EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

Vol. 17 No. 4 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 17(4) pp.83-89 (2024)*
DOI: **10.21608/EAJBSA.2024.399218**

Egypt. Acad. J. Biolog. Sci., 17(4):83-89 (2024)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/

Scanning Electron Microscopy of Cuticular Deformation in *Periplaneta americana* and *Blatella germanica* (Blattodea) Induced by Nanoemulsion of *Pimpinella anisum* Essential Oil

Radwa M. Azmy
Department of Entomology, Faculty of Science, Ain Shams University
*E-mail: radwa.azmy@sci.asu.edu.eg

ARTICLE INFO
Article History
Received:20/11/2024
Accepted:23/12/2024
Available:27/12/2024

Keywords:
Cuticle penetration, Mode of action, Epicuticle, Wax layer, Nanoemulsion.

ABSTRACT

The increasing concerns about safety push the researchers to provide natural alternatives to synthetic insecticides because of the harmful impact of the synthetic insecticides on the non-target living organisms including humans. Insecticides containing essential oils showed that they are promising to control economic and medical insect pests and nanotechnology allowed the formulation of essential oils for proper use. Nanoinsecticides containing essential oils represent a harmless alternative for pest management with different modes of action. The aim of this research is to study the cuticle deformation in *Periplaneta americana* and *Blatella germanica* (Blattodea) induced by nanoemulsion of *Pimpinella anisum* L. essential oil using the Scanning electron microscopy. *Periplaneta Americana*, the American cockroach, and *Blatella germanica,* the German cochroach, are important medical insect pests because they are able to mechanically transmit many pathogens and they are present in places where humans exist. These two species are a proper model to study the suggested mode of actions of the nanoinsecticides to better understand the effect of thenanoinsecticide on the insect pest.

INTRODUCTION

Cockroaches are a public health worry because they mechanically transmit many parasites and pathogens (Pai *et al.,* 2003). They are members of Order Blattodea which are associated with human inhabitation, such as homes, schools, bakeries, grocery stores and other sites where food, moisture, dark and damp cracks exist.

Periplaneta americana and *Blatella germanica* are medically important insects belong to order Blattodea, they can transmit different pathogens mechanically and they are important hygiene indicator as they contaminate the infested places with their exuviae and excrements (Yeom *et al.,* 2013) Protection of food sources and control of such disease vectors depend greatly on insecticides. However, the intense use of synthetic chemical insecticides generated many problems (Maiza *et al.,* 2013).

Furthermore, the public increasing concern about the negative effects of chemical insecticides in areas of food preparation, storage, and homes stimulated the exploration of botanical insecticides. Therefore, bioinsecticides containing essential oils represent a harmless alternative for insect pest management with different modes of action (Isman

2017). Essential oils are mixtures of novel volatile metabolites which contain few major components (Isman 2020). Attention to the promising use of essential oils for management of insect pests as a botanical insecticide grown rapidly in the past years (Isman 2020) and screened against insect pests under laboratory (Isman 2017). Many essential oils demonstrate lethal effects against cockroaches (Alzogaray *et al.*, 2013, 2011; Phillips and Appel, 2010).

Despite these promising properties, an important obstacle is the high volatility of essential oils and easy decomposition due to exposure to heat, light, and humidity (Turek and Stintzing, 2013). The nanoformulation of essential oils prevents degradation and evaporation, allowing a controlled release of the bioactive ingredients (de Oliveira *et al.*, 2014).

Nowadays, nanoemulsions of essential oils are greatly studied as insecticides against the all the different stages of insects. However, the mode of action of these natural insecticides is not definitely determined.

The cuticle or exoskeleton is the outermost layer of the insect body. It has several functions like sensory perception of the environment, protection against desiccation, support and locomotion (Wan *et al.*, 2016). Furthermore, the cuticle is the first barrier protecting the insect body against external compounds penetration.

The aim of this study is to investigate the cuticle deformation in wing cuticle of *Periplaneta americana* and *Blatella germanica* induced by nanoemulsion of *Pimpinella anisum* L. essential oil using the scanning electron microscopy. Clarification of the effect of the nanoemulsion of the essential oils on the cuticle may interpret the entry mode of the nanoemulsion containing the bioactive compounds which are toxic to the target insect pests.

