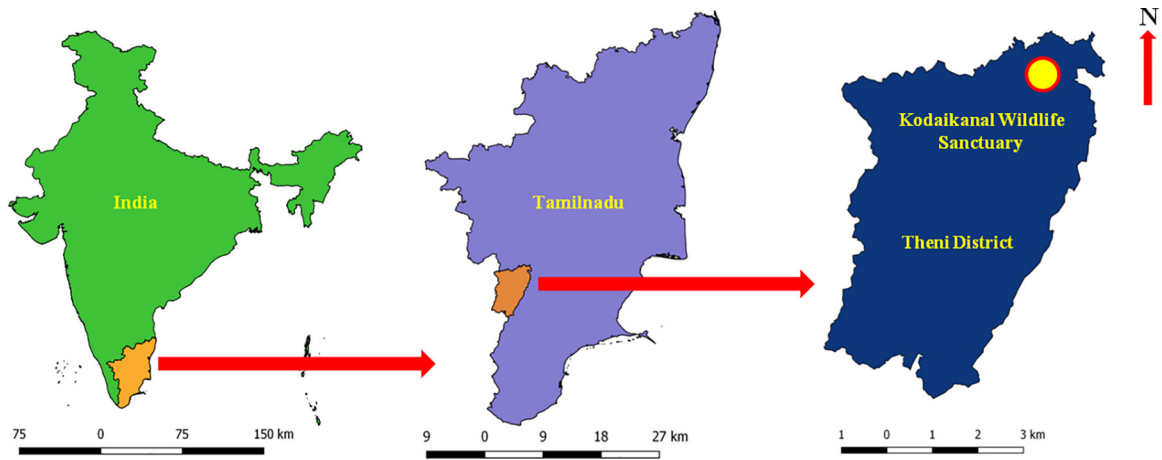


Fig. 1. Study area: KWS (Latitude 10°7'–10°28'N and Longitude 77°16'–77°46'E), Theni District, Tamilnadu, India.



Fig. 2. Plant processing: (A) Experimental plant *Blumea mollis* (Asteraceae) leaves, (B) shade dried leaves powdered, (C) Condensed *Blumea mollis* leaf aqueous extract (Bm-LAE) in a Petri plate.

Table 1  
Species-wise abundance of mosquito larvae collected from KWS, Tamilnadu, India.

| S.No. | Mosquito species                | No. of Larvae | Relative Abundance (%) | Mean ± SE    | Range | N   |
|-------|---------------------------------|---------------|------------------------|--------------|-------|-----|
| 1.    | <i>Ae. Aegypti</i>              | 250           | 13.89                  | 15.63 ± 0.84 | 12–22 | 16  |
| 2.    | <i>Ae. albopictus</i>           | 109           | 6.06                   | 6.81 ± 0.33  | 5–9   | 16  |
| 3.    | <i>Ae. pseudoalbopictus</i>     | 23            | 1.28                   | 1.44 ± 0.18  | 1–3   | 16  |
| 4.    | <i>Ae. pseudotoemiatus</i>      | 25            | 1.39                   | 1.56 ± 0.20  | 1–3   | 16  |
| 5.    | <i>Ae. stegomyia annandalei</i> | 27            | 1.50                   | 1.69 ± 0.21  | 1–4   | 16  |
| 6.    | <i>Ae. Vittatus</i>             | 30            | 1.67                   | 1.88 ± 0.22  | 1–4   | 16  |
| 7.    | <i>An. culiciformis</i>         | 140           | 7.78                   | 8.75 ± 1.04  | 4–17  | 16  |
| 8.    | <i>An. jeyporiensis</i>         | 93            | 5.17                   | 5.81 ± 0.44  | 3–9   | 16  |
| 9.    | <i>An. maculatus</i>            | 71            | 3.94                   | 4.44 ± 0.45  | 2–8   | 16  |
| 10.   | <i>An. Mirans</i>               | 30            | 1.67                   | 1.88 ± 0.22  | 1–4   | 16  |
| 11.   | <i>An. stephensi</i>            | 163           | 9.06                   | 10.19 ± 1.04 | 5–19  | 16  |
| 12.   | <i>An. subpictus</i>            | 26            | 1.44                   | 1.62 ± 0.18  | 1–3   | 16  |
| 13.   | <i>Ar. subalatus</i>            | 61            | 3.39                   | 3.81 ± 0.46  | 1–8   | 16  |
| 14.   | <i>Cx. culex mimeticus</i>      | 21            | 1.17                   | 1.31 ± 0.12  | 1–2   | 16  |
| 15.   | <i>Cx. epidesmus</i>            | 20            | 1.11                   | 1.25 ± 0.11  | 1–2   | 16  |
| 16.   | <i>Cx. mimuloides</i>           | 22            | 1.22                   | 1.38 ± 0.18  | 1–3   | 16  |
| 17.   | <i>Cx. mimulus</i>              | 98            | 5.44                   | 6.13 ± 0.56  | 4–11  | 16  |
| 18.   | <i>Cx. minutissimus</i>         | 36            | 2.00                   | 2.25 ± 0.31  | 1–4   | 16  |
| 19.   | <i>Cx. nigropancatus</i>        | 19            | 1.06                   | 1.19 ± 0.10  | 1–2   | 16  |
| 20.   | <i>Cx. nilgirisus</i>           | 25            | 1.39                   | 1.56 ± 0.18  | 1–3   | 16  |
| 21.   | <i>Cx. pallidothorax</i>        | 46            | 2.56                   | 2.88 ± 0.20  | 2–4   | 16  |
| 22.   | <i>Cx. pseudovishnui</i>        | 79            | 4.39                   | 4.94 ± 0.50  | 2–8   | 16  |
| 23.   | <i>Cx. quinquefasciatus</i>     | 172           | 9.56                   | 10.75 ± 1.34 | 3–20  | 16  |
| 24.   | <i>Cx. Vishnui</i>              | 60            | 3.33                   | 3.75 ± 0.40  | 2–7   | 16  |
| 25.   | <i>Oc. Grenii</i>               | 34            | 1.89                   | 2.13 ± 0.22  | 1–4   | 16  |
| 26.   | <i>Or. flavithorax</i>          | 30            | 1.67                   | 1.88 ± 0.20  | 1–3   | 16  |
| 27.   | <i>Ur. annandalei</i>           | 29            | 1.61                   | 1.81 ± 0.20  | 1–3   | 16  |
| 28.   | <i>Ur. Campestris</i>           | 23            | 1.28                   | 1.44 ± 0.18  | 1–3   | 16  |
| 29.   | <i>Ur. Novobscura</i>           | 38            | 2.11                   | 2.38 ± 0.18  | 1–4   | 16  |
| Total |                                 | 1800          | 100                    | 3.88 ± 0.18  | 1–22  | 464 |

One-way ANOVA; df = 28; F = 53.13; N = Number of sample; p < 0.05.

