Toxicity effect of green synthesized nanoparticle silver nitrate (AgNO₃) and titanium oxide (TiO₂) against mosquitoes

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Abstract

Most widely affecting species include Culex quinquefasciatus, A. albopictus and A. aegypti which serve as vectors for many hematophagous diseases. Various control measures have been used to control the mosquitoes but most important is the use of synthetic insecticides. This research focused on the use of botanical extracts and green synthesized silver nanoparticles of four different plants i.e. Moringa Olifera, Zingiber officinale, Syzygium aromaticum and Datura stramonium. Percent mean mortality was calculated. The results of the study indicated that on maximum concentration and all-out exposure time (96 hours) of application of plant extracts and green synthesized silver nanoparticles on mosquitoes Moringa Olifera based plant extract give highest percent mortality that was 79% and Silver nanoparticles of that plant also give highest percent mortality that was 92%.

Keywords: Bio pesticide; Nano-particle; Mosquitoes

1. Introduction

Due to the diversity of their forms and habitats, insects have seriously affected humans for a long time. Among them, mosquitoes play an important role, and the most widely affected species include Culex quinquefasciatus, A. albopictus, and A. aegypti, which are many blood-thirsty diseases Media (10). Mosquito populations and the spread of mosquito-borne pathogens are sensitive to temperature and hydrological variability (11). With climate change, the concentration of greenhouse gases in the atmosphere continues to increase. (In Scientific Basis 2001, higher temperatures and changes in the hydrological cycle are expected to affect mosquito population dynamics and the spread of mosquito-borne diseases. High temperatures may create many controversies about how vector-borne diseases are transmitted (13,14). However, future hydrological changes may determine transmission rates more importantly than global warming. IN order to combat these problems, scientists are looking for alternatives from natural resources to control the mosquitoes. Recently, bio pesticides such as plant extracts and green synthesized nano-products have received much attention as potentially useful bioactive compounds against insect pests and mosquitoes (1). Bio pesticides have the less risk to people and environment than synthetic pesticides (2). Plant contains the complex and variable mixtures of different bioactive compounds with different modes of action, offered by plants, may lessen the chance of resistance in mosquito populations (3). Plant extracts have been used traditionally in human communities throughout the world for making numerous products ranging from pharmaceuticals to insecticides (4) and for controlling and managing of insect pests (5). Several bioactive chemicals and secondary metabolites present in the plants like steroids, phenols, alkaloids, tannins, limonoids and terpenoids that found as a promising agent for insect control (6) that may work as insecticides, anti-feedants, repellents, fecundity reduction and respiration inhibition (7). Green synthesis of silver nanoparticles is
the subdivision of nanotechnology. Recently, biosynthetic strategies employing either biological microorganisms or fungus or vegetation extract have emerged as an easy and feasible opportunity to greater complex chemical synthetic techniques to attain nanomaterials (8). The green formation of silver nanoparticles by plant material provide better and advance results than the chemical method of formation, because the green formation of AgNPs is cost effective, easily managed and environmental friendly (9).

2. Material and methods

2.1. Selected plants

In this current research work, four plants leaves were used for the synthesis of crude extract and green silver nanoparticles.

2.2. Preparation of plant extracts

Systematic cleaning of the above healthy plant parts to remove dust and impurities. All plant parts are dried in a cool place for about three weeks and comminuted into a fine powder with the help of an electric mixing grinder. The powder was sieved through a 20-mesh sieve to obtain a suitable particle size range. The dried powder (100 g) was weighed and immersed in 500 ml of a 99% ethanol solvent to prepare a 100% stock solution. The solution was left on a rotary shaker at 220 rpm for 48 hours. After 48 hours, the plant material-based solution was filtered through Whatman No.1. 1 piece of filter paper and collect the extract in an Erlenmeyer flask, and store the stock solution in a refrigerator at 4-5 °C.

