Toxicity of Plant-Based Silver Nanoparticle Against Potential Disease Vector *Musca domestica* (Diptera: Muscidae) in Tabuk, Saudi Arabia

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**ABSTRACT**

The synanthropic house fly, *Musca domestica* is a major vector for the transmission of deadly diseases from one place to another. It plays a primary role in some diseases and community spread against human and animal origin. As the eradication and control measures are challenging through chemical or synthetic insecticides. The repeated exposure to insecticides developed resistance and undesired effects. A study to find the novel insecticides from natural plant-based origin were developed from *Lavandula angustifolia*, *Citrus limon*, *Mentha piperita*, *Azadirachta indica* and AgNPs against *M. domestica* at five different concentrations of each extract. The obtained results revealed good mortality from *Citrus limon* extract through dipping and feeding techniques with LC$_{50}$ values were 296.145 ppm (contact bioassay) and 380.187 ppm (feeding bioassay) after 24 h post-treatment. The plant-based nanoparticles developed from *A. indica* revealed good activity at a minimal concentration to reach its lethal effects and that LC$_{50}$ 67.056 ppm (contact bioassay) 110.279 ppm (feeding bioassay). Thus, the developed nano-insecticides are considered effective insecticidal against *M. domestica* and could save the world from the vector-transmitted disease in the present environment.

**INTRODUCTION**

*Musca domestica* L. (Diptera: Muscidae) housefly is a well-known pest among domestic, medical, and veterinary, which causes irritation, and food spoilage and is a carrier for some pathogenic organisms. It flies, feeds on foodstuffs, decayed matters, and human-animal wastes thus is present all over the world in the environment. It breeds and becomes a vector for pathogens such as bacteria, protozoa, and viruses (Palacios et al. 2009). Most food-borne diseases such as cholera, shigellosis and salmonellosis spread through this vector (Olsen et al. 2001). Adults transmit disease by direct contact with pathogens and disperse towards foods and contaminate through the propagation of microbes by their sponging mouthparts, hairy legs, lower body, and sticky parts of the feet (De-Jesus et al. 2004). House flies are responsible for the spread of diseases such as amoebic dysentery, shigellosis, salmonellosis, cholera, roundworms, hookworms, pinworms, tapeworms and many other animal and human diseases (Sasaki et al. 2000; Shono and Scott 2003).

Chemical insecticides which are used to kill insects have governed noble mortality for a shorter duration, but over-usage leads to resistance development among their populations (Edwin et al. 2016; Aziz et al. 2020).
Thus, developed resistance against insects fails to control in later stages. Houseflies developed resistance to pyrethroids, organochlorines, spinosad and organophosphates (Scott et al. 2000; Khan et al. 2021). The continuous usage has become an environmental issue such as pollution in the air, water, land areas, and non-fertile environment for plant growth, which become a problem for future generations. Through the food chain, it attains the human life cycle indirectly.

The development of new insecticides of plant-based origin or botanical insecticides is safe and reliable (Sultana et al. 2016; Mohammed et al. 2021). Natural resources are endowed with an immeasurable source of bioactive compounds. They can be effectively produced and utilized even by a layman. It can have peculiar action against insects and is easily degradable without any toxic effect on other organisms. Thus, it is safe for the present and future generations. The essential oil produced from plants also acts as a good insecticide (Isman, 2006).

Nanotechnology is emerging assimilation in biology enhancing the bioactive compounds for its action. The green synthesis of biocompatible nanomaterials is cost-effective, eco-friendly, and has high efficiency against insect pests (Aziz et al. 2020). It is safe because of its minimal usage of the desired compound and reaching the target site. Plant-based nanoparticles are stable for their lower contamination and easier production by the industry (Mohammad inejad et al. 2016). Thus, the present study was designed to evaluate the insecticidal effects of diverse plant extract and its synthesized silver nanoparticles (AgNPs) on M. domestica under laboratory conditions.

MATERIALS AND METHODS

Insect Rearing:

By using a sweeping net, the specimens of M. domestica were collected in September 2019 from a pesticides-free area of a sheep slaughterhouse in Tabuk Governorate, Saudi Arabia. They were transferred to the laboratory for identification and colonization in a wooden cage (16×16×16 cm). The colonies were maintained at 14:10 (light: dark) at 26 ± 2°C and 70-80% humidity. In order to produce a homogenised insect population, the insects were reared according to the method described by Sawicki (1964). Briefly, the adults were fed with 10% sucrose solution and reared in wire-frame cages (75x75x75 cm), with mesh covering the top and were supplemented with an artificial diet containing milk powder (600 gm), wheat bran (1000 gm), and yeast (30 gm) suspended with 1L of water for egg laying (Saleem et al. 2009; Cickova et al. 2012).