MATERIALS AND METHODS

Extraction of *Pimpinella anisum* Essential Oil:

Extraction of the oil from the seeds of *Pimpinella anisum* was done through hydrodistillation for three hrs using a Clevenger device according to Angioni *et al.* (2006).

Preparation of the NANOEMULSION:

The nanoemulsion of *Pimpinella anisum* essential oil was prepared using distilled water and tween 20 according to Duarte *et al.* (2015) at the Electron Microscope Unit in faculty of Science, Ain Shams University.

Droplet Size Distribution & Polydispersity Index of The Nanoemulsion:

Dynamic light scattering technique was applied to measure the droplet size according to Sugumar et al. (2014) using Malvern-UK, 4700 analyzer at the Egyptian Petroleum Research Institute.

Stability of the Nanoemulsion:

Thermodynamic stability of the nanoemulsion was tested by storing at 4 ºC and 25 ºC for four weeks. The nanoemulsion was centrifuged at 10,000 rpm for 20 min, and then was observed separation according to Ghosh et al. (2013) for any cracking or phase.

Insects:

The fore wings of *Periplaneta americana* and *Blatella germanica* were dipped in to the nanoemulsion of *Pimpinella anisum* L. essential oil of concentration 5% (w/w) for 24 hours.

Scanning Electron Microscopy:

Scanning electron microscopy was carried out in the Applied Center for Entomonematodes, Faculty of Agriculture, Cairo University.

The control and treated specimens were mounted on aluminum stubs. Then, the specimens were coated with 200 A° gold using a sputter coater. Finally, scanning electron micrographs of the normal wing and any cuticular changes in treated wings were taken.

RESULTS

Characterization of the Nanoemulsion:

Droplet Size Distribution & Polydispersity Index:

Droplet size distribution of the nanoemulsion of *Pimpinella anisum* (Fig. 1), with the peak value was 292 nm and the measurement of the polydispersity index was 0.24.

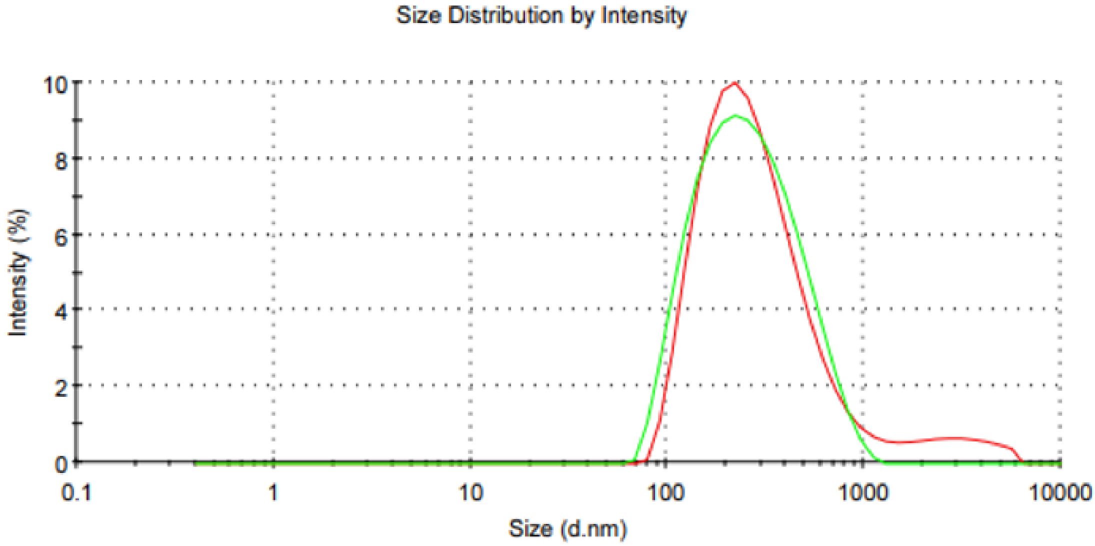

Fig. 1: Droplet size distribution of the nanoemulsion of *Pimpinella anisum* essential oil.

Stability of the Nanoemulsion:

There was no observed sign of instability of the nanoemulsion, including creaming or phase separation. The nanoemulsion was stable after centrifugation at 10,000 rpm for 20 min and also when stored at 4 ºC for a month.