2.3. Preparation of green synthesis nanoparticles

Ten grams of the dried crude extract powder was dissolved in 250 ml of sterile distilled water, and the solution was boiled for 5 minutes. This solution / extract was filtered using What man filter paper No.1. A 1 mM silver nitrate (AgNO₃) solution was prepared by adding 1 mM silver nitrate to 100 ml of double distilled water and mixing well until the silver nitrate (AgNO₃) was dissolved. Place 80 ml of silver nitrate (AgNO₃) solution on a hot plate magnetic stirrer and add 20 ml of a suitably prepared plant extract dropwise. The solution was boiled for 5 minutes. A changing color of the solution was observed, which resulted in a brown solution, indicating the formation of silver nanoparticles. The solution was placed in a falcon tube and centrifuged at 5000 rpm for 15 minutes. The solution of green synthetic silver nanoparticles was converted into a tray form, excess solution was removed from the Falcon tube, and the precipitate was transferred to a porcelain plate (100 mm). The porcelain plate was placed in an oven at 50 °C for 24 hours to dry green synthetic silver nanoparticles. After drying, the granules were ground with a handgun mortar. Store the powder in an Eppendorf tube and label it with the appropriate name. Eppendorf tubes are covered with aluminum foil to ensure the stability of green synthetic silver nanoparticles.

2.4. Collection and rearing of the mosquitoes

Collected the mosquito larvae from areas of standing water stations, ponds and abandoned tires. The larvae were transferred to a plastic storage tank containing about 500 ml of water and reared under controlled conditions. The temperature was maintained at 25-27 °C and the relative humidity was 85%. Place the plastic storage tank with its mouth open in a glass feeding cage. The openings of these glass-fed cages are covered with muslin for ventilation, and then food is transferred to the mosquito larvae. Replace the water in the plastic storage tank every 4 days. Feed the larvae until the larvae develop. With the help of a manual glass pipette, p was collected from a plastic storage tank and transferred to a separate storage tank, which was then placed in another adult-fed glass feeding cage. Adults were fed a 10% sugar solution for three days.

2.5. Mortality by plant extracts and green synthesized Nanoparticles

Plant extracts and green synthesized silver nanoparticles (AgNPs) with three different concentrations and control for each treatment was used against mosquitoes. The toxicity was performed by the standard method. For making the serial wise concentrations of each different plant extracts and their green synthesized silver nanoparticles, the powder of the highest concentration of each treatment were dissolved into 1ml distill water and further added it into 499 ml water for making stock solution. Then this stock solution further diluted into water for making serial wise concentrations for each treatments of plants extracts and green synthesized silver nanoparticles. Fifty larvae of each instar were used for every concentration. each concentration was replicated 5 times with ten individual larvae in a single replication. The mortality data was recorded after 24, 48, 72 and 96 h after applications of treatment.
2.6. Statistical analysis/data analysis
Percent mean mortality was calculated using statistical software. Mortality data was corrected with Abbot’s formula.

3. Results

Table 1 shows that effect of four plant extracts with different concentrations at different time periods against mosquito. *Moringa olifera, Zingiber officinale, Syzygium aromaticum and Datura stramonium* shows maximum mortality after 96 hrs. 79, 76, 66 and 74 % mortality respectively at maximum concentrations.

**Table 1 Toxicity of plant extracts against Mosquitoes**

| Treatments          | Conc. (%) | Mean % mortality |
|---------------------|-----------|------------------|
|                     |           | 24h  | 48h  | 72h  | 96h  |
| *Moringa olifera*   |           |      |      |      |      |
| 4                   | 15        | 17   | 21   | 28   |
| 6                   | 18        | 21   | 27   | 32   |
| 10                  | 30        | 42   | 49   | 50   |
| 12                  | 35        | 46   | 56   | 52   |
| 16                  | 39        | 56   | 69   | 79   |
| *Zingiber officinale*|          |      |      |      |      |
| 4                   | 16        | 22   | 26   | 23   |
| 6                   | 22        | 25   | 35   | 40   |
| 10                  | 36        | 30   | 42   | 54   |
| 12                  | 46        | 46   | 50   | 65   |
| 16                  | 52        | 56   | 68   | 76   |
| *Syzygium aromaticum*|         |      |      |      |      |
| 4                   | 12        | 16   | 22   | 23   |
| 6                   | 19        | 25   | 33   | 36   |
| 10                  | 19        | 26   | 38   | 46   |
| 12                  | 17        | 23   | 42   | 56   |
| 16                  | 20        | 28   | 49   | 66   |
| *Datura stramonium* |           |      |      |      |      |
| 4                   | 16        | 19   | 28   | 33   |
| 6                   | 24        | 25   | 38   | 44   |
| 10                  | 24        | 23   | 42   | 55   |
| 12                  | 26        | 28   | 50   | 62   |
| 16                  | 23        | 29   | 51   | 74   |

