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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<sub>50</sub> 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 vectortransmitted 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.

development The 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 synthesis biocompatible green of nanomaterials is cost-effective, ecofriendly, 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 М. 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 the laboratory transferred to for identification and colonization in a wooden cage ( $16 \times 16 \times 16$  cm). The colonies were maintained at 14:10 (light: dark) at  $26 \pm 2^{\circ}C$ 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 (AgNO<sub>3</sub>)  $10^{-3}$ , 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 in 98.5 ml of deionized water and Triton-x100 (0.5 ml) as an emulsifying agent. This gives the concentration of 1000ppm, which can be diluted for further experiments.

#### **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 five replications. done for 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 & Shapiroc-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<sup>®</sup>17 software. Probit analysis was used to find the lethal concentrations required to kill 50% (LC<sub>50</sub>) 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  $LC_{50}$  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 and 500ppm. Adults treated through feeding bioassay revealed 85% of mortality at a concentration of 500 ppm.

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Treatment	LC50 (ppm) (95% LCL-UCL)	LC90 (ppm) (95% LCL-UCL)	Slope	$\begin{array}{c} \chi^2\\ (d.f=4) \end{array}$	
Lavandula angustifolia	394.315 (354.033-450.653)	1164.118 (899.587-1723.142)	2.725	7.74 <i>n.s</i>	
Citrus limon	296.145 (212.696-430.377)	872.488 (792.326-2869.42)	2.731	9.45 n.s	
Mentha piperita	346.801 (320.455-377.861)	774.703 (660.903-968.884)	3.671	0.50 <i>n.s</i>	
Azadirachta indica	514.465 (432.234-638.289)	3313.483 (2105.718-6833.802)	1.584	1.04 <i>n.s</i>	
A. indica -derived AgNPs	67.056 (31.450-124.581)	588.697 (479.176-5709.497)	1.358	8.92 <i>n.s</i>	

**Table 1.** Insecticidal activity of Indigenous plant extract and AgNPs against *M. domestica* through contact bioassay

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)

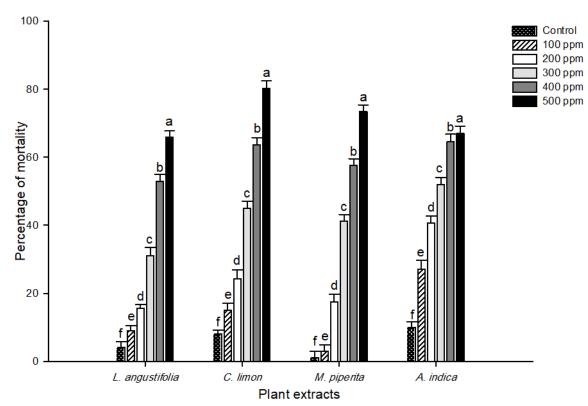


Fig. 1. Mortality percentage of *M. domestica* treated with different concentration through contact bioassay.

#### **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<sub>50</sub> 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.

Treatment	LC50 (ppm) (95% LCL-UCL)	LC90 (ppm) (95% LCL-UCL)	Slope	$\begin{array}{c} \chi^2\\ (d. f=4) \end{array}$
Lavandula angustifolia	458.327 (388.286-585.102)	2193.649 (1383.017-4913.038)	1.884	2.25 n.s
Citrus limon	380.187 (336.542-442.851)	1354.453 (993.532-2206.620)	2.322	6.05 <i>n.s</i>
Mentha piperita	433.666 (375.849-528.445)	1733.641 (1186.061-3250.37)	2.129	0.94 <i>n.s</i>
Azadirachta indica	497.304 (426.166-623.885)	1876.183 (1265.189-3635.423)	2.222	1.76 <i>n.s</i>
A. indica -derived AgNPs	110.279 (84.798-151.25)	2056.185 (989.595-7124.128)	1.008	7.79 <i>n.s</i>

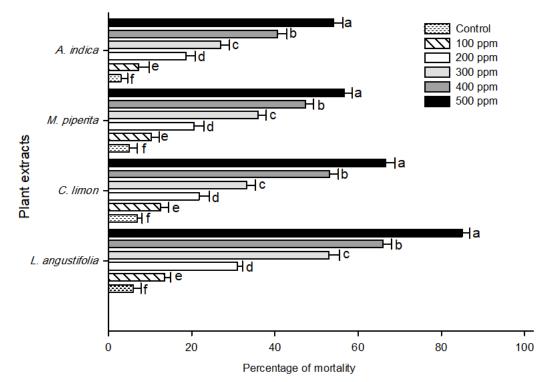
Control = no mortality

 $LC_{50}$  = 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. 2.** Mortality percentage of *M. domestica* treated with different concentration through Feeding bioassay

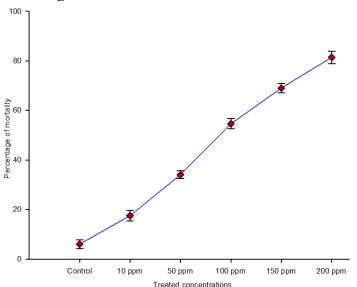
The *A. indica* mediated AgNPs were characterized through UV visible spectroscopy, EDAX and TEM observation were revealed in our earlier findings (Aziz 2021).