**Table 2**  
Month-wise abundance and diversity of mosquito larvae collected from KWS, Tamilnadu, India.

| S.No.               | Month          | Mean ± SE   | Range  | N   | Shannon Wiener Index |            | Simpsons Index |
|---------------------|----------------|-------------|--------|-----|----------------------|------------|----------------|
|                     |                |             |        |     | H                    | Variance H |                |
| Moth-wise abundance |                |             |        |     |                      |            |                |
| 1.                  | December- 2018 | 5.67 ± 0.49 | 167–22 | 116 | 3.03                 | 0.001      | 16.237         |
| 2.                  | January- 2019  | 4.03 ± 0.34 | 167–18 | 116 | 3.05                 | 0.001      | 16.727         |
| 3.                  | February- 2019 | 3.39 ± 0.30 | 167–16 | 116 | 3.02                 | 0.002      | 16.098         |
| 4.                  | March- 2019    | 2.43 ± 0.22 | 167–13 | 116 | 3.04                 | 0.003      | 15.735         |

One-way ANOVA; df = 3; F = 14.93; N = Number of sample; H =  $(N \log N - \text{filogfi}/N)$  (or)-(PilogPi): Shannon-Weiner diversity index; Variance H =  $(N \log N - \text{filogfi}/N)$  (or)-(PilogPi): Shannon-Weiner diversity index variance;  $p < 0.05$ .

**Table 3**  
Genus-wise abundance and diversity of mosquito larvae collected from KWS, Tamilnadu, India.

| S. No. | Genus                | Abundance (Mean ± SE) | Range | N   | Shannon Wiener Index |            | Simpsons Index |
|--------|----------------------|-----------------------|-------|-----|----------------------|------------|----------------|
|        |                      |                       |       |     | H                    | Variance H |                |
| 1.     | <i>Aedes</i>         | 4.83 ± 0.56           | 1–22  | 96  | 1.3645               | 0.0001     | 3.8514         |
| 2.     | <i>Anopheles</i>     | 5.45 ± 0.42           | 1–19  | 96  | 1.3259               | 0.0002     | 3.5707         |
| 3.     | <i>Armigeres</i>     | 3.81 ± 0.47           | 1–8   | 16  | 1.2945               | 0.0031     | 3.5260         |
| 4.     | <i>Culex</i>         | 3.40 ± 0.26           | 1–20  | 176 | 1.3253               | 0.0002     | 3.5912         |
| 5.     | <i>Ochlerotatus</i>  | 2.13 ± 0.22           | 1–4   | 16  | 1.3424               | 0.0038     | 4.0071         |
| 6.     | <i>Orthopodomyia</i> | 1.88 ± 0.20           | 1–3   | 16  | 1.3512               | 0.0041     | 4.1038         |
| 7.     | <i>Uranotaenia</i>   | 1.88 ± 0.12           | 1–4   | 48  | 1.3579               | 0.0008     | 3.9111         |

One-way ANOVA; df = 6; F = 7.722; N = Number of sample; H =  $(N \log N - \text{filogfi}/N)$  (or)-(PilogPi): Shannon-Weiner diversity index; Variance H =  $(N \log N - \text{filogfi}/N)$  (or)-(PilogPi): Shannon-Weiner diversity index variance;  $p < 0.05$ .

dangerous medical pests in half of the world and humans are severely suffered by chikungunya, dengue, yellow fever and zika (WHO, 2009; Chen and Wilson, 2010; WHO Zika virus report, 2016a). *Anopheles* is a major HVM of malaria, Japanese encephalitis, etc., in the Asian continent, especially India (Krishnappa et al., 2012b; Thenmozhi et al., 2016; Hasan et al., 2014; Mathivanan et al., 2014; Govindarajan and Benelli, 2016a). Around 120 million people are suffered from lymphatic filariasis, Japanese encephalitis, etc., by *Culex* and 44 million peoples have chronic manifestation. It is the second most important VBDs in India, accounting for 40% of the global prevalence of infection and it invariably spreads in both

urban and rural areas (Govindarajan, 2011; Tyagi et al., 2014; Govindarajan et al., 2015; Thenmozhi et al., 2016).

Synthetic chemical pesticides (SCPs) are the most common effective methods of control strategy against HVMs. Recently, unadvisable application of SCPs are increased and statistically significant negative health issues on aquatic and terrestrial organisms (Govindarajan and Rajeswary, 2015; Ndakidemi et al., 2016; Karthika et al., 2017; Abinaya et al., 2018; Divya et al., 2018). Globally, 355,000 deaths were recorded every year with the association of pesticide poisoning (Carvalho, 2017). Therefore, we need to search for unconventional, eco-recyclable indigenous approaches

**Table 4**  
Average physicochemical characteristics of mosquito breeding habitats and relationship between mosquito larvae at KWS, Tamilnadu, India.

| S. No. | Physico-chemical parameters     | December- 2018 | January- 2019 | February- 2019 | March- 2019 | One-way ANOVA<br>F and P value | Larval Abundance<br>R and P value |
|--------|---------------------------------|----------------|---------------|----------------|-------------|--------------------------------|-----------------------------------|
|        |                                 | Mean ± SE      | Mean ± SE     | Mean ± SE      | Mean ± SE   |                                |                                   |
| 1.     | Ammonia mg/L                    | 0.0 ± 0.0      | 0.1 ± 0.0     | 0.1 ± 0.0      | 0.1 ± 0.0   | 1651.6 and 0.05                | −0.270** and 0.001                |
| 2.     | Calcium mg/L                    | 38.3 ± 0.1     | 40.3 ± 0.1    | 47.1 ± 0.2     | 50.8 ± 0.1  | 2102.4 and 0.05                | −0.258** and 0.001                |
| 3.     | Chloride mg/L                   | 128.3 ± 0.1    | 131.6 ± 0.1   | 138.6 ± 0.2    | 145.5 ± 0.2 | 2665.9 and 0.05                | −0.264** and 0.001                |
| 5.     | Conductivity (μS/cm)            | 530.7 ± 0.6    | 570.8 ± 0.8   | 610.8 ± 2.1    | 656.0 ± 3.4 | 692.6 and 0.05                 | −0.251** and 0.001                |
| 4.     | Dissolved O <sub>2</sub> (mg/L) | 15.4 ± 0.0     | 12.5 ± 0.1    | 10.3 ± 0.1     | 7.8 ± 0.1   | 1502.5 and 0.05                | 0.270** and 0.001                 |
| 6.     | Fluoride mg/L                   | 0.1 ± 0.0      | 0.2 ± 0.0     | 0.3 ± 0.0      | 0.3 ± 0.0   | 548.0 and 0.05                 | −0.238** and 0.001                |
| 7.     | Humidity                        | 68.0 ± 0.2     | 42.4 ± 0.3    | 70.0 ± 1.0     | 80.0 ± 0.2  | 936.1 and 0.05                 | −0.090** and 0.053                |
| 8.     | Iron mg/L                       | 1.3 ± 0.0      | 1.8 ± 0.0     | 2.0 ± 0.0      | 2.5 ± 0.0   | 1312.9 and 0.05                | −0.263** and 0.001                |
| 9.     | Magnesium mg/L                  | 10.4 ± 0.0     | 11.1 ± 0.0    | 11.7 ± 0.0     | 12.4 ± 0.0  | 2996.6 and 0.05                | −0.275** and 0.001                |
| 10.    | Nitrate mg/L                    | 33.2 ± 0.1     | 35.6 ± 0.0    | 39.4 ± 0.1     | 42.8 ± 0.1  | 2303.5 and 0.05                | −0.269** and 0.001                |
| 11.    | Nitrite mg/L                    | 7.9 ± 0.0      | 8.7 ± 0.0     | 9.7 ± 0.0      | 10.1 ± 0.0  | 1625.7 and 0.05                | −0.264** and 0.001                |
| 12.    | p <sup>H</sup>                  | 7.0 ± 0.0      | 7.0 ± 0.0     | 6.9 ± 0.0      | 6.8 ± 0.0   | 77.5 and 0.05                  | 0.093** and 0.045                 |
| 13.    | Phosphate mg/L                  | 4.7 ± 0.0      | 4.8 ± 0.0     | 4.9 ± 0.0      | 5.2 ± 0.0   | 482.5 and 0.05                 | −0.246** and 0.001                |
| 14.    | Rainfall mm                     | 360.0 ± 2.5    | 208.2 ± 0.2   | 98.0 ± 1.2     | 43.0 ± 0.4  | 7160.3 and 0.05                | 0.294** and 0.001                 |
| 15.    | Sulphate mg/L                   | 28.6 ± 0.0     | 31.7 ± 0.0    | 32.3 ± 0.0     | 34.2 ± 0.1  | 2445.1 and 0.05                | −0.278** and 0.001                |
| 16.    | Temperature °C                  | 28.0 ± 0.1     | 30.3 ± 0.3    | 31.0 ± 0.3     | 32.0 ± 0.4  | 36.6 and 0.05                  | −0.116* and 0.012                 |
| 17.    | Total Hardness mg/L             | 33.4 ± 0.1     | 37.8 ± 0.1    | 39.8 ± 0.0     | 41.4 ± 0.1  | 1215.1 and 0.05                | −0.261** and 0.001                |
| 18.    | Total Dissolved Solids mg/L     | 235.9 ± 1.0    | 274.1 ± 1.4   | 340.7 ± 3.2    | 417.5 ± 0.7 | 1844.8 and 0.05                | −0.261** and 0.001                |
| 19.    | Total salinity ppt              | 0.5 ± 0.0      | 1.9 ± 0.0     | 2.7 ± 0.0      | 3.2 ± 0.0   | 1853.0 and 0.05                | −0.270** and 0.001                |
| 20.    | Turbidity NTU                   | 21.3 ± 0.1     | 27.6 ± 0.2    | 30.0 ± 0.0     | 31.6 ± 0.1  | 1864.8 and 0.05                | −0.268** and 0.001                |