Table 2 shows that effect of four synthesized AgNPs with different concentrations at different time periods against mosquito. *Moringa olifera AgNPs, Zingiber officinale AgNPs, Syzygium aromaticum AgNPs and Datura stramonium AgNPs* shows maximum mortality after 96 hrs. 92, 81, 68 and 86 % mortality respectively at maximum concentrations.
Table 2 Toxicity of synthesized AgNPs of different plants extracts against Mosquitoes.

| Treatments                  | Conc. (µg/ml) | Mean % mortality | 24h | 48h | 72h | 96h |
|-----------------------------|---------------|------------------|-----|-----|-----|-----|
| Moringa Olifera AgNPs       | 4             | 19               | 23  | 25  | 25  | 33  |
|                             | 6             | 25               | 21  | 27  | 27  | 52  |
|                             | 10            | 36               | 41  | 49  | 49  | 65  |
|                             | 12            | 39               | 46  | 59  | 59  | 79  |
|                             | 16            | 39               | 66  | 87  | 87  | 92  |
| Zingiber officinale AgNPs   | 4             | 16               | 21  | 27  | 27  | 30  |
|                             | 6             | 20               | 26  | 35  | 35  | 44  |
|                             | 10            | 28               | 35  | 42  | 42  | 54  |
|                             | 12            | 32               | 43  | 50  | 50  | 65  |
|                             | 16            | 44               | 52  | 73  | 73  | 81  |
| Syzygium aromaticum AgNPs   | 4             | 16               | 21  | 25  | 25  | 33  |
|                             | 6             | 19               | 25  | 33  | 33  | 39  |
|                             | 10            | 23               | 28  | 39  | 39  | 48  |
|                             | 12            | 31               | 33  | 52  | 52  | 61  |
|                             | 16            | 30               | 38  | 49  | 49  | 68  |
| Datura stramonium AgNPs     | 4             | 19               | 19  | 28  | 28  | 36  |
|                             | 6             | 28               | 35  | 45  | 45  | 54  |
|                             | 10            | 37               | 48  | 52  | 52  | 62  |
|                             | 12            | 42               | 51  | 59  | 59  | 72  |
|                             | 16            | 47               | 55  | 73  | 73  | 86  |

Table 3 Toxicity of synthesized TiO₂NPs of different plants extracts against Mosquitoes.

| Treatments                  | Conc. (µg/ml) | Mean % mortality | 24h | 48h | 72h | 96h |
|-----------------------------|---------------|------------------|-----|-----|-----|-----|
| Moringa olifera TiO₂NPs     | 4             | 17               | 19  | 21  | 21  | 30  |
|                             | 6             | 20               | 22  | 26  | 26  | 45  |
|                             | 10            | 28               | 39  | 44  | 44  | 55  |
|                             | 12            | 34               | 41  | 56  | 56  | 69  |
|                             | 16            | 38               | 56  | 67  | 67  | 78  |
| Zingiber officinale TiO₂NPs | 4             | 13               | 17  | 21  | 21  | 27  |
|                             | 6             | 18               | 22  | 31  | 31  | 41  |
|                             | 10            | 24               | 33  | 38  | 38  | 47  |
|                             | 12            | 27               | 39  | 48  | 48  | 56  |
|                             | 16            | 34               | 42  | 53  | 53  | 61  |
Table 3 shows that effect of four synthesized TiO$_2$NPs with different concentrations at different time periods against mosquito. *Moringa olfera* AgNPs, *Zingiber officinale* AgNPs, *Syzygium aromaticum* AgNPs and *Datura stramonium* AgNPs shows maximum mortality after 78 hrs. 92, 61, 65 and 76 % mortality respectively at maximum concentrations.