The *A. indica* mediated silver nanoparticles which were treated against *M*.

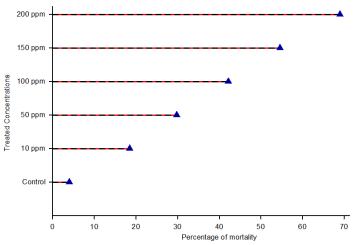
*domestica* larvae through contact bioassay showed good activity at the lowest concentration. The activity increased with an increase in concentration. The *M. domestica* larvae treated at different concentrations such as 10, 50, 100, 150 and 200 ppm showed good mortality and the maximum percentage of 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 AgNO<sub>3</sub> nanoparticles. The mortality obtained was clearly shown in Figure 4.

The lethal concentration to obtain LC<sub>50</sub> 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<sub>50</sub> and LC<sub>90</sub>. 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**. Mortality percentage of *M. domestica* treated with different concentration of silver nanoparticles synthesized from *A. indica* through contact bioassay.



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

#### DISCUSSION

The by disease caused М. domestica was increased and they transmit numerous human and animal intestinal namely amoebic dysentery, diseases shigellosis, salmonellosis, cholera and helminthic (Sasaki et al. 2000). Also, in humans it forms various skin diseases such as cutaneous diphtheria, mycoses, yaws and leprosy (Keiding 1986); larvae ingested causes intestinalmyiasis, with symptoms of nausea, pain and vomiting (Hill 2005). The through control measures 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, nonhazardous inexpensive insecticides for future management. Plants and plantderived compounds have potential against insecticides, this was due to the presence of phytochemical diverse 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 and beneficial insects of predators (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. lemon* 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, nanosynthesized 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-Monairyet 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

- Anjum, S.I.; Yousaf, M.J.; Ayaz, S.; Siddiqui, B.S. 2010. Toxicological evaluation of chlorpyrifos and neem extract (Biosal b) against 3rd instars larvae of *Drosophila melanogaster*. Journal of Animal and Plant Sciences, 20, 9-12.
- AttaullahZahoor, M.K.; Zahoor, M.A.; Mubarik, M. S.; Rizvi, H.; Majeed, H.N.; Zulhussnain, M.; Ranian, K.; Sultana, K.; Imran, M.; Qamer, S. 2020. Insecticidal, biological and biochemical response of *Musca domestica* (Diptera: Muscidae) to some indigenous weed plant extracts. *Saudi Journal of biological sciences*, 27,106–116.
- Aziz, A.T. 2021. A comparative study and characterization of *Azadirachta indica* mediated nano-insecticide and ethanolic extract against mosquito vectors – Arabian and Indian strains. *Entomological Research*, https://doi.org/10.1111/ 1748-5967.12549.
- Aziz, A.T., Alshehri, M.A.; Alanazi, N.A.; Panneerselvam, C.; Trivedi, S.; Maggi, F.; Sut, S.; Dall'Acqua, S. 2020. Phytochemical analysis of *Rhazya stricta* extract and its use in fabrication of silver nanoparticles effective against mosquito vectors and microbial pathogens. *Science* of the Total Environment, 700, 1344433.
- Cickova, H.; Kozanek, M; Takac, P. 2012. Improvement of survival of the

housefly (*Musca domestica* L.) larvae under mass-rearing conditions. *Bulletin of Entomological Research*, 103, 119-25.

- De-Jesus, A.J.; Olsen, A.R.; Bryce, J.R.; Whiting, R.C. 2004. Quantitative contamination and transfer of *Escherichia coli* from foods by houseflies, *Musca domestica* L. (Diptera: Muscidae). *International Journal of Food Microbiology*, 93, 259-262.
- E.; Vasantha-Srinivasan, P. S.; Edwin, Senthil-Nathan, S.; Thanigaivel, A.; Ponsankar, A.; Pradeepa, V.; Selin-Rani, S.; Kalaivani, K.; Hunter, W.B.; Abdel-Megeed, A.; 2016. Duraipandiyan, V.Antidengue efficacy of bioactive andrographolide from Andrographis paniculata (Lamiales:Acanthaceae) against the primary dengue vector Aedes aegypti (diptera: Culicidae). ActaTropica, 163, 167-178.
- El-Monairy, O.M.; Abdel-Meguid, A.D.;
  Emara, M.M. 2020. Efficacy of Methanol Leaf Extract, Biosynthesized Silver and Chitosan Nanoparticles Using Nerium oleander against Musca domestica. Egyptian Academic Journal of Biological Sciences F. Toxicology and Pest Control, 12, 35-45.
- Hill, D.S. 2005. Pests of stored food stuffs and their controls. Springer India Pvt. Ltd.: New Delhi, India. 250– 253.
- Isman, M.B.; Machial, C. M. 2006. Pesticides based on plant essential oils: from traditional practice to commercialization. *Advances in Phytomedicine*, 3, 29-44.
- Keiding, J. 1986. The housefly: biology and control. World Health Organization Vector Control Series, 63.