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

**Table 5**  
Larval density and container index of mosquito larvae collected from KWS, Tamilnadu, India.

| S. No.                | Different containers | A   | B   | C    | D        | E       |
|-----------------------|----------------------|-----|-----|------|----------|---------|
| <b>December- 2018</b> |                      |     |     |      |          |         |
| 1.                    | Rockypool            | 34  | 28  | 109  | 389.29   | 82.35   |
| 2.                    | Stream slow flowing  | 16  | 12  | 19   | 158.33   | 75.00   |
| 3.                    | Water storage tank   | 6   | 6   | 163  | 2716.67  | 100.00  |
| 4.                    | Tree hole            | 37  | 14  | 69   | 492.86   | 37.84   |
| 5.                    | Bamboo stump         | 15  | 9   | 12   | 133.33   | 60.00   |
| 6.                    | Stagnant pools       | 53  | 24  | 126  | 525.00   | 45.28   |
| 7.                    | Animal foot print    | 68  | 12  | 58   | 483.33   | 17.65   |
| 8.                    | Leaf axil            | 42  | 8   | 35   | 437.50   | 19.05   |
| 9.                    | Flower bracts        | 35  | 7   | 32   | 457.14   | 20.00   |
| 10.                   | Spring pool          | 6   | 2   | 35   | 1750.00  | 33.33   |
| <b>January- 2019</b>  |                      |     |     |      |          |         |
| 1.                    | Rockypool            | 28  | 16  | 85   | 531.25   | 57.14   |
| 2.                    | Stream slow flowing  | 12  | 8   | 32   | 400.00   | 66.67   |
| 3.                    | Water storage tank   | 6   | 6   | 115  | 1916.67  | 100.00  |
| 4.                    | Tree hole            | 28  | 11  | 40   | 363.64   | 39.29   |
| 5.                    | Bamboo stump         | 12  | 7   | 18   | 257.14   | 58.33   |
| 6.                    | Stagnant pools       | 44  | 18  | 88   | 488.89   | 40.91   |
| 7.                    | Animal foot print    | 51  | 10  | 31   | 310.00   | 19.61   |
| 8.                    | Leaf axil            | 36  | 6   | 22   | 366.67   | 16.67   |
| 9.                    | Flower bracts        | 27  | 5   | 17   | 340.00   | 18.52   |
| 10.                   | Spring pool          | 6   | 2   | 19   | 950.00   | 33.33   |
| <b>February- 2019</b> |                      |     |     |      |          |         |
| 1.                    | Rockypool            | 21  | 15  | 66   | 440.00   | 71.43   |
| 2.                    | Stream slow flowing  | 8   | 7   | 21   | 300.00   | 87.50   |
| 3.                    | Water storage tank   | 6   | 6   | 113  | 1883.33  | 100.00  |
| 4.                    | Tree hole            | 24  | 9   | 29   | 322.22   | 37.50   |
| 5.                    | Bamboo stump         | 9   | 6   | 21   | 350.00   | 66.67   |
| 6.                    | Stagnant pools       | 36  | 15  | 68   | 453.33   | 41.67   |
| 7.                    | Animal foot print    | 41  | 9   | 21   | 233.33   | 21.95   |
| 8.                    | Leaf axil            | 29  | 5   | 19   | 380.00   | 17.24   |
| 9.                    | Flower bracts        | 24  | 4   | 18   | 450.00   | 16.67   |
| 10.                   | Spring pool          | 5   | 2   | 17   | 850.00   | 40.00   |
| <b>March- 2019</b>    |                      |     |     |      |          |         |
| 1.                    | Rockypool            | 18  | 13  | 49   | 376.92   | 72.22   |
| 2.                    | Stream slow flowing  | 6   | 6   | 21   | 350.00   | 100.00  |
| 3.                    | Water storage tank   | 6   | 6   | 67   | 1116.67  | 100.00  |
| 4.                    | Tree hole            | 21  | 8   | 24   | 300.00   | 38.10   |
| 5.                    | Bamboo stump         | 7   | 4   | 16   | 400.00   | 57.14   |
| 6.                    | Stagnant pools       | 33  | 15  | 46   | 306.67   | 45.45   |
| 7.                    | Animal foot print    | 37  | 8   | 20   | 250.00   | 21.62   |
| 8.                    | Leaf axil            | 24  | 4   | 14   | 350.00   | 16.67   |
| 9.                    | Flower bracts        | 21  | 4   | 12   | 300.00   | 19.05   |
| 10.                   | Spring pool          | 4   | 2   | 13   | 650.00   | 50.00   |
| Total                 |                      | 942 | 359 | 1800 | 23530.18 | 1961.85 |

A: No. of containers searched.

B: No. of containers positive.

C: Total no. of larvae collected.

D: Total larval density.

E: Total container index.

