Larvicidal activity of acetone extract and green synthesized silver nanoparticles from Allium sativum L.(Amaryllidaceae) against the dengue vector Aedes aegypti L.(Diptera: Culicidae)

by Nasir, S., Walters, K.F., Pereira, R.M., Waris, M., Chatha, A.A., Hayat, M. and Batool, M.

Nasir, S., Walters, K.F., Pereira, R.M., Waris, M., Chatha, A.A., Hayat, M. and Batool, M. (2022) 'Larvicidal activity of acetone extract and green synthesized silver nanoparticles from Allium sativum L.(Amaryllidaceae) against the dengue vector Aedes aegypti L.(Diptera: Culicidae)', *Journal of Asia-Pacific Entomology*, 25 (3).

Journal of Asia-Pacific Entomology 25 (2022) 101937

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Full length article

Larvicidal activity of acetone extract and green synthesized silver nanoparticles from *Allium sativum* L. (Amaryllidaceae) against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae)

Shabab Nasir [a], Keith F.A. Walters [b,*], Roberto M. Pereira [c], Muhammad Waris [a], Awais Ali Chatha [a], Munawar Hayat [a], Marriam Batool [a]

[a] Department of Zoology, Government College University, Faisalabad, Pakistan
[b] Centre for Integrated Pest Management, Harper Adams University, Newport, Shropshire TF10 8NB, UK
[c] Department of Entomology and Nematology, University of Florida, USA

ARTICLE INFO

Keywords:
Dengue
Aedes aegypti
Larvicide
Allium sativum
Mosquito larvae
Silver nanoparticles

ABSTRACT

Mosquito vectors of major human diseases are currently controlled using chemical and biological products. Extensive insecticide use has led to resistance development and human/environmental health risks, and alternative sustainable control options are needed; in this study, activity of an extract of garlic (*Allium sativum*; Amaryllidaceae), and silver nanoparticles (AgNPs) synthesized from the extract, were evaluated against 2nd and 3rd instar larvae of the yellow fever mosquito, *Ae. aegypti* (Diptera**:** Culicidae). Synthesis of AgNPs was confirmed using UV–Vis spectroscopy, and characterised using powdered X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy. Larvae were exposed to five concentrations (50, 100, 150, 200, 250 ppm) of garlic extract or synthesized AgNPs, with distilled water and silver nitrate solution (1 mM) as controls. The mortality of larvae was recorded after 6, 12, 24, 36, and 48 h following addition of the respective extracts.

Dose- and time-dependent toxicity were recorded in both treatment groups with no mortality in control groups. Exposure to AgNPs at 250 ppm for 48 h yielded 100% mortality for both larval instars, with corresponding LC_{50} values of 44.77 (2nd) and 62.82 ppm (3rd). Exposure to garlic extract resulted in similar 48-hour mortality (99 ± 0.77% (2nd) and 98 ± 1.10% (3rd), but consistently higher LC_{50} values after all exposure times compared to AgNPs (e.g. 48-hour exposure: 108.42 ppm (2nd), 129.11 ppm (3rd), suggesting that AgNPs may potentially be used at lower concentrations for *Ae. aegypti* control.

Introduction

Malaria, dengue fever, Zika virus, yellow fever and Chikungunya are transmitted among vertebrates (including humans) by mosquitoes, causing significant concern for public health (Becker et al., 2010). It is estimated that 390 million dengue virus infections occur per year with an 8 fold increase in reported cases over the last two decades, and 3.9 billion people at risk of infection (Brady et al., 2012; Bhatt et al., 2013; WHO, 2022). Although this risk extends across 129 countries, 70% occurs in Asia (Bhatt et al., 2013; Brady et al., 2012). Population growth, climate change, deforestation, habitat change, extensive use of insecticides, increased activities such as trade, migration and travel by humans have all contributed to spread of these vector-borne diseases (Jones et al., 2008). *Aedes aegypti* (Diptera: Culicidae) is one of the main vectors, affecting the lives of hundreds of millions of people every year (Bhatt et al., 2013).

Aedes aegypti is an anthropophilic species which thrives in urban areas and can spread rapidly in the tropical and subtropical regions of the world (Akram et al., 2009). Horizontal transmission (i.e. transmitted by person-person infection) is a major cause of disease spread although in some cases, vertical transmission from mothers to offspring in the womb can also occur (Sardar et al., 2015; Ferreira-de-Lima and Lima-Camara, 2018). Due to the lack of effective vaccines against the viral diseases vectored by *Ae. aegypti*, control of transmission relies heavily on

Abbreviations: AgNPs, Silver nanoparticles; LC_{50}, Lethal Concentration; ppm, parts per million.

* Corresponding author.
E-mail address: kwalters@harper-adams.ac.uk (K.F.A. Walters).

https://doi.org/10.1016/j.aspen.2022.101937
Received 16 December 2021; Received in revised form 19 May 2022; Accepted 25 May 2022
Available online 27 May 2022

the management of mosquito populations (Pattnaik et al., 2020). Female mosquitoes lay eggs along the edges of bodies of fresh water and after hatching, the larvae and pupae develop in an aquatic environment, emerging as free flying adults. The limited mobility of the aquatic immature stages results in larvicides being important tools for the control of mosquito populations, including synthetic insecticides such as temephos (an organophosphorus larvicide often used to treat aquatic stages of disease vectors), growth inhibitors, biological control programmes utilizing *Bacillus thuringiensis* subspecies *israelensis* (Bti) or plant extracts (Tiwary et al., 2007). However, sustained, widespread and intensive use of insecticides may cause both resistance in the vector population and environmental pollution (Mathivanan et al., 2000), and alternative agents for mosquito control are urgently required.

Plant extracts have been investigated as natural larvicidal components for bioinsecticides. Several plant species contain compounds such as saponins, isoflavonoids, tannins, terpenes, steroids, etc., which can act as ovicides, larvicides and adulticides (Rahuman et al., 2009). As these secondary phytochemicals offer a range of modes of action, plant extracts can contribute to integrated management strategies that reduce the potential for resistance development in target populations. As exposure to some plant-derived bioactive chemicals results in high larval mortality in several mosquito species, therefore, plants have been studied as a source of potent natural, biodegradable, potentially more ecologically acceptable and specific larvicidal agents (Pavela, 2016; Waris et al., 2020).