4. Conclusion

Table 1 shows that effect of four plant extracts with different concentrations at different time periods against 3$^{rd}$ instar larvae of mosquito. *Moringa olfera*, *Zingiber officinale*, *Syzygium aromaticum* and *Datura stramonium* shows maximum mortality after 96 hrs. 79, 76, 66 and 74 % respectively at maximum concentrations. Table 2 shows that effect of four synthesized AgNPs with different concentrations at different time periods against 3$^{rd}$ instar larvae of mosquito. *Moringa olfera* AgNPs, *Zingiber officinale* AgNPs, *Syzygium aromaticum* AgNPs and *Datura stramonium* AgNPs shows maximum mortality after 96 hrs. 92, 81, 68 and 86 % mortality respectively at maximum concentrations. Table 3 shows that effect of four synthesized TiO$_2$NPs with different concentrations at different time periods against mosquito. *Moringa olfera* TiO$_2$NPs, *Zingiber officinale* TiO$_2$NPs, *Syzygium aromaticum* TiO$_2$NPs and *Datura stramonium* TiO$_2$NPs shows maximum mortality after 78 hrs. 92, 61, 65 and 76 % mortality respectively at maximum concentrations. So results show that the effect of silver green synthesis nano particles result in more mortality than the simple plant extract. In which *Moringa olfera* AgNPs based nanoparticles gives more mortality then the other silver based nanoparticles, so it proves to be very effective against the mosquitoes then the other plant extract based nanoparticles.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict or interest among the authors.

References

[1] Andrade-Ochoa S, Sánchez-Aldana D, Chacón-Vargas KF, Rivera-Chavira BE, Sánchez-Torres LE, Camacho AD, Nevárez-Moorillón GV. Oviposition deterrent and larvicidal and pupaecidal activity of seven essential oils and their major components against Culex quinquefasciatus Say (Diptera: Culicidae): synergism–antagonism effects. Insects. 2018; 9(1): 25.

[2] Saxena S, Pandey AK. Microbial metabolites as eco-friendly agrochemicals for the next millennium. Applied Microbiology and Biotechnology. 2001; 55(4): 395-403.

[3] Mdoe FP, Cheng SS, Lyaruu L, Nkwengulila G, Chang ST, Kweka EJ. Larvicidal efficacy of Cryptomeria japonica leaf essential oils against Anopheles gambiae. Parasites & Vectors. 2014; 7(1): 426.

[4] Alouani A, Ababsia T, Rahal I, Rehim N, Boudjelida H. Activity evaluation of botanical essential oils against immature mosquitoes of Culex pipiens (Diptera: Culicidae). Journal of Entomology and Zoology Studies. 2017; 5(4): 829-834.
[5] Abad MKR, Besheli BA. Insecticidal potential of essential oil from the leaves of Citrus aurantium L. against Oryzaephilus surinamensis (F.), Lasioderma serricorne (L.) and Sitophilus oryzae (L.). Journal of Entomology and Zoology Studies. 2016; 4(5): 865-869.

[6] Abutaha N, Al Mekhlafi FA, Al Keridis LA, Farooq M, Nasr FA, Al Wadaan M. (2018). Larvicidal potency of selected xerophytic plant extracts on Culex pipiens (Diptera: Culicidae). Entomological Research. 2018; 48(5): 362-371.

[7] El-Bokl MM. Toxicity and bioefficacy of selected plant extracts against the mosquito vector Culex pipiens L. (Diptera: Culicidae). Environment. 2016; 6: 7.

[8] Logeswari P, Silambarasan S, Abraham J. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society. 2015; 19(3): 311-317.

[9] Bhuvaneswari R, Xavier RJ, Arumugam M. Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (Anopheles stephensi and Aedes aegypti). Journal of King Saud University-Science. 2016; 28(4): 318-323.

[10] Guruprasad NM, Jalali SK, Puttaraju HP. Wolbachia-a foe for mosquitoes. Asian Pacific Journal of Tropical Disease. 2014; 4(1): 78-81.

[11] Kettle DS. Medical and Veterinary Entomology. Wallingford, UK: CAB International. 1995.

[12] Intergovernmental Panel on Climate Change (2001) Climate Change 2001: The Scientific Basis. Geneva: IPCC 2001.

[13] Intergovernmental Panel on Climate Change. Climate Change 2001: Impacts, Adaptation, and Vulnerability. Geneva: IPCC 2001.

[14] Pascual M, Ahumada JA, Chaves LF, Rodó X, Bouma M. Malaria resurgence in the East African highlands: Temperature trends revisited. PNAS 103. 2006; 5829-5834.