Plant Collection and Extraction:

The healthy plant leaves from Lavandula angustifolia Mill, Citrus limon (L.) Burm.f., Mentha piperita L. and A. indica A. Juss were collected from Al Manshiya, Tabuk region, Saudi Arabia during morning hours. The plants were authenticated by Taxonomist at the University of Tabuk, Saudi Arabia. The fresh leaves were washed, chopped finely, and subjected to Soxhlet extraction through acetone at a boiling point of 56°C for 8 hours. The crude extract was concentrated and removed the excess solvent through a rotary vacuum evaporator. The extract was stored at 4°C for further assays.

Synthesis of Neem-Based Silver Nanoparticle and Characterization:

About 1ml of A. indica extract was added with 1 ml of silver nitrate (AgNO₃) 10⁻³, 97.5 ml of distilled water and 0.5ml of Triton-x100. The solution was incubated at room temperature until the color changed into grey, implying nanoparticle synthesis (Roni et al. 2013). The synthesized nanoparticles were subjected to various biophysical characterization, which was also studied in our earlier research (Aziz 2021).

Preparation of Standard Solution:

The standard solution was prepared by adding 1 ml of the selected plant extract

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Toxicity of Plant-Based Silver Nanoparticle Against Potential Disease Vector Musca domestica

Dipping Technique:

In this method early second and third instar larvae were used for the experiment. Twenty larvae (second and third instar) were dipped into the selected plant extracts *L. angustifolia, C. limon, M. piperita,* and *A. indica* (100, 200, 300, 400 and 500 ppm) and AgNPs (10, 50, 100, 150 and 200 ppm) with a dip net. Control was tap water. After 30 seconds the treated larvae were introduced to food (yeast, dry milk powder, wheat bran and water) in a 1:1:1 ratio containing jar. They were further noted for the survival of the life cycle and emergence of adults. This experiment was carried out in a confined laboratory environment at 14:10 (light: dark), 28±2°C and 70–80% humidity. Five replicates were maintained in each dose.

Feeding Technique:

The toxicity of the nanomaterial and plant extract at different concentrations was studied through an artificial diet. The diet was prepared with milk powder (600 gm), wheat bran (1000 gm) and yeast (30 gm) mixed with one liter of water. The experiment was designed in such a way that 25gm of artificial feed was mixed with 2.5 ml of water with the selected plant extracts *L. angustifolia, C. limon, M. piperita, A. indica* (100, 200, 300, 400 and 500 ppm) and AgNPs (10, 50, 100, 150 and 200 ppm). The emerged larvae were subjected, and the emerged adults were noted. The experiments were carried out under 14:10 (light: dark) and maintained at 28±2°C and 70–80% humidity. The experiments were done for five replications. Feeding experiments were carried out according to (Kristensen and Jespersen 2003).

Statistical Analysis:

All mortality was subjected to ANOVA. The Completely Randomized Design (CRD) is used for the experiment. Before applying ANOVA, the normality of the data was tested by applying two tests (Kolmogrov-Smirnov & Shapiro-Wilk), which show that the data is continuous and normally distributed. Tukey’s multiple range test (P < 0.05) was used to analyze the significant differences between treated and control groups by Minitab®17 software. Probit analysis was used to find the lethal concentrations required to kill 50% (LC50) of larvae 24 h post-treatment.

RESULTS

Contact Bioassay:

The acetone extracted leaves of *Lavandula angustifolia, Citrus limon, Mentha piperita* and *A. indica* against *M. domestica* showed varied activity by variance in their mortality. The mortality rate of larvae through contact bioassay revealed their potential against treated larvae. The treated larvae of *M. domestica* with different concentrations displayed good activity. The percentage of mortality increased with an increase in concentration. The mortality of *M. domestica* larvae treated with *C. limon* extract through contact bioassay revealed their activity was maximum at a concentration of 500 ppm and that the LC50 value is 296.145 ppm. Results obtained was shown in Table 1 and Figure 1. The percentage was 9, 15.5, 31, 53 and 66% at 100, 200, 300, 400, 500 ppm. Adults treated through feeding bioassay revealed 85% of mortality at a concentration of 500 ppm.
Table 1. Insecticidal activity of Indigenous plant extract and AgNPs against *M. domestica* through contact bioassay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LC(_{50}) (ppm) (95% LCL-UCL)</th>
<th>LC(_{90}) (ppm) (95% LCL-UCL)</th>
<th>Slope</th>
<th>(\chi^2) (d.f.=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lavandin angustifolia</em></td>
<td>394.315 (354.033-450.653)</td>
<td>1164.118 (899.587-1723.142)</td>
<td>2.725</td>
<td>7.74 n.s</td>
</tr>
<tr>
<td><em>Citrus limon</em></td>
<td>296.145 (212.696-430.377)</td>
<td>872.488 (792.326-2869.42)</td>
<td>2.731</td>
<td>9.45 n.s</td>
</tr>
<tr>
<td><em>Mentha piperita</em></td>
<td>346.801 (320.455-377.861)</td>
<td>774.703 (660.903-968.884)</td>
<td>3.671</td>
<td>0.50 n.s</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>514.465 (432.234-638.289)</td>
<td>3313.483 (2105.718-6833.802)</td>
<td>1.584</td>
<td>1.04 n.s</td>
</tr>
<tr>
<td><em>A. indica</em> -derived AgNPs</td>
<td>67.056 (31.450-124.581)</td>
<td>588.697 (479.176-5709.497)</td>
<td>1.358</td>
<td>8.92 n.s</td>
</tr>
</tbody>
</table>