Silver nanoparticles (AgNPs) synthesized from extracts of various plants represent a promising alternative to the direct use of plant-derived bioactive chemicals as larvicides (Athanassiou et al., 2018). Green nanoparticles can be synthesized using low cost, bio-compatible methods, and plant material is suitable for the synthesis of AgNPs as it can contain secondary metabolites that act as reducing, capping and stabilizing agents (Benelli et al., 2017) leading to associated reduced reaction times (Shaalan et al., 2005). For example, phytochemical analysis has confirmed that garlic (*Allium sativum*) is rich in secondary metabolites such as alkanes, aromatics, phenols, ethers, amines and amides, which are known to be involved in the reduction and capping of silver and copper ions (Benelli et al., 2017; Mikaili et al., 2013). In addition garlic extract assisted AgNPs have been shown to display significant insecticidal activity together with positive antibacterial, anti-biofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity properties (Vijayakumar et al., 2019).

Phyto-synthesized AgNPs have the ability to penetrate through the exoskeleton into the mosquito's cells causing mortality after binding to proteins or DNA. They also cause mutation in DNA and deformation of enzymes but with limited effect on non-target species such as beneficial arthropods and fish (Subramaniam et al., 2015). Silver nanoparticles exhibit high efficacy because of favourable surface area to volume ratio due to their minute size (1–100 nm) and are effective even at very low concentration (Benelli et al., 2017).

In recent years, Pakistan and other Asian countries have experienced multiple arbovirus outbreaks resulting from unsatisfactory sanitary conditions and mosquito-friendly climate change with shorter winters and longer summers, leading to increasing vector populations. Due to the warmer climate, many arboviral diseases such as chikungunya, dengue and malaria, have reached pandemic levels, with the rapidly increasing cases of chikungunya and dengue reported in Pakistan causing significant concern (Rauf et al., 2017). Recent research into larval control of *Ae. aegypti* with green AgNPs has demonstrated a high level of efficacy, and created new avenues of investigation into improved, more sustainable pest control (Waris et al., 2020). This study was conducted to evaluate the larvicidal potential of a common medicinal plant extract (*A. sativum*) and its synthesized AgNPs against 2nd and 3rd larval stages of *Ae. aegypti*.

Materials and methods

Preparation of A. sativum (Garlic) acetone extract

Fresh, healthy *A. sativum* bulbs were collected from the campus of Government College University, Faisalabad (GCUF) and their identification confirmed by Prof. Dr. Naeem Iqbal. Cloves from the bulbs were carefully separated and washed with distilled water before being surface dried with tissue paper, cut into fine slices and maintained away from direct sunlight on a plastic tray in the laboratory (25–35 °C) for 23 days (Kalu et al., 2010). The air dried slices were then ground into a fine powder using an electric grinder (Anex, Germany). A bulb extract was made using a slightly modified standard simplex centroid experimental design method (Satyavani et al. 2011). Fifty grams of garlic powder and analytical grade acetone (250 ml) were added to the inner tube of a Soxhlet apparatus (J.P. Selecta, S.A.; 75 cm (L) × 25 cm (W) × 90 cm (H); volume used 500 ml), and heated gently at 55.5–56.5 °C for 8 h (16 cycles). The garlic extract was kept overnight in an incubator at 40 °C to allow the remaining acetone to evaporate, then stored at 4 °C for further use (Vogel, 1978).

Synthesis of A. sativum (garlic) silver nanoparticles

A solution of silver nitrate (1 mM; Sigma Aldrich, USA) was prepared in an Erlenmeyer flask in the dark. *A. sativum* plant extract in acetone (10 ml) was added to 90 ml of the silver nitrate solution, followed by 2–3 drops of 1% NaOH (to adjust the pH to 8), while the solution was mixed using a magnetic stirrer for one week. A change to a yellow–brown color indicates the formation of AgNPs. The solution was cooled to room temperature and then centrifuged three times at 5000 rpm for 20 min to obtain nanoparticle pellets. Purified suspension was prepared by dissolving the nanoparticle pellets in double distilled water and was frozen at – 4 °C for further use (Satyavani et al., 2011).

Characterization of A. sativum (garlic) silver nanoparticles

The characteristics of green synthesized AgNPs. were investigated at the High-Tech Central Laboratory, GCUF.

UV–Vis spectroscopy

The surface plasmon resonance (SPR) band of the green AgNPs synthesized from the extract of *A. sativum bulbs* was investigated using a Cary 60 double-beam spectrophotometer (Spectramax, M3 Molecular Devices; Agilent Technologies, USA) with 1-nm resolution and the measurement range from 200 to 800 nm. Dilutions (10x) were prepared with distilled water from colloidal solutions obtained from the synthesis process (Raut et al., 2009). Samples (1 ml) of the suspension were collected periodically to monitor the completion of bio reduction of silver in aqueous solution.

Fourier transform infrared spectroscopy (FTIR)

Infrared spectroscopy analyses were performed on a Spectrum BX FT-IR System (PerkinElmer, USA) with ATR Miracle Pike coupling at 4 cm^{-1} resolution and conditions ranging from 500 to 4000 cm^{-1}. Fresh purified sample (1–2 ml in aqueous form) was used for FTIR analysis. As the sample was in paste form, both the extract and the synthesized nanoparticles were dried in an oven at 60 °C for 24 h to obtain the dry powder required for FTIR (Vivek et al., 2011).

Powdered X-ray diffraction (XRD)

Synthesized AgNPs in suspension were centrifuged at 10,000 rpm at 4 °C for ten minutes to obtain a pellet of pure nanoparticles. For XRD analysis, a 1 g sample of the AgNPs was loaded in the sample holder of

the instrument, which was then run using a 40 kV voltage and 30 mA current with CuK α radiation to determine the structure and size of the particles (Shankar et al., 2004). Both AgNP characteristics were calculated through diffracted intensities. The scanning was completed within the range of 0°–80° 2θ in Copper:Potassium (CuK) α radiation with time constant 2 sec by using a Rigaku, Ultima IV, and X-ray diffractometer system (Rigaku Corporation, USA).

Scanning electron microscopy (SEM)

Scanning electron microscopy was performed using a Phenom ProX scanning electron microscope (Thermo Fisher Scientific) equipped with an EDX-detector operating at 10 kV and Prosuite-EDS software, and carbon pin. Thin films of the AgNPs were prepared on a carbon-coated copper grid by dropping a small amount of the sample onto the grid then removing excess solution using absorbent paper, before allowing them to dry under a mercury lamp for 5 min (Santoro et al., 2017). The grain size was then calculated from the SEM image using image J-software (Collins, 2007; Schneider et al., 2012).

