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The Effect of Ethanolic Extract of Stinging Nettle (*Urtica Dioica*) on the Larval Stages of Greater Wax Moth (*Galleria mellonella*) Lepidoptera

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Abstract

The present study was carried out at the Duhok University College Laboratory Centre in the Agricultural Engineering Sciences from September 2021 to June 2022, to evaluate the toxic effect of stinging nettle ethanolic extracts of stinging nettle (*Urtica dioica*) at different concentrations on the larval stages of the Greater wax moth (*Galleria mellonella*, Lepidoptera: Pyralidae). The Soxhlet extraction method was used in this study, with five concentrations (0.5, 1, 1.5, 2.5, and 3.5 mg/ml) of the ethanolic plant extract after 24, 48, and 72 hours of exposure. The third and fifth instar larvae of the insect were examined under the conditions (at 30±1°C and 70±5% R.H). There was a highly significant influence ($P < 0.001$) on concentrations, affected mortality statistically significant ($P < 0.05$). The results of the stinging nettle extracted at 3.5 mg/ml after 72 hours of exposure were obtained and the highest mortality rate recorded in the 3rd and 5th instar larvae which were 96.66 and 86.66% respectively.

The ethanolic Nettle Stinging extract was testified to cause morphological deformations in both larval instars of the insect.

Keywords: *Urtica dioica*, *Galleria mellonella.* Extraction method, mortality rate

INTRODUCTION

One of the most significant pests of honeybee colonies is the Greater Wax Moth (GWM) (*Galleria mellonella* Linnaeus), which belongs to the family Pyralidae (order Lepidoptera) (Al-sinjari, 2017). GWM larvae are extremely destructive, economically important and destroying storage beeswax combs in a matter of minutes. They penetrate and chew their way through combs, feeding on wax, pollen, and cocoons of bee larvae (**Ellis** *et al*., 2013). Pathogens of major bee diseases, such as foulbrood, can also be transferred from hive to hive by adults and GWM larvae (Beyene and Woldatsadik, 2019). Many insecticides are used to control and exterminate insect pests, but in many cases, the pests would acclimate themselves to the toxins and develop resistance (Lynch *et al*., 1999). Plant-chemical products are important for the life and development of plants, some of which are called secondary products or natural products. These products are partially made in the plant cell, and they are considered as defensive means against the animals and insects that attack the plants so protected (Perveen *et al.,* 2008). Botanical insecticides, which employ plant extracts as active components, are less harmful to humans and the environment than synthetic pesticides (Dadang and Prijono, 2009). These substances can affect the insect's nutritional behaviour, act as repellents or inhibitors, slow the insect's growth and development, or turn it infertile (Al-Joary *et al.,* 2021). Stinging nettle *Urtica dioica* L. is an herbaceous perennial herb flower plant that is native to many warmer temperate regions of the world, including Asia, Europe, North America, and North Africa (Bisht *et al.,* 2012). This species belongs to Urticaceae botanical family that grows annually in the wild (Ori *et al.,* 2014). As there are few studies on the effects of *Urtica dioica* extract on insects, and also few studies about the effect of this plant on GWM in Iraq, this study was suggested, aiming to create alternatives of chemical insecticides from natural resources (plants) by using *Urtica dioica* extract to control *Galleria mellonella* L.

MATERIALS AND METHODS

Insect rearing

The infected hive by the Greater Wax Moth *Galleria mellonella* L. was obtained from the University of Agriculture Engineering Science / Duhok and other samples from Seje village/ Sumel region in August 2021. To obtain a pure culture, infested wax cubes (feeding medium) were cut and transferred to a clean 5L capacity plastic container. The merged moths were taken to new containers fortified with uninfected waxes and left to copulate and lay eggs. After hatching, the larvae were monitored to obtain the desired instars for the following assay. Rearing and treatments were carried out under incubator conditions (at $30\pm1C^{\circ}$ and $70\pm5\%$ R.H), the maintenance of the colonies was carried out by re-generating them after each generation (Al-Sinjari, 2017). The larval stages that had been used in this study were larvae in the 3^{rd} and 5^{th} instar.

Plant collection

Nettle (*Urtica dioica* L.), which is a member of the Urticaceae family, was collected from Derishke and Qumurey village/ Kani Massi sub-district/ Kurdistan Region/ Iraq in May 2021. After collection, the plants were washed (to remove dust) with tap water, the plants were dried in shadow and the entire plant (except the root) was ground to fine powder by an electric mill cutter, then the powder obtained powder was kept in closed plastic bags and placed at room temperature until the time of use (**Abdulmajeed** and AL Chalabi, 2011).

Method for Plant Extraction

The extraction of bioactive chemicals from *U. dioica* has been developed utilising both traditional procedures and various innovative techniques, and ethanol was the most productive organic solvent used, leading to the greatest extraction yield. The plant extract was prepared according to Banat *et al.* (2013) with slight variation. For one cycle of the Soxhlet apparatus, 20 g of plant powder (*U. dioica*) was placed in the Soxhlet continuous extraction apparatus. The oil was extracted from it with 300 ml of 70% ethanol (60-80C ° boiling point). The yielded solution was later filtered and stored in a refrigerator until separated by a vacuum rotary evaporator.