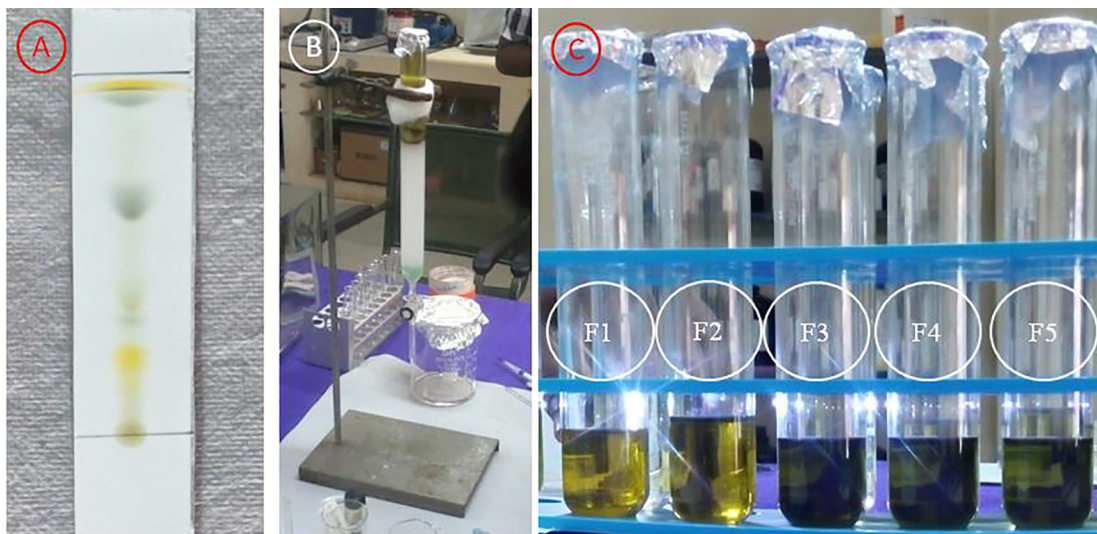
**Table 6**  
Qualitative analysis of phyto-chemical screening different leaf extracts of *B. mollis*.

| S. No. | Phyto-chemical screening | <i>Blumea mollis</i> -various extracts |     |     |     |     |
|--------|--------------------------|--|-----|-----|-----|-----|
|        |                          | ACE                                    | DME | EAE | ETL | AQU |
| 1.     | Alkaloids                | -                                      | -   | -   | -   | +   |
| 2.     | Anthroquinones           | +                                      | -   | +   | +   | +   |
| 3.     | Carbohydrates            | -                                      | +   | -   | -   | -   |
| 4.     | Coumarins                | +                                      | +   | +   | -   | +   |
| 5.     | Flavonoids               | -                                      | -   | -   | +   | +   |
| 6.     | Phenolics                | +                                      | -   | +   | +   | +   |
| 7.     | Resins                   | -                                      | +   | -   | -   | +   |
| 8.     | Saponins                 | -                                      | +   | -   | +   | +   |
| 9.     | Tannins                  | +                                      | +   | +   | -   | +   |
| 10.    | Triterpenes              | +                                      | -   | +   | -   | +   |

ACE: Acetone; DME: Dichloromethane; EAE: Ethyl acetate; ETL: Ethanol; AQU: Aqueous.

+ = noted for presence of phyto-chemical group.

- = noted for absence of phyto-chemical group.



**Fig. 3.** Various fractional units: (A) TLC plate, (B) CC unit, (C) different fractions (F1: fraction one, F2: fraction two, F3: fraction three and F5: fraction five).

that should be required for prohibiting HVMs. Naturally available phyto-compounds (PCs) are more preferable and it has bio-viable safer agents for controlling HVMs using in many parts of the world (Mathivanan, et al., 2010; Elumalai and Krishnappa, 2014; Krishnappa and Elumalai, 2012b; Elumalai et al., 2013; Govindarajan and Benelli, 2016b). Plants have consisted of plenty of PCs; it has prospective toxic effects on various life stages of HVMs. India has rich floral diversity country, the flora has a wide variety of PCs and more than 2000 herbaceous species have known as both medicinal and entamotoxic properties (Krishnappa and Elumalai, 2014; Krishnappa and Elumalai, 2013; Govindarajan et al., 2016c,d,e; Govindarajan and Benelli, 2016c,d,e; Krishnappa et al., 2019). Present investigation is the first-hand report on abundance, density, diversity, water physico-chemical characteristics at HVMs breeding habitats of KWS and larval toxicity of (*B. mollis* leaf aqueous extract-mediated silver nanoparticles) *Bm*-AgNPs against selected HVMs.

## 2. Materials and methods

### 2.1. Study areas

The survey were carried out between December- 2018 to April-2019 on the water-holding areas supporting HVMs breeding and it includes different water holding 10 sites (Bridge area, kaludhai oodai, kondaikuthi oodai, lower falls, upper falls, valukkparai, vanathiparai, vengayaparai, watch tower 1 and watch tower 2) in protected area, KWS, Theni District, Tamilnadu, India (Latitude 10°7'–10°28' N and Longitude 77°16'–77°46' E) (Fig. 1). The vegetation of the study locations consists of residual native forests and grassy areas. The vertebrate species includes fishes, amphibians, reptiles, birds, and mammals. A lot of natural and artificial water bodies are here and it mainly supports to breeding sites HVMs.

### 2.2. Mosquito collection and meteorological data acquisition

Different water bodies like that rocky pool, stream slow-flowing, water storage tank, tree hole, bamboo stump, stagnant ponds, animal footprint, leaf axil, flower bracts and spring pool were examined for the presence of HVMs larvae. The surveys were carried out every week interval of four months. The larval samples were carefully collected from different habitats in dawn (6–9 am)

and dusk (6–9 pm) hours and followed by a standard larval sampling protocol (Silver, 2008; WHO, 2013; Senthamarai Selvan et al., 2015). Weather data like rainfall, relative humidity and temperature were taken into consideration throughout the study period. This was obtained from the Regional Meteorological Centre (RMC), Chennai and Tamilnadu Agricultural University (TNAU), Coimbatore.

### 2.3. Physicochemical characteristics

Water samples were collected in small sterilized 250 mL glass containers at selected HVMs breeding sites. The bottles were covered with perforated caps and foil sheet, labeled it properly. The collected water samples (Within 3 hrs) were carried carefully to the laboratory for analyzing purposes. The breeding water characteristics were recorded using a standard procedure (APHA, 2005). The temperature was regularly measured at the time of sample collection (Senthamarai Selvan et al., 2016).

### 2.4. Mosquito density and diversity analysis

The abundance of container breeding HVM was calculated by larval density and container index. Overall the collected HVMs larval diversity and dominant species were calculated by Shannon-Weiner's (1949) diversity index and Simpson's (1949) dominance index, respectively. Larval density is the study of numerical strength of a species in relation to the total number of individuals of all the species and can be calculated as:

$$\text{Larval density} = \frac{\text{Total number of larvae collected}}{\text{Total number of Positive containers}} \times 100$$

$$\text{Container index} = \frac{\text{Total number of positive containers}}{\text{Total number of containers inspected}} \times 100$$

### 2.5. Plant processing and spectral analysis

The fresh floral leaves (Fig. 2A) of *Blumea mollis* (Asteraceae) were collected at Koothur Village (Latitude 11.7794°N, Longitude 78.2034°E), Mayiladuthurai District, Tamilnadu, India. The floral leaves washed with tap water, allowed to shade dried on blotting paper spread at room temperature. The dried plant material