Mosquito collection and rearing

Aedes aegypti larvae and pupae were collected from indoor breeding sites in Faisalabad, Punjab, Pakistan (31° 25′ 7.3740′’ N; 73° 4′ 44.7924′’ E; 192 m above sea level) using a mosquito dipper (BioQuip, USA), and identified to species (Qasim et al., 2014).

For laboratory stock cultures, larvae and pupae were cultured at water temperatures of 27 ± 2℃ (Ahmed et al., 2016), in batches of 300 in stainless steel trays (35x30x5 cm). Two drops of 0.02% yeast suspension were provided daily to each batch when the first two instars were present, and ground fish feed was fed to later instars (Arivoli et al., 2011). Larval instars present in each tray were determined by time after egg hatch, and verified using morphological characteristics from a subsample of 30 larvae (Bar and Andrew, 2013). Adults were reared in glass cages (60x60x60 cm) with ad libitum access to both sucrose solution (10%) and the blood of white rats (for egg development).

Bioassay

The larvicidal activity of either the *A. sativum* extract, or green AgNPs synthesized from the extract, each diluted to five different concentrations, 50, 100, 150, 200 and 250 ppm in distilled water, was evaluated.. There were two controls in which larvae were released into either distilled water or silver nitrate solution (1 mM).

Glass beakers, each containing 200 ml of one of the five *A. sativum* extract concentrations (treatments) described above, or one of the controls, were maintained at 27 ± 2℃ throughout the experiment. Twenty actively swimming 2nd instar larvae of *Ae. aegypti* were released into each beaker and sequential assessments were made to record mortality, the first after 6 h, then repeated at 12, 24, 36 and 48 h after the start of the experiment. The WHO (2005) bioassay protocol was used to calculate mortality rates of larvae. Each treatment and both controls were replicated five times. A similar procedure was used to assess mortality following exposure of 3rd instar larvae.

The experimental approach was also used to test similar concentrations of green AgNPs synthesized from the garlic extract, with both assays running simultaneously.

Statistical analysis

Statistical analysis was conducted using Minitab-17 statistical software (Minitab LLC, USA). The percentage mortality data was subjected to Probit analysis before calculating lethal concentrations (LC_{50}) of the larvae, and dose- and time-dependent survival curves from the regression lines, following the standard method described by WHO (2005).

Results

UV–Vis spectroscopy of A. sativum (garlic) AgNPs

The SPR band of green silver nanoparticles (AgNPs) synthesized from the extract of *A. sativum* bulbs showed maximum absorbance at 430 nm which confirmed synthesis of AgNPs due to reduction of Ag ions by the phytochemicals of *A. sativum* (Fig. 1).

FTIR spectroscopy

FTIR spectroscopy was used to verify the coating of the plant extract on synthesized AgNPs. In the FTIR spectrum of *A. sativum* bulbs the 3375 cm^{-1} wavelength corresponds to the stretching of the hydroxyl group O–H, the 2933 cm^{-1} to the stretching of the C–H bonds, and the band at 1642 cm^{-1} is assigned to carbonyls of different functional groups. Similarly, the 1107 cm^{-1} band showed the stretching and vibrations of the primary amines C-N (Fig. 2).

Powdered X-ray diffraction

The dry powder XRD pattern of the AgNPs synthesized from the *A. sativum* bulb extract is shown in (Fig. 3). Four peaks at 2θ values of (38°, 44.5°, 65°, 77°) corresponding to 110, 200, 221 and 310 planes of silver were observed for this plant.

SEM analysis

The SEM image shows the morphological characteristics including size, shape and surface of the AgNPs synthesized from the extract of *A. sativum* bulbs (Fig. 4a). The particles were roughly spherical in shape with a mean diameter of 30 nm size, and ranging from 22.3 nm to 34.4 nm.

Collating the results of the work characterizing the *A. sativum* silver nanoparticles, the predicted structure is illustrated in Fig. 4b.

Larvicidal activity of A. sativum bulb extracts and synthesised AgNPs

Both the acetone bulb extract and the *A. sativum* synthesized AgNPs showed a dose- and time-dependent toxic effect against the 2nd and 3rd instar larvae of *Ae. aegypti* (Figs. 5, 6). No mortality was recorded in the control groups.

Exposure to AgNPs synthesized from *A. sativum* at a concentration of 250 ppm for 48 h was followed by 100% mortality, irrespective of the larval instar tested. However, the corresponding LC_{50} values for different time intervals (6, 12, 24, 36 and 48 h) for 2nd instar larvae were 222.02, 175.57, 115.03, 69.12 and 44.77 ppm, with higher values

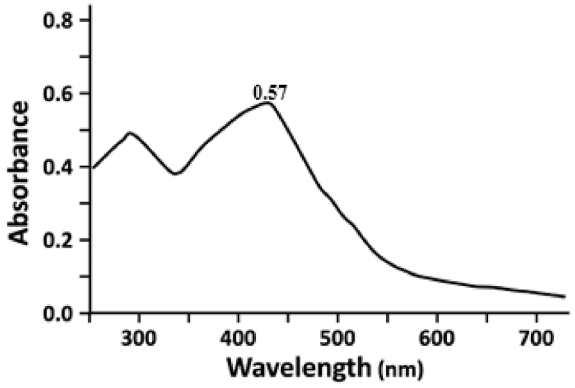

Fig. 1. UV–Vis spectrum of AgNPs synthesized using *Allium sativum* bulb extract (AgNPs 1 mM 1:10 v/v deionized water). 0.57 = maximum absorbance (at 430 nm).

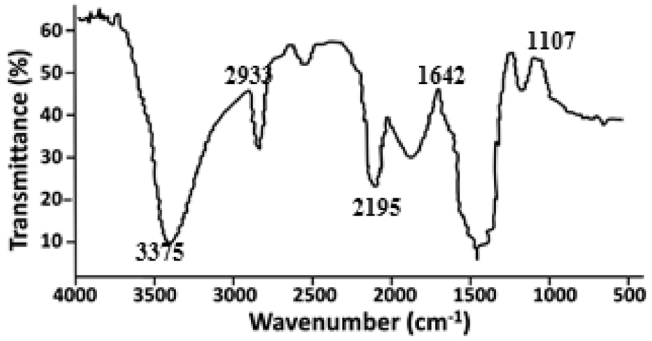

Fig. 2. Fourier transform infrared spectrum of AgNPs synthesized using *Allium sativum* bulb extract.