- Khamesipour, F.; Lankarani, K. B.; Honarvar, B.; Kwenti, B. 2018. A systematic review of human pathogens carried by the housefly (*Musca domestica* L.). *BMC Public Health*, 18, 1-5.
- Khan, H.A.L. 2021. Permethrin resistance associated with inherited genes in a near-isogenic line of *Musca domestica*. *Pest Management Science*, 77, 963-969.
- Kristensen, M.; Jespersen, J. B. 2003. Larvicide resistance in *Musca* domestica (Diptera: Muscidae) populations in Denmark and establishment of resistant laboratory strains. *Journal of Economic Entomology*, 96, 1300-1306.
- Mansour, S.A.; Bakr, R.F.R.; Mohamed, R.I.; Hasaneen, N.M. 2011. Larvicidal activity of some botanical extracts, commercial their insecticides and binary mixtures against the housefly, domestica L. Musca Open Toxinology Journal, 4, 11-13.
- Mohammad inejad, R.; Karimi, S.; Iravani, S.; Varma R.S. 2016. Plantderived nanostructures: types and applications. *Green Chemistry*, 18, 20-52.
- Mohammed, A.A.; Ahmed, F.A.; Kadhim, J.H.; Salman, A.M. 2021. Susceptibility of adult and larval stages of housefly, *Musca domestica* to entomopathogenic fungal biopesticides. *Biocontrol Science and Technology*, https:// doi.org/10.1080/ 09583157. 2021. 1917513.
- Morey, R.A. 2016. Evaluation of crude extracts of *Citrus limon* and *Ocimum basilicum* against *Musca domestica* L. *In Proceeding of National Conference on Environment and Development*, 69-72.
- Olsen, A.R.; Gecan, J.S.; Ziobro, G.C.; Bryce, J.R. 2001, Regulatory

action criteria for filth and other extraneous materials: V. Strategy for evaluating hazardous and nonhazardous filth. *Regulatory Toxicology and Pharmacology*, 33, 363-392.

- Palacios, S.M.; Bertoni, A.; Rossi, Y.; Santander, R.; Urzúa, A. 2009.Efficacy of Essential Oils from Edible Plants as Insecticides against the House Fly, *Musca Domestica* L. *Molecules*, 14, 1938-1947.
- Regnault-Roger, C.; Vincent, C.; Arnason, J.T. 2012. Essential oils in insect control: Low-risk products in a high-stakes world. *Annual Review* of Entomology, 57, 405-424.
- Roni, M.; Murugan, K.; Panneerselvam, C.;
  Subramaniam, J.; Hwang, J.S.
  2013. Evaluation of leaf aqueous extract and synthesized silver nanoparticles using *Nerium oleander* against *Anopheles stephensi* (Diptera: Culicidae).
  Parasitology Research. 112, 981-90.
- Saleem, M.A.; Ashfaq, M.A.R.; Shakoori, A.R. 2009. In vivo Effect of Spinosad on Proteases of Insecticide-Resistant and Susceptible Strains of Musca domestica. Pakistan Journal of Zoology, 41, 455-462.
- Sasaki, T.; Kobayashi, M.; Agui, N. 2000. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of *Escherichia coli* O157: H7 to food. *Journal of Medical Entomology*, 37, 945-9.
- Sawicki, R.M. 1964. Some general considerations of housefly rearing techniques. *Bulleten of WHO*, 31, 535-537.
- Scott, J.G.; Alefantis, T. G.; Kaufman, P.E.; Rutz. D.A. 2000. Insecticide resistance in house flies from caged-layer poultry facilities. *Pest*

Management Science, 56, 147-153.

- Shono, T.; Scott, J.G. 2003. Spinosad resistance in the housefly, *Musca domestica*, is due to a recessive factor on autosome 1. *Pesticide Biochemistry and Physiology*, 75, 1-7.
- Sultana, K.; Zahoor, M.K.; Sagheer, M.; Nasir, S.; Zahoor, M.A.; Jabeen, F.; and Riaz, B. 2016. Insecticidal activity of weed plants, *Euphorbia prostrata* and *Chenopodiastrum murale* against stored grain insect pest *Trogoderma granarium*

Everts, 1898 (Coleoptera: Dermestidae). *Turk Entomoloji Dergisi*, 40, 291-301.

Thanigaivel, A.; Vasantha-Srinivasan, P.; Edwin, E.S.; Ponsankar, A.; Selin-Rani, S.; Chellappandian, M.; Kalaivani, K.; Senthil-Nathan, S.; Benelli, G. 2018. Development of an eco-friendly mosquitocidal agent from *Alangium salvifolium* against the dengue vector *Aedes aegypti* and its biosafety on the aquatic predator. *Environmental Science and Pollution Research*, 25, 10340-10352.