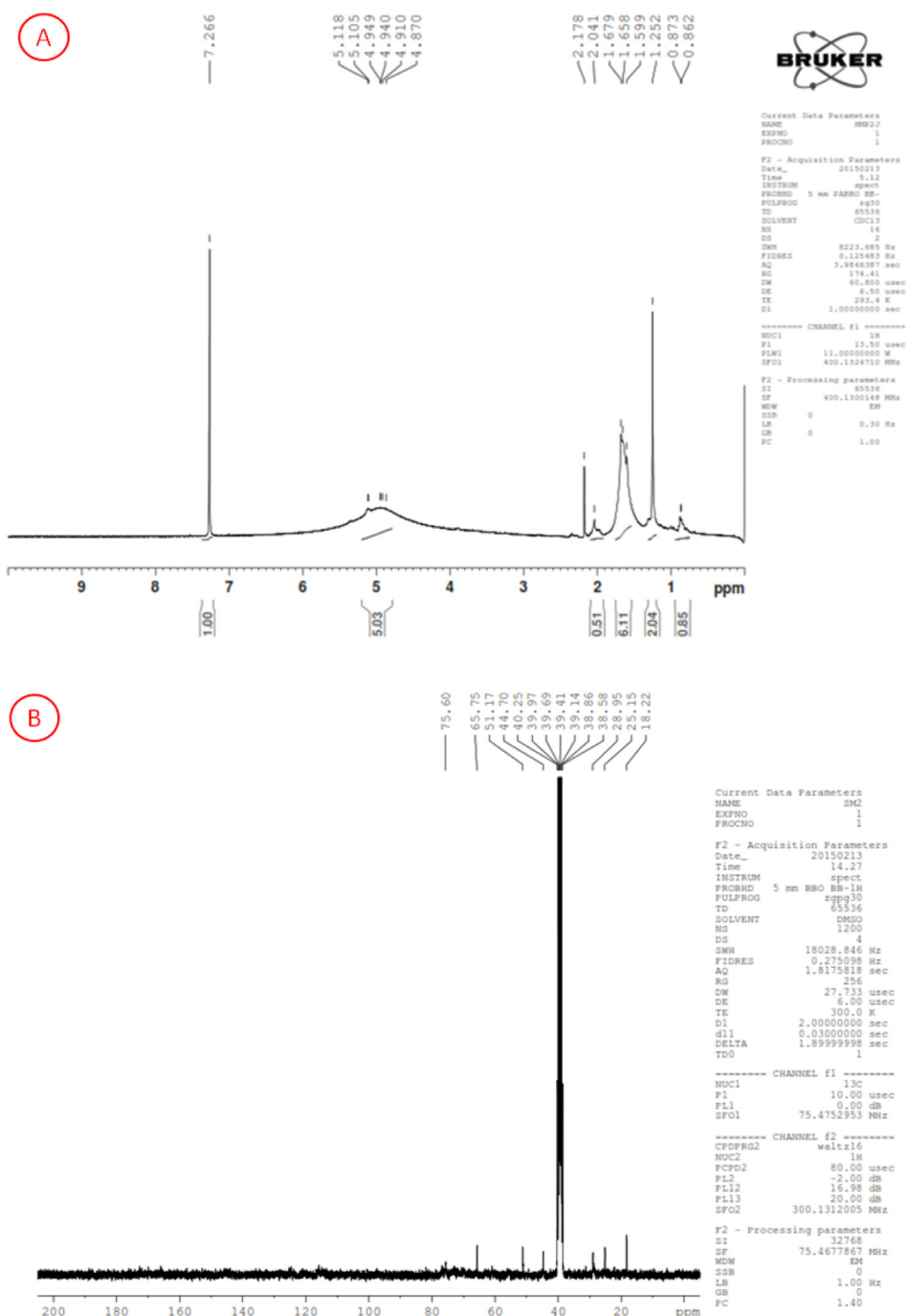


Fig. 4. NMR spectrum of *Blumea mollis* leaf aqueous extract. (A) <sup>1</sup>H NMR, (B) <sup>13</sup>C NMR.

was powdered (Fig. 2B), which extracted with different solvents by adapting a standard protocol Vogel, 1978. The extract was condensed (Fig. 2C), which stored in aseptic amber bottle vials. Phyto-chemicals of leaves were screened by prescribed methodology Nweze et al. (2004) and Senthikumar and Reetha (2009). *Bm*-LAE bio-efficient of PCs were explored by TLC and CC run with different solvent systems in which aqueous and ethyl acetate 9:1 ratio provided maximum fractions and NMR spectroscopy can provide a principal of additional information about peptide in solution. The *Bm*-AgNPs were illustrated by various spectral (UV-vis, FTIR and XRD) and microscopic (SEM) analysis.

## 2.6. Insect rearing and larval toxicity

The HVMs larvae collected from KWS and carefully reared in the insectariums. Fresh 3rd instars larvae were carried to assess the larval toxic bioassay. *Bm*-LAE of AgNPs was evaluated by WHO (2005) protocol and maintained five replications. 3rd instars larvae were transferred to reusable transparent glass container 500 mL capacities in which added 449 mL of chlorine-free H<sub>2</sub>O + 1 mL of emulsifier. The appropriate volume (1–10 µg/mL) of diluted *Bm*-LAE of AgNPs was added against desired HVMs. Larval toxic bioassay started with the lowest concentration and an equal number of control groups maintained without *Bm*-LAE of AgNPs.



Fig. 5. Visual observations of (A) silver nitrate solution, (B) leaf aqueous extract and (C) synthesized AgNPs.

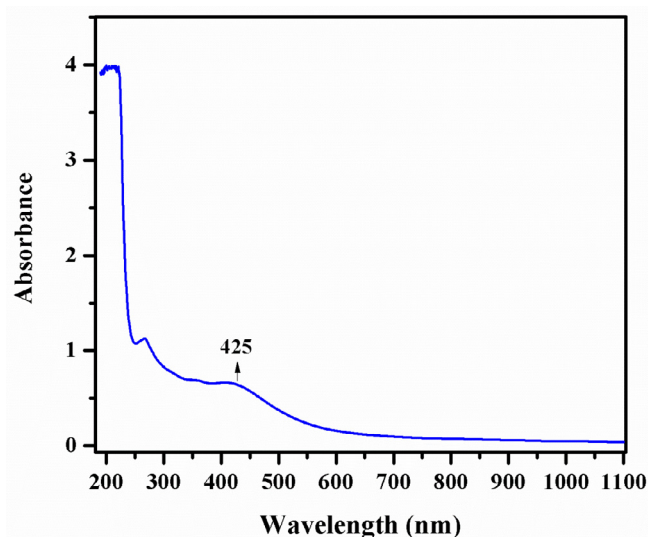


Fig. 6. UV –vis spectrum obtained of AgNPs.

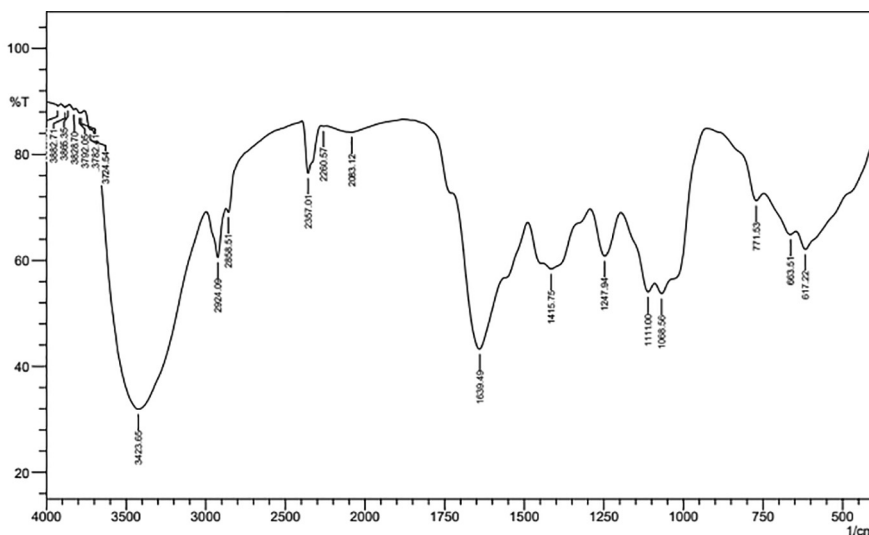


Fig. 7. FTIR spectrum of AgNPs.