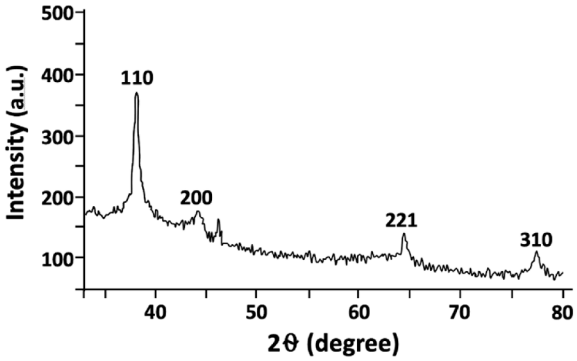

Fig. 3. Powdered X-ray diffraction pattern of AgNPs synthesized from *Allium sativum* bulb extract.

recorded for the 3rd instar larvae of 258.70, 208.50, 120.75, 91.88 and 62.82 ppm respectively.

Exposure to garlic bulb extract resulted in consistently higher LC_{50} values than were recorded for its synthesized AgNPs after all exposure times, suggesting that AgNPs may have the potential to be used at lower concentrations for *Ae. aegypti* control than the plant extract itself (Figs. 5, 6). After each exposure time investigated, an LC_{50} of 365.83, 289.74, 228.74, 196.45 and 108.42 ppm were recorded for 2nd instar larvae, much higher than the equivalent values for AgNPs. Similarly, equivalent values for 3rd instar larvae (415.08, 339.46, 262.42, 224.14 and 129.11) were also higher in tests using the plant extract. However, as mortality in both instars were dose- and time- dependent, after 48 h exposure to a concentration of 250 ppm, garlic bulb extract resulted in 99% mortality of the 2nd instar larvae (similar to AgNPs), and 98% for the 3rd instar larvae (only slightly lower than recorded with the nanoparticles).

Discussion

Synthesis of green AgNPs offers advantages over some other pest management methods in terms of cost, their ease of production and large scale synthesis, and as a new option for integrated systems that promote more sustainable pest control (Malabadi et al., 2012). In this study, the phytochemicals found in the *A. sativum* bulb extract reduced the silver ions to form AgNPs with UV–Vis spectroscopy confirming the synthesis, thus reflecting the successful use of the protocol in earlier work utilizing a wide range of other plant species (Ahmed et al., 2016). The dry powder XRD pattern showed four peaks corresponding to four planes that can be indexed according to the facets of the face centered cubic crystal structure of silver. These findings were in line with Satyavani et al. (2011) who reported three peaks at similar 2θ values. The FTIR spectrum obtained in the current work indicated that biofabrication of different functional groups (O–H, C–H, carbonyls and C-N), which were present in the bulb extract, caused the stabilization of the green AgNPs. This illustrated the role of different functional groups in the synthesis and stabilization of the AgNPs (Priyadarshini et al., 2012; Ahmed et al., 2016; Jinu et al., 2017). The synthesized AgNPs, reflected the shape of those reported in earlier studies (Bouqellah et al., 2019; Saha and Bandyopadhyay, 2019). However, although they were within the overall size range recorded previously, a slightly lower mean size was noted.

The rapid increase in the production of nanomaterials for diverse uses (including pest management) and associated release into the environment, has resulted in a need to improve our currently limited understanding of their toxic effects on development and physiology of both non-target and target organisms. Externally, nanoparticles can affect integrity of the cuticle and pigmentation, and internally gene expression (thus lipid, carbohydrate and protein metabolism), the impairment of development and reproduction of insects, as well as inducing immune responses (Shahzad and Manzoor, 2019). In insects, nanoparticles are known to penetrate the exoskeleton (Rai et al., 2014), entering into the intracellular space, before nanomaterials bind to phosphorus from DNA or sulfur from proteins, leading to denaturation of organelles and enzymes (Benelli, 2016; Tunçsoy, 2018). The generation of reactive oxygen species (ROS) is considered one of the most harmful cellular effects induced by exposure to nanoparticles and three main mechanisms have been proposed to explain the induction of intra- and extracellular ROS in organisms (Tunçsoy, 2018). Despite these effects studies have revealed only limited impact of AgNPs on non-target species such as beneficial arthropods and fish (Subramaniam et al., 2015).

It is known, however, that several kinds of nanoparticles trigger oxidative stress in arthropod tissues, including commercial silver nanoparticles (Mao et al., 2018; Nair et al., 2011, 2012; Yasur and Usha-Rani, 2015; Dziewięcka, et al., 2016). Mao et al. (2018) have demonstrated that AgNPs trigger the accumulation of ROS dipteran tissue leading to ROS-mediated apoptosis, DNA damage, and autophagy at sublethal doses, while Ahamed et al. (2010) also demonstrated up-regulation of heat shock protein HSP70 expression by AgNPs and induced oxidative stress. DNA is also highly susceptible to oxidative

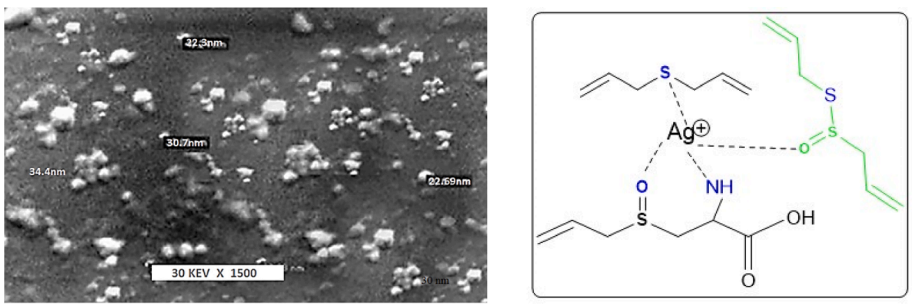

Fig. 4. (A) SEM image (x1500 magnification; voltage = 30 KEV) of single and clumped AgNPs synthesized from *Allium sativum* bulb extract, showing sizes of representative particles, and (B) their predicted structure.