Bioassay

Four concentrations were prepared from the stock solution (0.5, 1, 1.5, 2.5 mg/ml) using dechlorinated tap water and were used for spraying the larvae. *Galleria mellonella.* Due to their large size and fluidity in dealing with them, the third and fifth instar larvae of the GWM were isolated, and each ten larvae were placed in an 80 cm^3 plastic bag shortly before treatment (Birah *et al.*, 2008). The surface treatment technique was used for the 3^{rd} and 5^{th} instar larvae of the GWM and the effect of different concentrations of plant extract was studied by exposing three replications (each of 10 larvae) of each concentration. For the control groups, they were treated with tap water by exposing the same replications under the same laboratory conditions. The treated insects were examined after 15 minutes, then, they were placed in Petri dishes and stored in the incubator to ensure their survival and to replace any abnormal insects, if any mortality rates were recorded for larvae of the insect after 24, 48 and 72 hours, After that, the following calculation was used to correct the mortality rates using Abbott (1925) equation:

M%= M% in certain dose- control M% / (100 - % control mortality) * 100

where M = Mortality

Statistical analysis

Data were analyzed using the analysis of variance technique in the form of two-way ANOVA, using the Minitab software package 17. Subsequently, the Tukey test was used to compare means.

RESULT AND DISCUSSION

The effect of *U. dioica* on the 3rd instar larvae

The purpose of this experiment was to evaluate the insecticidal activity of ethanolic extracts of *U. dioica* against *Galleria mellonella* third instar larvae. Table (1) shows the effectiveness of plant extracts on larval mortality exposed to various doses over various periods, the percentage of mortality increased as the dose of plant extract increased. The highest mortality rate was 93.33% at 3.5 mg/ml, while the lowest mortality percentage of 13.33% was observed after 24 hrs of exposure at 0.5 mg/ml dose.

Table 1: The effect of *U. dioica* extract on the third instar larvae of *Galleria mellonella* at different exposure periods.

Concentrations mg/ml	% Mortality		
	24 hrs	48 hrs	72 hrs
Control	10.00 b	13.33 b	13.33 b
0.5	13.33 b	16.66 b	20 b
1	16.66 b	20.00 b	26.66 b
1.5	86.66 a	86.66 a	86.66 a
2.5	90,00 a	90,00 a	90.00 a
3.5	93.33 a	93.33 a	96.66 a

	Dose	Time	Dose*Time
***P* value**	$P < 0.001$	$P > 0.05$	$P > 0.05$

Means that do not share a letter in any of the columns are statistically significant (P < 0.05).

When the concentration increased to 3.5 mg/ml, the mortality rate increased to 93.33%, while the lowest mortality rate (16.66%) was obtained when the concentration was 0.5 mg/ml after 48 hours of exposure. A concentration of 3.5 mg/ml was shown to increase mortality in the control group by 20% to 96.66% after 72 hours of exposure. Statistical analysis showed that there was a significant effect of concentrations on the mortality rate ($P < 0.001$. The dead larvae that had been treated with a concentration of 1 mg/ml of *U. dioica* compared to the control larva shown in **Fig. (1).** The cause of larval death is due to the biochemical analysis of nettle plant extracts (PEs) as a natural bioinsecticide that revealed that the nettle extracts were insect repellents and are often used as a prophylactic measure (**Popescu** *et al.*, 2014). In addition, the poisonous effect of the nettle extracts may be due to the containing of some chemical compounds, especially formic acid, which can kill the larval instar (Coile, 1999).

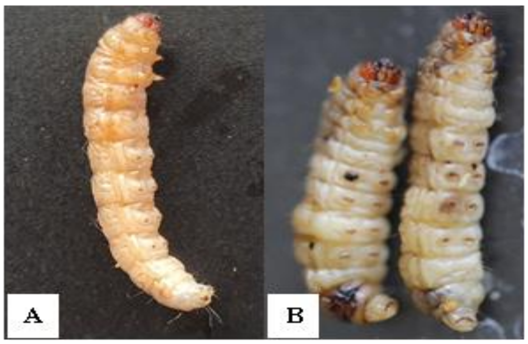

Figure 1: B: Dead third instar larvae of *G. mellonella* treated with 1 mg/ml of *U. dioica* ethanolic extract. **A**: Larva of control treatment. (Canon: EOS 600D).

Fawzy *et al*. (2017) investigated the influence of natural compounds on *G. mellonella* larvae, the highest effect (26.67%) was recorded in peppermint ethanolic extract, which was recorded at 4% concentration, followed by China's propolis and cinnamon (30.33% and 30.08%, respectively) at 4% concentration. In comparison to the present study, the effect of *U. dioica* extract was more lethal than the effect of the above PEs. The toxicity of *Azadirachta indica* and *Ocimum basilicum* extracts at a concentration of 15% against larvae of *G. mellonella*. They induced the highest mortality rate (100%) among larvae, while the low larval mortality (42.82%) was obtained from *Vebascum sinatticum* after 48 h of exposure period (Beyene and Woldatsadik, 2019).