Control = no mortality  
LC\(_{50}\) = lethal concentration killing 50 % of the insects  
LC\(_{90}\) = lethal concentration killing 90 % of the insects  
\(\chi^2\) = chi-square  
d.f. = degrees of freedom  
n.s. = not significant (\(\alpha=0.05\))

Fig. 1. Mortality percentage of *M. domestica* treated with different concentration through contact bioassay.

**Feeding Bioassay:**

The larvae treated with *C. limon* extract showed mortality of 15, 24.3, 15, 63.7 and 80.3% at 100, 200, 300, 400 and 500 ppm and the LC\(_{50}\) value is 380.187 ppm. The maximum percentage of mortality was noted at higher concentrations. The mortality percentage directly depends on the concentration of the extract. The feeding bioassay implied a maximum of 66.6 percent at 500 ppm.

The acetone leaf extract of *M. piperita* revealed a mortality of 73.5% at 500ppm through contact bioassay. The feeding bioassay of adults revealed a maximum percentage of 56.7 at a higher
concentration. The A. Indica leaf extract against M. domestica larvae through contact bioassay revealed 67% at 500 ppm concentration. Through the feeding bioassay method, the adults of M. domestica displayed 54.1 percent at the highest concentration. Results obtained was shown in Table 2 and Figure 2.

Table 2. Insecticidal activity of Indigenous plant extract and AgNPs against M. domestica through feeding bioassay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LC50 (ppm) (95% LCL-UCL)</th>
<th>LC90 (ppm) (95% LCL-UCL)</th>
<th>Slope</th>
<th>$\chi^2$ (d.f.=f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavandula angustifolia</td>
<td>458.327 (388.286-585.102)</td>
<td>2193.649 (1383.017-4913.038)</td>
<td>1.884</td>
<td>2.25 n.s</td>
</tr>
<tr>
<td>Citrus limon</td>
<td>380.187 (336.542-442.851)</td>
<td>1354.453 (993.532-2206.620)</td>
<td>2.322</td>
<td>6.05 n.s</td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>433.666 (375.849-528.445)</td>
<td>1733.641 (1186.061-3250.37)</td>
<td>2.129</td>
<td>0.94 n.s</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>497.304 (426.166-623.885)</td>
<td>1876.183 (1265.189-3635.423)</td>
<td>2.222</td>
<td>1.76 n.s</td>
</tr>
<tr>
<td>A. indica -derived AgNPs</td>
<td>110.279 (84.798-151.25)</td>
<td>2056.185 (989.595-7124.128)</td>
<td>1.008</td>
<td>7.79 n.s</td>
</tr>
</tbody>
</table>

Control = no mortality
LC50 = lethal concentration killing 50 % of the insects
LC90 = lethal concentration killing 90 % of the insects
$\chi^2$ = chi-square
d.f. = degrees of freedom
n.s. = not significant (α=0.05)

Fig. 2. Mortality percentage of M. domestica treated with different concentration through Feeding bioassay

The A. indica mediated AgNPs were characterized through UV visible spectroscopy, EDAX and TEM observation were revealed in our earlier findings (Aziz 2021).

The A. indica mediated silver nanoparticles which were treated against M.
domestica larvae through contact bioassay showed good activity at the lowest concentration. The activity increased with an increase in concentration. The M. domestica larvae treated at different concentrations such as 10, 50, 100, 150 and 200 ppm showed good mortality and the maximum percentage of activity was obtained at 200 ppm of 81.4. Results were displayed in Figure 3. The feeding bioassay of adults revealed 69 % of mortality at 200 ppm concentration. The result obtained was higher in A. indica-mediated AgNO3 nanoparticles. The mortality obtained was clearly shown in Figure 4.