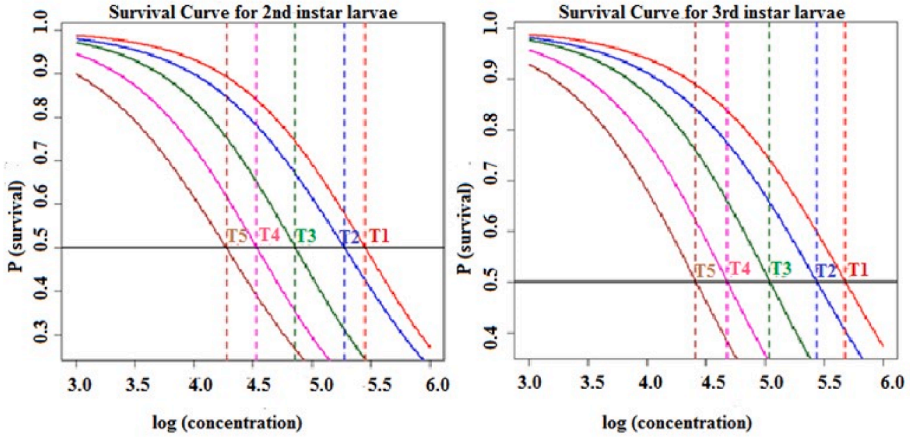

Fig. 5. Time-dependent survival curves of 2nd and 3rd instar *Ae. aegypti larvae* following exposure to five concentrations (50, 100, 150, 200 and 250 ppm in distilled water) of green synthesized AgNPs from *A. sativum* bulb extract. T1 = 6 h; T2 = 12 h; T3 = 24 h; T4 = 36 h; T5 = 48 h. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

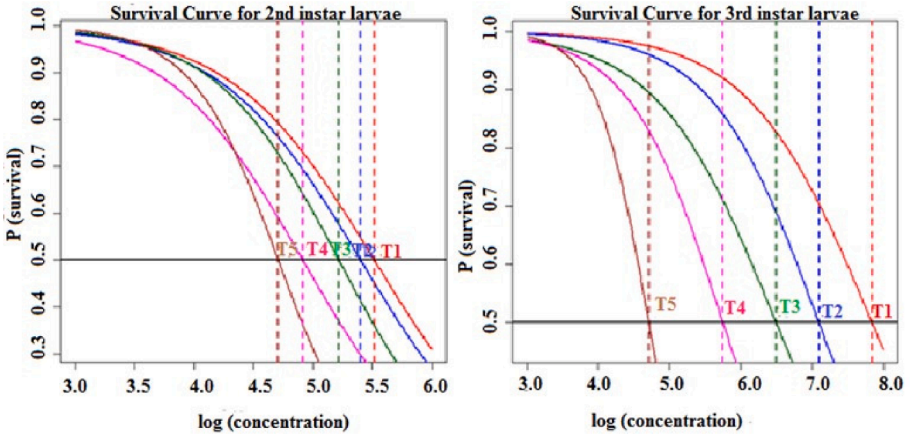

Fig. 6. Time-dependent survival curves of 2nd and 3rd instar *Ae. aegypti larvae* following exposure to five concentrations (50, 100, 150, 200 and 250 ppm in distilled water) of *A. sativum* bulb extract. T1 = 6 h; T2 = 12 h; T3 = 24 h; T4 = 36 h; T5 = 48 h.

damage and the genetic properties of nanomaterials include several mechanisms linked to DNA damage (Tunçsoy, 2018). Few studies of genotoxicity of nanoparticles in invertebrates are available, however, and published work has focused on aquatic invertebrates rather than insects (Gagné et al., 2008; Gomes et al., 2012). Nanoparticles may bind to and inhibit the activity of the enzyme acetylcholinesterase (AChE), which degrades acetylcholine (AChE), an essential neurotransmitter in the central nervous system (CNS) of insects. In common with organophosphate and carbamate pesticides, a range of metals (Regoli, 1995) are known to inhibit AChE activity, and both, AChE, and α and β carboxylesterase activities decrease in *Ae. Aegypti, Aedes albopictus* and *Culex pipiens pallens* when exposed to AgNPs (Fouad et al., 2018; Ga'al et al., 2018; Parthiban et al., 2019). In mosquitoes epithelial cell damage in the midgut of *Ae. aegypti* has also been detected following exposure to AgNPs (Kalimothu et al., 2017), and in other species AgNPs induce decreases in esterase, and phosphatase enzymes in larval *A. albapictus* (Ga'al et al., 2018).

Due to these different toxicity mechanisms, an objective of this study was to facilitate comparison of synthesized garlic AgNPs with published data evaluating the potential insecticidal activity of other candidate AgNPs (synthesized from other plant species) to support selection of the best formulations for further development work. As such two instars that were commonly tested in other studies were selected for use in the current work. Within the dose range used in this study, larvicidal efficacy increased directly with exposure time, with a corresponding decrease in LC_{50} values. A higher mortality was observed for 2nd instar larvae than 3rd instars in initial assessments, a trend also reported by Singh et al. (2016). Iqbal et al. (2018) tested seven indigenous plants including *A. sativum* and *Zingiber officinale* (Zingeraceae) for their larvicidal efficacy against *Culex quinquefasciatus* (Say), reporting a significant concentration-dependent correlation with larval mortality. Thus their study reflected the findings of the current work.

Significant insecticidal activity against mosquito larvae has been reported following exposure to extracts of several plant species. For example, our results showed more than 98% mortality of 2nd instar larvae of *Ae. aegypti* following exposure to garlic extract for 48 h, with LC_{50} of 108.42 ppm. In comparison, *Aloe vera* (Asphodelaceae) leaf extracts displayed lower toxicity against *Ae. aegypti*, with LC_{50} values for 1st to 4th fourth instars of 162.74, 201.43, 253.30 and 300.05 ppm respectively, but it was concluded that that the material may offer potential as a control agent (Subramaniam et al., 2012). Essential oils from *Allium cepa* (Amaryllidaceae) were also found to be active against 4th stage larvae of *Culex pipiens* within the same time period after initial exposure (48 h) that was tested in the current study but again lower toxicity was recorded (Habeeb et al., 2009).