2.7. Statistical analysis

The larval mortality data were counted every 6 h for 24 h, and the percent mortality was calculated by Abbott’s (1925) formula and probit analysis assessed by Finney (1971). The total larval mortality, abundance, density, diversity, species richness, etc., were calculated by using the IBM-SPSS Statistics version 25.0 version.

3. Results and discussion

3.1. A species-wise abundance of mosquito larvae

An HVM survey was conducted at KWS for larvae that were collected during the selected study period, which identified in the species level. A total of 29 species belongs to 7 genera of 1800 individuals were identified (Table 1). Among the 29 species, 5 species were highly abundances in the protected water habitat they were *Culex*, *Anopheles* and *Aedes* genus. *Ae. aegypti* found 250 larvae, relative abundance 13.89% and its Mean ± SE were 15.63 ± 0.846 and followed by *Cx. quinquefasciatus*, *An. stephensi*, *An. culiciformis* and *Ae. albopictus*: No. of larvae, relative abundance % and Mean ± SE values were 172, 9.56, 10.75 ± 1.346; 163, 9.06, 10.19 ± 1.046; 140, 7.78, 8.75 ± 1.043 and 109, 6.06, 6.81 ± 0.332 respectively. In south India, rainfall has attained a maximum in November and December simultaneously, proportionate to the HVMs breeding sites and HVMs population also increased in many localities. A similar trend have been noticed in previous research and it well with support to the present study (Basset, 2001; Basset et al., 2012; Gilbert et al., 2014).

3.2. Month-wise, Genus-wise abundance and diversity

The Month-wise abundance and diversity of HVMs larvae were collected from four different months from December- 2018 to March- 2019. Maximum abundance values 5.67 ± 0.49, Shannon Wiener Index: H and Variance H values were 3.03 and 0.001 respectively, Simpsons Index values were 16.237 and Species Richness 29 were observed on December- 2018 and followed by January- 2019, February- 2019 and March- 2019. The data calculated by One-way ANOVA; degrees of freedom = 3; F value = 14.93; p < 0.05. We examined a total of 7 genera were recorded at KWS, the Genus-wise abundance of HVMs larvae values were 5.45, 4.83, 3.81, 3.40, 2.13, 1.88 and 1.88 examined against *Anopheles*, *Aedes*, *Armi-*

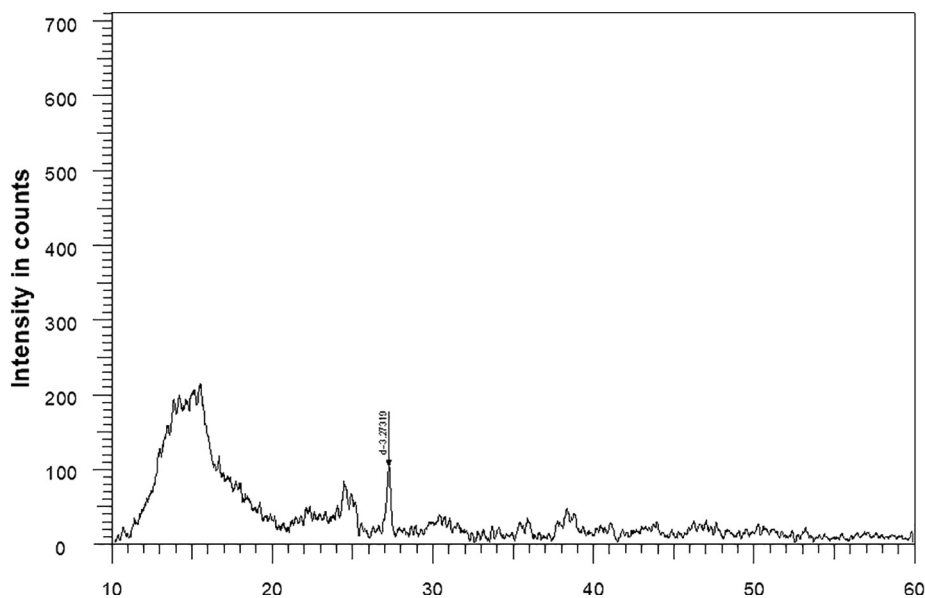


Fig. 8. XRD analysis AgNPs.

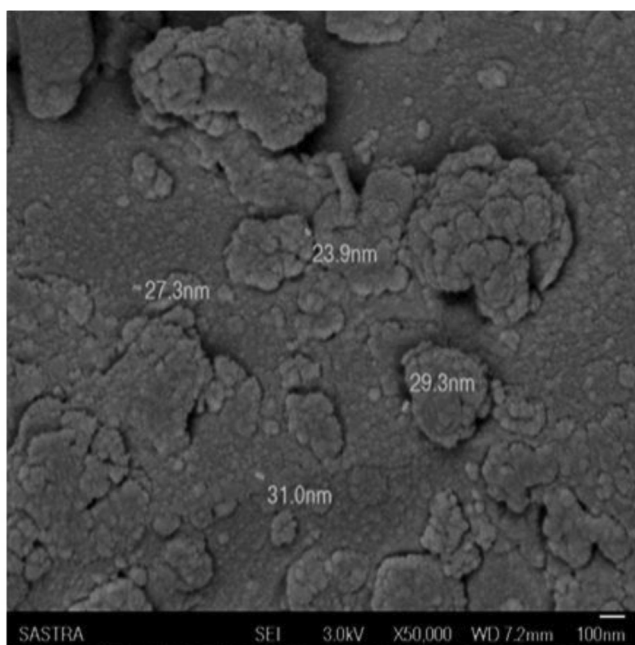


Fig. 9. SEM image of AgNPs.

geres, *Culex*, *Ochlerotatus*, *Orthopodomyia* and *Uranotaenia* respectively. The Genus-wise diversity of HVMs larvae were evaluated by Shannon Wiener Index: H/Variance H/Simpsons Index values were 1.3645/0.0001/3.8514, 1.3259/0.0002/3.5707, 1.2945/0.0031/3.5260, 1.3253/0.0002/3.5912, 1.3424/0.0038/4.0071, 1.3512/0.0041/4.1038 and 1.3579/0.0008/3.9111 observed against *Aedes*, *Anopheles*, *Armigeres*, *Culex*, *Ochlerotatus*, *Orthopodomyia* and *Uranotaenia* respectively. The data were calculated into One-way ANOVA; degrees of freedom = 6; F value = 7.722;  $p < 0.05$  (Tables 2 and 3). The present study demonstrated that the Month-wise abundance and diversity of HVMs at KWS gradually build up during the rainy season, which reached as the maximum in December month followed by January as well as highly declined in April 2019. The percentage of HVMs and related VBDs were preferably increased during the selected months and water physicochemical

factors may also play a critical role at larval stages of HVMs habitats (Rajesh et al., 2015; Vijayakumar et al., 2014; Senthamarai Selvan et al., 2015 and 2016). Moreover, Genus-wise abundance and diversity of HVMs at KWS, among the collected genera the *Culex* were recorded predominantly, followed by *Anopheles* and *Aedes* in fresh water habitat. The similar trends of Genus-wise abundance and diversity of HVMs investigation were previously estimated (Suganthi et al., 2014; Makeesh Kumar et al., 2015).