Study of the Forewing Surface of *Periplaneta americana* and *Blatella germanica*:

The untreated forewing of *Periplaneta americana* appeared normal with its characterized brown colour when observed by the naked eye, and the treated wing did not appear to be different from the control wing.

Scanning electron micrographs of the normal forewing of *Periplaneta americana* showed normal appearance with cuticular scales (Fig. 2A, B). Scanning electron microscopy analysis revealed that treatment with the nanoemulsion of *Pimpinella anisum* essential oil had no significant effect on the forewing of *Periplaneta americana* (Fig. 2 C, D).

The dorsal surface of untreated tegmina forewing of *Blatella germanica* appeared normal with its characterized brown colour when observed by the naked eye, while the treated wing appeared to be softer and lighter in colour than the control wing.

Scanning electron micrographs of the normal forewing of *Blatella germanica* showed normal appearance (Fig. 3A) with the wing integument covered by the cuticular scales with smoother distal edges (Fig. 3B). Scanning electron microscopy analysis revealed that treatment of the tegmina forewing of *Blatella germanica* resulted in damage of the wax layer on the surface of the wing with presence of remnants of the nanoemulsin on the surface (Fig 3C) and high magnification revealed malformation of the cuticular scales on the surface and the definition of the individual scales is lost and they are entirely damaged (Fig 3 D).

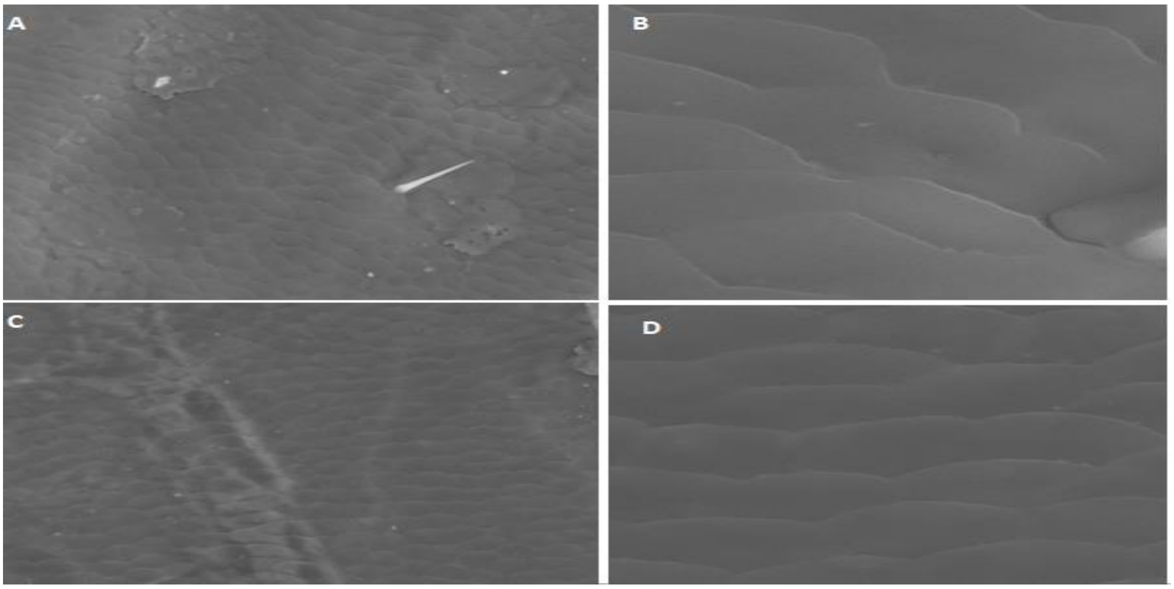

Fig. 2: Scanning electron images of dorsal surface of the tegmina fore wing of *Periplaneta americana*. A: portion of the distal end of untreated wing 500 X; B: more magnification (2 KX); C: portion of the distal end of treated wing with nanoemulsion of *Pimpinella anisum* essential oil (500 X); D: more magnification (2 KX) with no significant change from the untreated wing.