More widely, larvicidal activity of plant extracts from a range of botanical families has been investigated. Green synthesized AgNPs using *Calotropis gigantea* (Apocynaceae) resulted in lower LC_{50} values for 2nd (2.25) and 3rd instar (7.30) *Ae. Aegypti* than in this study but with notably higher variation recorded (Priya et al., 2014). Evaluation of fruit

extract of *Sapindus emarginatus* (Sapindaceae) against 3rd instar larvae of *Ae. aegypti* showed larvicidal activity with LC_{50} values of 92.9 ppm (Surendran et al. (2009)). Jawale et al. (2010) found that *Cestrum nocturnum* (Solanaceae) methanol extract was a highly active larvicide against the same mosquito species, with 100% larval mortality after 24 h at concentrations of 45 µg/mL and 25 µg/mL. Conversely, generally poor larvicidal activity of hexane, ethyl acetate and methanol leaf extracts of *Ageratum houstonianum* (Asteraceae) has been reported against the third instar larvae of *Ae. aegypti, Anopheles stephensi* and *C. quinquefasciatus* (Tennyson et al., 2015). However, against *A. stephensi* more than 80% mortality was observed at higher concentrations of all three extracts, and for *Ae. aegypti* and *C. quinquefasciatus*, following exposure to high concentrations of ethyl acetate extracts.

In summary the LC_{50} values obtained following both the 24 and 48 h exposures to garlic AgNPs in this study are at the lower end (or in some cases below) the range of published values for AgNPs synthesized from other plant species. As in previous studies, the synthesized nanoparticle treatments resulted in greater *Ae. aegypti* larval mortality following shorter exposure periods and at lower concentrations than the plant extract, supporting earlier conclusions that plant synthesized nanoparticles were more potent than plant extracts themselves (Priyadarshini et al., 2012; Jinu et al., 2017)., In these respects they display significant potential as alternative control agent for disease vectors. As such they provide the basis for potentially useful additions to sustainable pest management systems for insect vectors of human diseases such as mosquitoes, warranting further investigation, including of the potential for lowering the concentrations used in field.

Conclusions

Garlic can be easily obtained or grown in many world regions, and garlic bulb extracts contain phytochemicals that can be used to synthesize AgNPs at low cost, using a simple process that historically has produced a large range of larvicidal products. Although insecticidal activity of the garlic extract alone was recorded against 2nd and 3rd instar larvae of *Ae. aegypti*, AgNPs synthesized from this extract resulted in greater mortality following shorter exposure periods and at lower concentration. These nanoparticles may be used in the production of biological nano pesticides, contributing to the development of more sustainable control strategies for populations of mosquito vectors of important diseases that seriously affect poor human populations. Following further development, the application of these products to mosquito breeding habitats may represent an affordable and practical solution to mosquito-borne diseases in many areas of the world.

Funding source

The study was supported by Government College University, Faisalabad research funds. The funding committee was not involved in study design; collection analysis and interpretation of data; writing of the report or submission of the article for publication.

Data statement

The datasets used and/or analyzed during the current study are stored at the Government College University, Faisalabad, Pakistan and are available from the corresponding author on reasonable request.

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.

Acknowledgements

We thank Prof. Dr. Naeem Iqbal (Government College University, Faisalabad, Pakistan) for providing advice on plant taxonomy.

References

Ahamed, M., Siddiqui, M.A., Akhtar, M.J., Ahmad, I., Pant, A.B., Alhadlaq, H.A., 2010. Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. Biochem. Biophys. Res. Comm. 396 (2), 578–583.

Ahmed, S., Ahmad, M., Swami, B.L., Ikram, S., 2016. A review on plant extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J. Adv. Res. 7, 17–28.

Akram, W., Hafeez, F., Ullah, U.N., Kim, Y.K., Hussain, A., Lee, J.-J., 2009. Seasonal distribution and species composition of daytime biting mosquitoes. Entomol. Res. 39 (2), 107–113.

Arivoli, S., Ravindran, K.J., Raveen, R., Tennyson, S., 2011. Larvicidal activity of botanicals against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Intern. J. Zool. Res. 2, 13–17.

Athanassiou, C.G., Kavallieratos, N.G., Benelli, G., et al., 2018. Nanoparticles for pest control: current status and future perspectives. J. Pest Sci. 91, 1–15. https://doi.org/10.1007/s10340-017-0898-0.

Bar, A., Andrew, J., 2013. Morphology and Morphometry of Aedes aegypti Larvae. Ann. Rev. Res. Biol. 3, 1–21.

Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., Kaiser, A. (Eds.), 2010. Mosquitoes and Their Control. Springer Berlin Heidelberg, Berlin, Heidelberg.

Benelli, G., 2016. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol. Res. 115 (1), 23–34.

Benelli, G., Caselli, A., Canale, A., 2017. Nanoparticles for mosquito control: challenges and constraints. J. King Saud Univ. Sci. 29 (4), 424–435.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J. M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R.W., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. Nature 496 (7446), 504–507.

Bouqellah, N.A., Mohamed, M.M., Ibrahim, Y., 2019. Synthesis of eco-friendly silver nanoparticles using *Allium* sp. And their antimicrobial potential on selected vaginal bacteria. Saudi J. Biol. Sci. 26 (7), 1789–1794.

Brady, O.J., Gething, P.W., Bhatt, S., Messina, J.P., Brownstein, J.S., Hoen, A.G., Moyes, C.L., Farlow, A.W., Scott, T.W., Hay, S.I. 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLOS Neglected Tropical Diseases, 2012. 6(8): p. e1760. https://doi.org/10.1371/journal.pntd.0001760 (accessed 3 March 2022).

Collins, T.J., 2007. ImageJ for microscopy. Biotechniques 43 (1 Suppl), 25–30. https://doi.org/10.2144/000112517 (accessed 12 March 2022).

Ferreira-de-Lima, V.H., Lima-Camara, T.N., 2018. Natural vertical transmission of dengue virus in *Aedes aegypti* and*Aedes albopictus*: a systematic review Parasites & Vectors 11, 77 (2018). doi.org/10.1186/s13071-018-2643-9 (accessed 3 March 2022).

Fouad, H., Hongjie, L.i., Hosni, D., Wei, J., Abbas, G., Ga'al, H., Jianchu, M.o., 2018. Controlling *Aedes albopictus* and *Culex pipiens pallens* using silver nanoparticles synthesized from aqueous extract of Cassia fistula fruit pulp and its mode of action. Artif. Cells Nanomed. Biotech. 46 (3), 558–567.