### 3.3. Physico-chemical characteristics

The physicochemical parameters are represented in table 4. Totally twenty different physicochemical water parameters were assessed in study periods in different HVMs breeding habitats of KWS. One-way ANOVA was employed to calculate physicochemical parameters data, F values are shown in the table 4 and P values are statistically significant at 0.05%. Similarly, the F values of physicochemical factors were 1651.6, 2102.4, 2665.9, 1502.5, 692.6, 548.0, 936.1, 1312.9, 2996.6, 2303.5, 1625.7, 77.5, 482.5, 7160.3, 2445.1, 36.6, 1215.1, 1844.8, 1853.0 and 1864.8 observed against ammonia, calcium, chloride, dissolved O<sub>2</sub>, conductivity, fluoride, humidity, iron, magnesium, nitrate, nitrite, pH, phosphate, rainfall, sulphate, temperature, total hardness, total dissolved solids, total salinity and turbidity (Table 4). The physicochemical characteristics are correlated with HVMs breeding habitats; the R values have mentioned two different stars (single star and double stars). \*correlation is significant at the 0.05 level (2-tailed) and \*\*correlation is significant at the 0.01 level (2-tailed). Many of the physicochemical factors had negatively significant and some factors had positively significant to HVMs larval development in different breeding habitats. Seventeen physicochemical factors were negatively significant and its values were  $-0.270^{**}$ ,  $-0.258^{**}$ ,  $-0.264^{**}$ ,  $-0.251^{**}$ ,  $-0.238^{**}$ ,  $-0.090^{**}$ ,  $-0.263^{**}$ ,  $-0.275^{**}$ ,  $-0.269^{**}$ ,  $-0.264^{**}$ ,  $-0.246^{**}$ ,  $-0.278^{**}$ ,  $-0.116^{*}$ ,  $-0.261^{**}$ ,  $-0.261^{**}$ ,  $-0.270^{**}$  and  $-0.268^{**}$  against ammonia, calcium, chloride, conductivity, fluoride, humidity, iron, magnesium, nitrate, nitrite, phosphate, sulphate, temperature, total hardness, total dissolved solids, total salinity and turbidity. Three physicochemical factors were positively significant and its values were  $0.270^{**}$ ,  $0.093^{**}$  and  $0.294^{**}$  against dissolved O<sub>2</sub>, pH and rainfall. Its P values of larval abundance are represented in table 4. The



**Table 7**  
Larvicidal activity of AgNPs against various vector mosquitoes.

| S.No. | Mosquito species                | LC <sub>50</sub> (µg/ml) | 95% Fiducial Limit |       | LC <sub>90</sub> (µg/ml) | 95% Fiducial Limit |       | Slope | χ <sup>2</sup> |
|-------|---------------------------------|--------------------------|--------------------|-------|--------------------------|--------------------|-------|-------|----------------|
|       |                                 |                          | (µg/ml)            |       |                          | (µg/ml)            |       |       |                |
|       |                                 |                          | LCL                | UCL   |                          | LCL                | UCL   |       |                |
| 1.    | <i>Ae. aegypti</i>              | 29.34                    | 20.44              | 34.24 | 47.54                    | 41.43              | 51.52 | 4.233 | 8.301          |
| 2.    | <i>Ae. albopictus</i>           | 28.75                    | 20.68              | 33.25 | 47.30                    | 41.39              | 51.49 | 4.567 | 6.423          |
| 3.    | <i>Ae. pseudoalbopictus</i>     | 24.57                    | 18.49              | 30.17 | 44.75                    | 37.98              | 47.36 | 4.256 | 7.619          |
| 4.    | <i>Ae. pseudotoemiatu</i>       | 30.57                    | 21.76              | 34.78 | 47.95                    | 42.23              | 52.32 | 4.802 | 8.975          |
| 5.    | <i>Ae. stegomyia annandalei</i> | 23.73                    | 18.89              | 27.62 | 42.93                    | 36.75              | 47.65 | 3.931 | 6.602          |
| 6.    | <i>Ae. vittatus</i>             | 23.45                    | 18.82              | 27.73 | 42.49                    | 36.66              | 47.53 | 3.813 | 6.021          |
| 7.    | <i>An. culiciformis</i>         | 23.88                    | 18.90              | 27.79 | 43.10                    | 36.85              | 47.73 | 3.944 | 6.612          |
| 8.    | <i>An. jeyporiensis</i>         | 31.85                    | 22.72              | 35.65 | 48.58                    | 42.89              | 52.79 | 4.843 | 8.644          |
| 9.    | <i>An. maculatus</i>            | 24.34                    | 18.93              | 29.82 | 43.84                    | 36.92              | 47.87 | 3.602 | 6.332          |
| 10.   | <i>An. mirans</i>               | 30.54                    | 22.64              | 35.29 | 47.81                    | 41.71              | 51.87 | 4.253 | 8.356          |
| 11.   | <i>An. stephensi</i>            | 24.44                    | 18.93              | 29.82 | 44.10                    | 37.42              | 48.14 | 3.602 | 7.332          |
| 12.   | <i>An. subpictus</i>            | 18.17                    | 15.34              | 23.98 | 39.56                    | 33.28              | 44.91 | 3.722 | 6.816          |
| 13.   | <i>Ar. subalbatus</i>           | 28.63                    | 19.73              | 33.19 | 46.65                    | 40.92              | 50.96 | 4.252 | 6.542          |
| 14.   | <i>Cx. culex mimeticus</i>      | 24.78                    | 18.68              | 30.40 | 44.89                    | 38.24              | 48.47 | 4.258 | 7.632          |
| 15.   | <i>Cx. epidemus</i>             | 31.23                    | 22.25              | 35.45 | 48.45                    | 42.47              | 52.45 | 4.408 | 8.532          |
| 16.   | <i>Cx. mimuloides</i>           | 24.52                    | 17.46              | 30.72 | 44.38                    | 37.61              | 48.27 | 4.365 | 7.375          |
| 17.   | <i>Cx. mimulus</i>              | 25.79                    | 18.28              | 31.91 | 44.85                    | 38.74              | 49.17 | 4.129 | 7.392          |
| 18.   | <i>Cx. minutissimus</i>         | 27.45                    | 18.28              | 31.91 | 45.94                    | 40.44              | 50.57 | 4.144 | 8.392          |
| 19.   | <i>Cx. nigropunctatus</i>       | 26.11                    | 19.95              | 29.52 | 45.12                    | 39.13              | 49.38 | 3.853 | 7.565          |
| 20.   | <i>Cx. nilgircus</i>            | 26.34                    | 19.13              | 30.22 | 45.22                    | 39.47              | 49.57 | 3.325 | 7.602          |
| 21.   | <i>Cx. pallidothorax</i>        | 26.53                    | 18.49              | 30.17 | 45.75                    | 39.88              | 49.86 | 4.255 | 7.619          |
| 22.   | <i>Cx. pseudovishnui</i>        | 26.89                    | 18.62              | 30.22 | 45.82                    | 39.93              | 49.95 | 4.452 | 6.478          |
| 23.   | <i>Cx. quinquefasciatus</i>     | 27.79                    | 18.54              | 32.32 | 45.99                    | 40.62              | 50.82 | 4.129 | 8.542          |
| 24.   | <i>Cx. vishnui</i>              | 21.82                    | 16.57              | 25.85 | 40.43                    | 34.19              | 46.32 | 3.908 | 6.372          |
| 25.   | <i>Oc. grenii</i>               | 24.36                    | 17.45              | 30.72 | 43.98                    | 37.21              | 47.92 | 4.365 | 6.375          |
| 26.   | <i>Or. flavithorax</i>          | 29.66                    | 21.20              | 34.39 | 47.72                    | 41.64              | 51.80 | 4.498 | 8.532          |
| 27.   | <i>Ur. annandalei</i>           | 25.88                    | 19.81              | 32.70 | 45.08                    | 38.86              | 49.26 | 3.542 | 6.480          |
| 28.   | <i>Ur. campestris</i>           | 25.63                    | 19.65              | 32.44 | 44.92                    | 38.65              | 48.56 | 3.720 | 6.701          |
| 29.   | <i>Ur. novobscura</i>           | 28.32                    | 18.72              | 32.48 | 46.32                    | 40.88              | 50.87 | 4.144 | 8.392          |