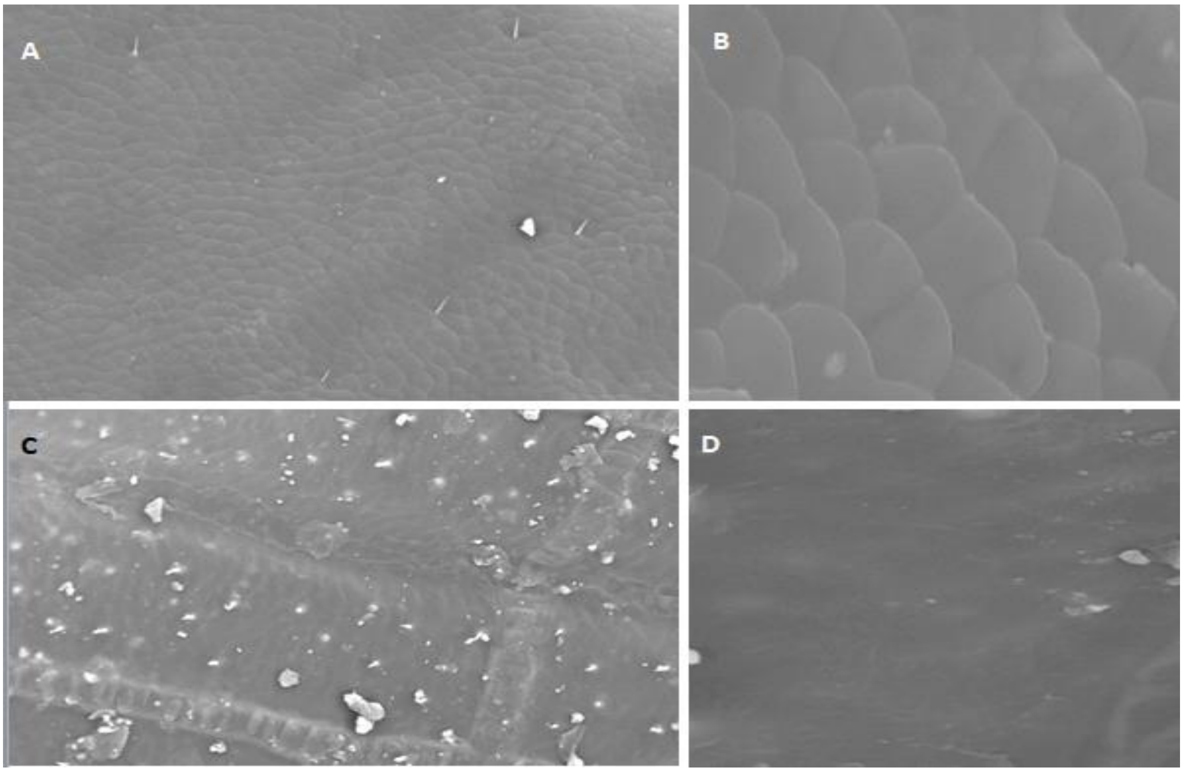

Fig. 3: Scanning electron images of dorsal surface of the tegmina fore wing of *Blatella germanica*. A: portion of the distal end of untreated wing 500 X; B: more magnification (2 KX); C: portion of the distal end of treated wing with nanoemulsion of *Pimpinella anisum* essential oil showing malformation of the integument with loosing of the waxy layer and remnants of the nanoemulsion (500 X); D: more magnification (2 KX) with damage and loosing of the definition of the cuticular scales.

DISCUSSION

Growing concerns about the medical and environmental impacts of synthetic chemical insecticides inspired the researchers for safer control strategies against medical insect pests such as the American and German cockroaches. The plant essential oils are considered as alternative candidates, the nanotechnology allowed innovation in formulation of essential oils into applicable nanoinsecticides.

Despite the enthusiastic research in this field, we still know little about the biological mode of action of the nanoinsecticides containing essential oils; deeper understanding of their biological interactions will develop optimal use of these botanical nanoinsecticides in pests control programs. Generally, three mechanisms correlated to resistance against insecticides have been suggested including the cuticular resistance, this mechanism involves decreasing the insecticides penetration into the insect pest body (Rattan, 2010; Xu *et al.*, 2020).