Ga'al, H., Fouad, H., Tian, J., Hu, Y., Abbas, G., Mo, J., 2018. Synthesis, characterization and efficacy of silver nanoparticles against *Aedes albopictus* larvae and pupae. Pestic. Biochem. Phys. 144, 49–56.

Gagné, F., Auclair, J., Turcotte, P., Fournier, M., Gagnon, C., Sauvé, S., Blaise, C., 2008. Ecotoxicity of CdTe quantum dots to freshwater mussels: Impacts on immune system, oxidative stress and genotoxicity. Aquat. Toxicol. 86 (3), 333–340.

Gomes, T., Araújo, O., Pereira, R., Almeida, A.C., Cravo, A., Bebianno, M.J., 2012. Genotoxicity of copper oxide and silver nanoparticles in the mussel Mytilus galloprovincialis. Mar. Environ. Res. 84, 51–59.

Habeeb, S.M., El-Namaky, A.H., Salama, M.A., 2009. Efficiency of *Allium cepa* and *Commiphora molmol* as a Larvicidal agent against fourth stage larvae of *C. pipiens* (Diptera: Culicidae). Am. Eurasian J. Agric. Environ. Sci. 5, 196–203.

Iqbal, J., Ishtiaq, F., Alqarni, A.S., Owayss, A.A., 2018. Evaluation of larvicidal efficacy of indigenous plant extracts against *Culex quinquefasciatus* (Say) under laboratory conditions. Turk. J. Agric. For. 42, 207–215.

Jawale, C., Kirdak, R., Dama, L., 2010. Larvicidal activity of *Cestrum nocturnum* on *Aedes aegypti*. Bangladesh J. Pharmacol. 5, 39–40.

Jinu, U., Rajakumaran, S., Senthil-Nathan, S., Geetha, N., Venkatachalam, P., 2017. Potential larvicidal activity of silver nanohybrids synthesized using leaf extracts of *Cleistanthus collinus* (Roxb.) Benth. ex Hook.f. and *Strychnosnux-vomica* L. *nux-vomica* against dengue, Chikungunya and Zika vectors. Physiol. Mol. Plant Pathol. 101, 163–171.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P., 2008. Global trends in emerging infectious diseases. Nature 451 (7181), 990–993.

Kalimuthu, K., Panneerselvam, C., Chou, C., Tseng, L.C., Murugan, K., Tsai, K.H., Alarfaj, A.A., Higuchi, A., Canale, A., Hwang, J.S., Benelli, G., 2017. Control of dengue and Zika virus vector *Aedes aegypti* using the predatory copepod *Megacyclops formosanus*: synergy with *Hedychium coronarium*-synthesized silver nanoparticles and related histological changes in targeted mosquitoes. Process. Safety and Environ. Prot. 109, 82–96.

Kalu, I.G., Ofoegbu, U., Eroegbusi, J., Nwachukwu, C.U., Ibeh, B., 2010. Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. J. Med. Plants Res. 4, 496–498.

Malabadi, R.B., Naik, S., Meti, N.T., Mulgund, G.S., Nataraja, K., Kumar, S.V., 2012. Silver nanoparticles synthesized by *in-vitro* derived plants and callus cultures of *Clitoria ternatea*; evaluation of antimicrobial activity. Res. Biotechnol. 3, 26–38.

Mao, B.H., Chen, Z.Y., Wang, Y.J., Yan, S.J., 2018. Silver nanoparticles have lethal and sublethal adverse effects on development and longevity by inducing ROS-mediated stress responses. Sci. Rep. 8 (1), 2445.

Mathivanan, T., Govindarajan, M., Elumalai, K., Krishnappa, K., Ananthan, A., 2000. Mosquito larvicidal and phytochemical properties of *Ervantania coronaria* (Family Apocynaceae). J. Vector Borne Dis. 47, 178–180.

Mikaili, P., Maadirad, S., Moloudizargari, M., Aghajanshakeri, S., Sarahroodi, S., 2013. Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds, Iran. J. Basic Med. Sci. 16, 1031–1048.

Nair, P.M.G., Choi, J., 2011. Identification, characterization and expression profiles of Chironomus riparius glutathione S-transferase (GST) genes in response to cadmium and silver nanoparticles exposure. Aquat. Toxicol. 101 (3), 550–560.

Nair, P.M.G., Choi, J., 2012. Modulation in the mRNA expression of ecdysone receptor gene in aquatic midge, Chironomus riparius upon exposure to nonylphenol and silver nanoparticles. Environ. Toxicol. Pharmacol. 33 (1), 98–106.

Parthiban, E., Ramachandran, M., Jayakumar, M., Ramanibai, R., 2019. Biocompatible green synthesized silver nanoparticles impact on insecticides resistant developing enzymes of dengue transmitted mosquito vector. SN Appl. Sci. 1. https://doi.org/10.1007/s42452-019-1311-9.

Pattnaik, A., Sahoo, B.R., Pattnaik, A.K., 2020. Current Status of Zika Virus Vaccines: Successes and Challenges. Vaccines 8, 266–285.

Pavela, R., 2016. History presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects - a review. Plant Protect. Sci. 52 (No. 4), 229–241.

Priya, S., Murugan, K., Priya, A., Dinesh, D., Panneerselvam, C., Durga Devi, G., Chandramohan, B., Mahesh Kumar, P., Barnard, D.R., Xue, R.-D., Hwang, J.-S., Nicoletti, M., Chandrasekar, R., Amsath, A., Bhagooli, R., Wei, H., 2014. Green synthesis of silver nanoparticles using *Calothropis gigantea* and their potential mosquito larvicidal property. Int. J. Pure and Appl. Zool. 2, 128–137.

Agalya Priyadarshini, K., Murugan, K., Panneerselvam, C., Ponarulselvam, S., Hwang, J.-S., Nicoletti, M., 2012. Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae) Parasitol. Res. 111 (3), 997–1006.

Qasim, M., Naeem, M., Bodlah, I., 2014. Mosquito (Diptera: Culicidae) of Murree Hills. Punjab. Pakistan. Pak J. Zoology 46, 523–529.

Rahuman, A.A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir, A.A., Elango, G., 2009. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res. 104 (6), 1365–1372.