LC<sub>50</sub>: 50% larval lethality occurring concentration.

LC<sub>90</sub>: 90% larval lethality occurring concentration.

µg/mL: Micrograms per millilitre.

χ<sup>2</sup>: Chi-square.

maximum numbers of HVMs populations were abundance in December which was based on the higher suitability of pH, temperature, water resources, Turbidity, Dissolved O<sub>2</sub>, humidity, etc. This finding elucidates that the water pH<sup>1</sup> 7, optimum temperature 25–30 °C and other physicochemical parameters were given more supports for a HVMs development for their breeding habitat. The water physicochemical and whether variability can be highly influenced in HVMs population (Suganthi et al., 2014; Ahmad et al., 2011; Egbuche et al., 2016). Nevertheless, after the rainy season, natural and artificial containers are the major substrates of supporting agents for the proliferation of HVMs. Especially, genes *Culex*, *Anopheles* and *Aedes* are dominated and widely distributed in selected sites. The finding of the present study compares with previous research observations that the reported a higher number of HVMs were obtained maximum at optimum physicochemical parameters which are more supported to HVMs (Sinka et al., 2011; Kaushal et al., 2014; Jerome et al., 2017).

### 3.4. Larval density and container index of mosquitoes

The different breeding habitats such as rocky pool, stream slow-flowing, water storage tank, tree hole, bamboo stump, stagnant pools, animal footprint, leaf axil, flower bracts and spring pool were observed in the different stations of the KWS based on the availability and non-availability of the breeding habitats. Totally 10 different types of 942 breeding sources were searched for HVMs larvae survey among them 359 were identified as HVMs breeding places within that 1800 larvae were collected. The high number of larval density and container index were recorded in the water storage tank during December- 2018 to March- 2019 followed by bamboo stump had very least larval density 1140.47 and least con-

tainer index were examined in the leaf axil 69.63 (Table 5). The different species of HVMs breeding habitats were searched. A total of 942 (10 different types of habitats) breeding containers were searched, in which 359 were positive containers and collected 1800 larvae. The similar types of reports were observed in a previous investigation (Getachew et al., 2015; Senthamarai Selvan and Jebanesan, 2016; Getachew et al., 2018).

### 3.5. Phytochemical screening

The phytochemical screening of *B. mollis* various extracts were evaluated. The presence of important phyto groups were noticed from *Bm*-LAE (Table 6).

### 3.6. TLC, CC and NMR analysis

The bio-effectiveness of *B. mollis* extracts were subjected to by TLC (Fig. 3A) and CC (Fig. 3B, C) analysis, the maximum of five fractions gathered from *Bm*-LAE. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral analysis of *Bm*-LAE was performed and its spectral peaks are displayed in the Fig. 4A, B. The following PCs identified from *Bm*-LAE: Atalantin, terpene; Atalaphylline; 6-Methoxy-3,3,12-trimethyl-3,1 2-dihydro-7H-pyrano[2,3-c]acridine-7-thione; Lunacrine, tryportant; Methylation; Noracronycine; Limonoid (Tetranortriterpenoid); Coumarins; Cycloepitalantin; Limonin; Dehydroatantantin; Sesquiterpene; Rutaevin.

### 3.7. AgNP synthesis and its characterization analysis

Silver ions significantly reduced to metallic nanosilver when the *Bm*-LAE was added to the solution. According to scientific reports,

AgNPs solution has turned from light yellowish to brown and change into dark brown after the reaction (Fig. 5A–C) and indicates the sign of AgNPs formation, which is strong agreement with earlier published works (Ramesh Kumar et al., 2014; Priya et al., 2014). The establishment of AgNPs was observed upon the colour change and UV–vis spectrum and it is shown in Fig. 6 (Panchawat and Sisodia, 2010; Jain et al., 2011). FTIR spectroscopy (Fig. 7) elucidate the identity of the biomolecules responsible for the reduction and efficient stabilization of the AgNPs (Prasad and Elumalai, 2011; Mukunthan et al., 2011; Esan et al., 2020). The crystalline nature of the AgNPs was confirmed by XRD analysis (Fig. 8). The morphological characterization of synthesized AgNPs size and shape are shown in Fig. 9. SEM analyses clearly showed the particle size ranged from 23 to 31 nm which magnified at 50000X. The particles appeared to be beads shaped it belongs to the category of NPs (Morejón et al., 2018; Anandan et al., 2019; Pilaquinga et al., 2019).

### 3.8. Larval toxicity of Bm-LAE of AgNPs

The Larval toxicity of Bm-AgNPs tested against the 3rd instar larvae of various HVMS and shown in Table 7. Bm-AgNPs provided maximum toxic effects its LC<sub>50</sub>/LC<sub>90</sub> values of 18.17/39.56, 23.45/42.49 and 21.82/40.43 µg/mL were observed on *An. subpictus* Cx. *vishnui* and *Ae. vittatus* respectively. Moreover, very least LC<sub>50</sub>/LC<sub>90</sub> values of 31.85/48.58 µg/mL were noticed on *An. jeyporiensis*. The various similar explanations were recorded in earlier reports (Ramesh Kumar et al., 2014; Velayutham et al., 2016; Govindarajan et al., 2016d; Govindarajan and Benelli, 2017).

## 4. Conclusion

Various anthropogenic activities and lack of public awareness about HVMS/VBDs, etc., have highly responsible for increased the population of HVMS much beyond their natural levels. As well as the nomadic communities every year migrates with farm animals and there is high level of interaction between humans, wildlife, livestock and HVMS vectors in the migration routes. Nowadays, environmental safety is the paramount significance, present study green synthesis AgNPs are considered as target specific, eco-friendly, inexpensive, highly biodegradable, readily available and effective alternate insecticidal agents to the synthetic chemical pesticides. India possesses vast plants diversity and is used in traditional medicines. Most surprisingly, despite the *B. mollis* being a miraculous plant, very few studies have been done. There is an urgent requirement for intensive studies on this plant to exploit it for the control of medical pests for human health importance across the world.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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