The current study aimed to study the impact of the nanoemulsion of Pimpinella anisum essential oil on the cuticle of *Periplaneta americana* and *Blatella germanica* using scanning electron microscopy technique.

The mean droplet size of the formulated nanoemulsion droplet was less than 30 nm so it belongs to the nano-scale according to Katas & Alpar (2006). The tiny size of the droplets of the nanoemulsion enhances fast and easy penetration through the larval cuticle. There was no any observed sign of instability such as phase separation or creaming. The Polydispersity index low value indicated the stability and uniformity of the droplet size which provide long-term stability of the nanoemulsion (Anjali *et al.*, 2010). The tween 20 as a surfactant is responsible for stabilization of the nanoemulsion, as it provides a mechanical barrier between the droplets to prevent accumulation (Anjali *et al.*, 2011).

Insect cuticle serves as a barrier and defense line against the penetration of foreign substances; Insect cuticle is divided into procuticle and epicuticle which is a lipid layer covering the outside surface (Moussian, 2013). The epicuticle is principally responsible for impermeability of water phase (Ahmad *et al.*, 2006).

The hydrophobic epicuticle is considered as a final barrier between the outside environment and the insect. In the cockroach there is a flat layer of wax on the surface secreted by the epidermal cells. The cuticular wax generally consists of complex mixtures of lipids that prevent desiccation (Mitov *et al.*, 2018).

The scanning electron micrographs of this study revealed that the nanoemulsion of the *Pimpinella anisum* demolished the wax layer of the wing cuticle of *Blatella germanica*, leading to loss of its hydrophobic property, so the nanoemulsion droplets were adsorbed on the surface of the wing and droplets of the nanoemulsion adhered on the surface as shown in **Fig 3C**, leading to the continued release and diffusion of the droplets into the insect body, allowing the bioactive compounds to reach their target sites. The disorganization of the wax layer will facilitate the transport of the solute by diffusion across the epicuticle as suggested by Lewis 1980. Basically, the hydrophobic property of the oils exerts mechanical effects on the insect such as disturbing the cuticular waxes and blocking spiracles, which cause suffocation and death (Ellse and Wall, 2014).

The effect of the nanoemulsion on the wing of *Periplaneta americana* was less obvious than on that of *Blatella germanica*, this may be due to the different thickness of wings of the two different species. It is suggested that the structural differences including thickness and architecture of the cuticle in the different insect species may explain the different resistance levels exhibited by the insect species against the same insecticide. In addition, the effect of nanoemulsion concentration must be considered, and the role of the surfactant must be taken in consideration. It is recommended to apply a higher concentration of the nanoemulsion on *Periplaneta americana*.

The present study sheds the light on the penetration through the insect cuticle, as it is one of the suggested modes of action of the nanoinsecticides containing essential oils to deepen the understand of how these nanoinsecticide interact with the insect body pest.

Declarations:

Ethical Approval: This research paper was approved by the research ethics committee from Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2024/11/1).

Funding: This work did not receive any funding.

Acknowledgements: Not applicable.

REFERENCES

Ahmad, M., Denholm, I., & Bromilow, R.H. (2006). Delayed cuticular penetration and enhanced metabolism of deltamethrin in pyrethroid-resistant strains of *Helicoverpa armigera* from China and Pakistan. *Pest Management Science*: formerly *Pesticide Science*, 62(9), 805-810.

Alzogaray, R.A., Sfara, V., Moretti, A.N., Zerba, E.N. (2013). Behavioural and toxicological responses of *Blattella germanica* (Dictyoptera: Blattellidae) to monoterpenes. *European Journal of Entomology*, 110, 247–252.

Angioni, A., Barra, A, Coroneo, V., Dessi, S., Cabras, P. (2006). Chemical composition, seasonal variability, and antifungal activity of Lavandula stoechas L. ssp. stoechas essential oils from stem/leaves and flowers. *Journal of Agriculture and Food Chem*istry, 54: 4364-4370.

Anjali, C., Khan, S., Goshen, K. and Magdassi, S. (2010). Formulation of water dispersible nanopermethrin for larvicidal applications. *Ecotoxicology and environmental safety*, 73: 1932-1936.

Anjali, C., Sharma, Y., Mukherjee, A. and Chandrasekaran, N. (2011). Neem oil (*Azadirachta indica*) nanoemulsion - a potent larvicidal agent against *Culex quinquefasciatus*. Wiley Online Library.