Rai, M., Kon, K., Ingle, A., Duran, N., Galdiero, S., Galdiero, M., 2014. Broad-spectrum bioactivities of silver nanoparticles: the emerging trends and future prospects. Appl. Microbiol. & Biotech. 98 (5), 1951–1961.

Rauf, M., Fatima-tuz-Zahra, S., Manzur, S., Mehmood, A., Bhatti, S., 2017. Outbreak of chikungunya in Pakistan. Lancet Infect. Dis. 17, 258.

Raut, R.W., Lakkakula, J.R., Kolekar, N.S., Mendhulkar, V.D., Kashid, S.B., 2009. Phyto synthesis of silver nanoparticles using *Gliricidia sepium* (Jaeq). Current Nanosci. 5, 117–122.

Regoli, F., Principato, G., 1995. Glutathione, glutathione–dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers. Aquat. Toxicol. 31 (2), 143–164.

Saha, M., Bandyopadhyay, P.K., 2019. Green biosynthesis of silver nanoparticle using garlic, *Allium sativum* with reference to its antimicrobial activity against the pathogenic strain of *Bacillus* sp. And *Pseudomonas* sp. infecting goldfish. *Carassius auratus*. Proc. Zool. Soc. 72 (2), 180–186.

Santoro, C., Kodali, M., Kabir, S., Soavi, F., Serov, A., Atanassov, P., 2017. Three-dimensional graphene nanosheets as cathode catalysts in standard and supercapacitive microbial fuel cell. J. Power Sources 356, 371–380.

Sardar, T., Rana, S., Chattopadhyay, J., 2015. A mathematical model of dengue transmission with memory. Comm. Nonlin. Sci. Num. Simul. 22 (1-3), 511–525.

Satyavani, K., Ramanathan, T., Gurudeeban, S., 2011. Plant mediated synthesis of biomedical silver nanoparticles by leaf extract of *Citrullus colosynthis*. Res. J. Nanosci. Nanotech. 1, 95–101.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis". Nat. Methods 9 (7), 671–675. https://doi.org/10.1038/nmeth.2089 (Accessed 3 March 2022).

Shaalan, E.-S., Canyon, D., Younes, M.W.F., Abdel-Wahab, H., Mansour, A.-H., 2005. A review of botanical phytochemicals with mosquitocidal potential. Environ. Int. 31 (8), 1149–1166.

Shahzad, K., Manzoor, F., 2019. Nanoformulations and their mode of action in insects: a review of biological interactions. Drug & Chem. Toxicol. 44, 1–11. https://doi.org/10.1080/01480545.2018.1525393 (Accessed 21 March 2022).

Shankar, S.S., Rai, A., Ahmad, A., Sastry, M., 2004. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J. Colloid Interface Sci. 275 (2), 496–502.

Singh, A., Mondal, R.P., Ghosh, A., Chandra, G., 2016. Studies on larvicidal activity of some plant extracts against Filarial vector *Culex quinquefasciatus*. J. Mosq. Res. 6, 1–6.

Subramaniam, J., Kovendan, K., Mahesh Kumar, P., Murugan, K., Walton, W., 2012. Mosquito larvicidal activity of *Aloe vera* (Family: Liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector. *Aedes aegypti*. Saudi J. Biol. Sci. 19 (4), 503–509.

Subramaniam, J., Murugan, K., Panneerselvam, C., Kovendan, K., Madhiyazhagan, P., Kumar, P.M., Dinesh, D., Chandramohan, B., Suresh, U., Nicoletti, M., Higuchi, A., Hwang, J.-S., Kumar, S., Alarfaj, A.A., Munusamy, M.A., Messing, R.H., Benelli, G., 2015. Eco-friendly control of malaria and arbovirus vectors using the mosquito fish *Gambusia affinis* and ultra-low dosages of *Mimuso pselengi* synthesized silver nanoparticles: towards an integrative approach. Environ. Sci. Pollut. Res. Int. 22 (24), 20067–20083.

Surendran, S.N., Kumaran, V., Sivarajah, R., Krishnarajah, S., Srikaran, S.R., Raghavendra, K., 2009. A note on the larvicidal efficacy of saponin constituted crude extracts of plant and animal origin against *Aedes aegypti*. L. J. Nat. Sci. Found. Sri Lanka. 37, 215–217.

Tennyson, S., Ravindran, J., Eapen, A., William, J., 2015. Larvicidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Asian Pac. J. Trop. Dis. 5, S73–S76.

Tiwary, M., Naik, S.N., Tewary, D.K., Mittal, P.K., Yadav, S., 2007. Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. J. Vector Borne Dis. 44, 198–204.

Tunçsoy, B.S., 2018. Toxicity of nanoparticles: A review. Accessed 21 March 2022 Adana BTU Fen Bilimleri Dergisi 1 (2). https://www.researchgate.net/publication/347839379.

Vijayakumar, S., Malaikozhundan, B., Saravanakumar, K., Durán-Lara, E.F., Wang, M.-H., Vaseeharan, B., 2019. Garlic clove extract assisted silver nanoparticle – Antibacterial, antibiofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. J. Photochem. Photobiol. B: Biol. 198, 2019. https://doi.org/10.1016/j.jphotobiol.2019.111558.

Vivek, M., Kumar, P.S., Steffi, S., Sudha, S., 2011. Biogenic silver nanoparticles by *Gelidiella acerosa* extract and their antifungal effects. Avicenna J. Med. Biotechnol. 3, 143–148.

Vogel, A.I., 1978. Textbook of practical organic chemistry, including qualitative organic analysis. The English Language Book Society and Longman, London, p. 1368.

Waris, M., Nasir, S., Abbas, S., Azeem, M., Ahmad, B., Khan, N.A., Hussain, B., Al-Ghanim, K.A., Al-Misned, F., Mulahim, N., Mahboob, S., 2020. Evaluation of larvicidal efficacy of *Ricinus communis* (Castor) and synthesized green silver nanoparticles against *Aedes aegypti* L. Saudi J. Biol. Sci. 27 (9), 2403–2409.

Who, 2005. World Health Organization: Guidelines for laboratory and field testing of mosquito larvicides. accessed 14 March 2022. https://apps.who.int/iris/handle/10665/69101.

WHO, 2022. Dengue and severe dengue. https://www.who.int/news-room/fact- sheets/detail/dengue-and-severe-dengue (accessed 3 March 2022).

Yasur, J., Usha-Rani, P., 2015. Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physiology. Chemosphere 124, 92–102.