The lethal concentration to obtain LC$_{50}$ against M. domestica through different extracts varied. The obtained results revealed that C. limon exhibited good activity at a lower concentration of 45.2 ppm in contact bioassay and 59.2 ppm through feeding bioassay. The experimental study revealed after 24 h of exposure showed good activity and the obtained results were calculated for its LC$_{50}$ and LC$_{90}$. At a concentration of 0-500 ppm through contact and feeding bioassay revealed good activity from C. limon. Through synthesized neem-based nanoparticles the activity was higher at the lowest concentration of 295.4 ppm and 83.6 ppm at contact and feeding bioassay.

![Fig. 3](image_url)

**Fig. 3.** Mortality percentage of M. domestica treated with different concentration of silver nanoparticles synthesized from A. indica through contact bioassay.

![Fig. 4](image_url)

**Fig. 4.** Mortality percentage of M. domestica treated with different concentration of silver nanoparticles synthesized from A. indica through feeding bioassay
DISCUSSION

The disease caused by *M. domestica* was increased and they transmit numerous human and animal intestinal diseases namely amoebic dysentery, shigellosis, salmonellosis, cholera and helminthic (Sasaki *et al.* 2000). Also, in humans it forms various skin diseases such as cutaneous diphtheria, mycoes, yaws and leprosy (Keiding 1986); larvae ingested causes intestinalmyiasis, with symptoms of nausea, pain and vomiting (Hill 2005). The control measures through chemical insecticides against *M. domestica* showed higher mortality at the initial stages but failed due to cross-resistance on repeated usage and this become an environmental hazard (Attaullah Zahoor *et al.* 2020).

Thus, resistance development and an uncontrolled environment have led scientists to develop eco-friendly, non-hazardous inexpensive insecticides for future management. Plants and plant-derived compounds have potential against insecticides, this was due to the presence of diverse phytochemical constituents (polyphenols, alkaloids, terpenoids, essential oils, steroids, lignins, fatty acids and sugars). The developed insecticides can have good activity and their availability in their environment also degraded after their activity, this directly helps for the survival of predators and beneficial insects (Regnault-Roger *et al.* 2020). Therefore, bio-pesticides play a vital role in controlling various insects (Anjum *et al.* 201; Thanigaivel *et al.* 2018).

The plant extract-based study of *Lavandula angustifolia*, *Citrus limon*, *Mentha piperita* and *Azadirachta indica* against *M. domestica* through contact bioassay revealed good activity in all extracts. This revealed the plant extracts exhibit good toxic effects against insect larvae. Similarly, the study made on *M. domestica* larvae through 11 plant extracts showed that the crude ethanoic extracts of *Annona squamosa* and *Calotropis procera* exhibited good insecticidal activity (Mansour *et al.* 2011). In contact bioassay, the activity obtained was maximum from *C. limon* extract. A study by Morey (2016) made against *M. domestica* through a crude extract of *Citrus limon* and *Ocimum basilicum* showed good activity. Through the feeding bioassay method, the mortality rate revealed was higher in *L. angustifolia* treated extract. The results showed the activity was higher due to the presence of toxic compounds in the extract. The toxic compound is bound to the ingested food, and this leads to the death of the insect.

Reports and results revealed *A. indica* has potential activity against insect pests which was used traditionally by many authors (Sultana *et al.* 2016; Aziz 2021). Thus *A. indica*-based nanoparticles was studied against *M. domestica* through contact bioassay and feeding bioassay. The results revealed the synthesized AgNPs nanoparticles are more effective against *M. domestica* at lower concentrations. Through contact bioassay, the mortality rate was 81.4% at a treated concentration of 200 ppm which implies the most efficient toxicant. In feeding bioassay, the mortality percentage was 69% at 200 ppm. When compared to the plant extracted insecticides, nano-synthesized insecticides are more active.

The extract was combined with silver nitrate to obtain nanoparticles due to the strong reducing agent and faster in reducing charged ions into a chargeless nanoparticle. Earlier studies on methanolic extracted *Nerium oleander* mediated silver nanoparticles showed good activity (El-Monairy *et al.* 2020). Thus, plant-mediated silver nanoparticles could be an alternative measure to control the population of *M. domestica* in the environment. It is evident from an earlier report the vector carries most of the microbial diseases (Khamesipour *et al.* 2018). This provides the environment free from contamination and spreadable of some diseases in society.

In conclusion, the insecticidal effect of *L. angustifolia* Mill, *C. limon* (L.) Burm.f., *M. piperita* L. and *A. indica* A. Juss
and its fabricated AgNPs were studied in laboratory conditions. The plant extract and AgNPs have been shown to be effective housefly control agents. Therefore, natural products and their fabricated nanomaterials are the best alternatives to chemical insecticides.

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REFERENCES