Conti, B., Canale, A., Bertoli, A., Gozzini, F., Pistelli, L. (2010). Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitology research*. 107, 1455–1461.

de Oliveira, J.L., Campos, E.V.R., Bakshi, M., Abhilash, P.C., Fraceto, L.F. (2014). Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: prospects and promises. *Biotechnology advances*, 32, 1550–1561.

Duarte, J.; Amado, J.; Oliveira, A.; Cruz, R.; Ferreira, A.; Souto, R.; Falcão, D.; Carvalh,o J. and Fernandes, C. (2015). Evaluation of larvicidal activity of a nanoemulsion of Rosmarinus officinalis essential oil. *Revista Brasileira de Farmacognosia*, 25(2):189– 192.

Ellse, L., Wall, R. (2014). The use of essential oils in veterinary ectoparasite control: a review. *Medical and Veterinary Entomology*, 28, 233–243.

Ghosh, V.; Mukherjee, A.; and Chandrasekaran, N. (2013). Formulation and characterization of plant essential oil based nanoemulsion: evaluation of its Larvicidal activity against *Aedes egypti*. *Asian Journal of Chemistry*, 25:S321- S323.

Isman, M.B. (2017). Bridging the gap: moving botanical insecticides from the laboratory to the farm. *Industrial crops and products*, 110, 10–14.

Isman, M.B., (2020). Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochemistry reviews*, 19 (2), 235–241

Katas, H. & Alpar, H.O. (2006). Development and characterisation of chitosan nanoparticles for siRNA delivery. *Journal of controlled release*, 115(2), 216-225.

Lewis, C.T. (1980). The penetration of cuticle by insecticides. In Cuticle techniques in arthropods (pp. 367-400). New York, NY: Springer New York.

Maiza, A., Aribi, N., Smagghe, G., Kilani-Morakchi, S., Bendjedid, M., Soltani, N. (2013). Sublethal effects on reproduction and biomarkers by spinosad and indoxacarb in cockroaches *Blattella germanica*. *Bulletin of Insectology,* 66, 11–2

Mitov, M., Soldan, V. & Balor, S. (2018). Observation of an anisotropic texture inside the wax layer of insect cuticle. *Arthropod structure & development*, 47(6), 622-626.

Moussian, B. (2013).The arthropod cuticle, in Arthropod Biology and Evolution, ed. by Minelli A, Boxshall G and Fusco G. Springer-Verlag Berlin Heidelberg, Heidelberg, pp. 221–252.

Pai, H.H., Ko, Y.C., Chen, E.R. (2003). Cockroaches (*Periplaneta americana* and *Blattella germanica*) as potential mechanical disseminators of Entamoeba histolytica. *Acta tropica*, 87, 355–359.

Phillips, A.K., Appel, A.G. (2010). Fumigant toxicity of essential oils to the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology*, 103, 781–790.

Rattan, R.S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop protection*, 29, 913–920.

Sugumar, S., Clarke, S., Nirmala, M., Tyagi, B., Mukherjee, A. and Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. *Bulletin of entomological research*, 104(03): 393–402.

Turek, C., Stintzing, F.C. (2013). Stability of essential oils: a review. *Comprehensive reviews in food science and food safety*. 12, 40–53.

Wan, C., Z.X. Hao, and Feng, X.Q. (2016) Structures, properties, and energy-storage mechanisms of the semi-lunar process cuticles in locusts. *Scientific reports,* 6(1), p.35219.

Xu, Y., Yang, X., Sun, X., Li, X., Liu, Z., Yin, Q. & Zhu, C. (2020). Transcription factor FTZ-F1 regulates mosquito cuticular protein CPLCG5 conferring resistance to pyrethroids in *Culex pipiens pallens*. *Parasites & vectors*, 13, 1-13.

Yeom, H.J., Kang, J.S., Kim, G.H., Park, I.K. (2013). Fumigant and contact toxicity of Myrtaceae essential oils and blends of their constituents against adults of German Cockroach (*Blatella germanica*) and their acetylcholinesterase inhibitory activity. *Pesticide Biochemistry and Physiology,* 107, 20