The effect on fifth instar larvae

The effect of *U. dioica* extract on larvae of the fifth instar of *G. mellonella* is demonstrated in **Table (2)**. After 24 hours of exposure, a high mortality rate (80%) was identified at a concentration of 3.5 mg/ml, while the lower mortality rate (40%) was reported at a concentration of 0.5 mg/ml, compared to the control larval mortality rate of 13.33 percent. After 48 hours of exposure, the mortality rate increased at a concentration of 3.5 mg/ml (83.33%), while it was the lowest (53.33%) at a concentration of 0.5 mg/ml, and the mortality rate slightly increased to 86.66% at a concentration of 3.5 mg/ml after 72 hours of exposure, while mortality was low (60%) at a concentration of 0.5 mg/ml. The impact of concentrations on mortality was statistically significant ($P < 0.001$), whereas the effects of time and interactions between times and concentrations were not significant ($P > 0.05$).

The dead dark-spotted fifth instar larva of *G. mellonella* was caused by its treatment with a concentration of 1.5 mg / ml of *U. dioica* extract is shown in **Fig. (2)**.

Table 2: The effect of *U. dioica* extract on the fifth instar larvae of *Galleria mellonella* at different exposure periods.

Concentrations mg/ml	% Mortality		
	24 hrs	**48 hrs**	**72 hrs**
Control	13.33 c	13.33 c	13.33 c
0.5	40.oo bc	53.33 ab	60.00 ab
1	53.33 ab	66.66 ab	66.66 ab
1.5	66.66 ab	66.66 ab	73.33 ab
2.5	76.66 a	80.00 a	80.00 a
3.5	80.00 a	83.33 a	86.66 a
	Dose	**Time**	**Dose*Time**
***P* value**	$P < 0.001$	$P > 0.05$	$P > 0.05$

Means that do not share a letter in any of the columns are statistically significant (P < 0.05).

Figure 2: B: Dead fifth instar larva of *G. mellonella* treated with 1.5 mg/ml of *U. dioica* ethanolic extract. **A**: Larva of control treatment. (Canon: EOS 600D).

The alcoholic extract of the leaves of the *E. camaldulensis* tree was more harmful than other PEs, including mint, clove, thyme, lemongrass oil and basil when tested on *G. mellonella* fifth instar. The results obtained revealed that the materials effectiveness of the examined varied. As a result, extremely effective oils (methyl salicylate, formic acid, clove, and basil oils) were used to protect wax combs normally stored in the apiary (Owayss and Abd-Elgayed, 2007). Eucalyptus oil (*Eucalyptus* spp.) has been the main component of eucalyptol and can successfully handle numerous plant pests, such as the lesser mealworm *Alphitobius diaperinus* (Pinto Junior *et al*., 2010) and the fall armyworm *Spodoptera frugiperda* (Souza *et al*., 2010). Typically, *U. dioica* was used in the form of ethanol extract, as bioinsecticides, and for foliar spray; it is supposed to repel insects (Marii *et al*., 2021).

The comparative effect of *U. dioica* extract on larvae of third and fifth instars after different period of exposure.

Table (3) shows the comparative effect of *U. dioica* extracts on the third and 5th instar larvae of *G. mellonella* after 24, 48 and 72 hours of exposure. Mortality was affected by *U. dioica* extracts, particularly at concentrations of 0.5 and 1 mg/ml. The highest percentage of mortality (96.66%) was found at a concentration of 3.5 mg/ml for larvae of the third instar after 72 hrs. exposure, while the lower percentage of mortality (13.33%) was found at a concentration of 0.5 mg/ml for the third instar larvae after 24 hrs of exposure.

Table 3: Comparative effect of *U. dioica* extract on the third and 5th instar larvae of *G. mellonella* after different time periods of exposure.

Concentrations mg/ml	% Mortality					
	After 24 hrs exposure		After 48 hrs exposure		After 72 hrs exposure	
	3rd instar larvae	5th instar larvae	3rd instar larvae	5th instar larvae	3rd instars larvae	5th instar larvae
Control	10.00	13.33	13.33	13.33	13.33	13.33
0.5	13.33	40.00	16.66	53.33	20.00	60.00
1	16.66	53.33	20.00	66.66	26.66	66.66
1.5	86.66	66.66	86.66	66.66	86.66	73.33
2.5	90.00	76.66	90.00	80.00	90.00	80.00
3.5	93.33	80.00	93.33	83.33	96.66	86.66

The larval mortality achieved in the present study is equivalent to the study done by Al-Sinjari (2017) who examined the toxic effect of Actara and ginger oil of *Zingiber officinale*, individually or in combination in larvae of G. mellonella in the 3rd and 5th instar, after 24 hours of exposure to the 1:3 mixing ratio. Exposure to Actara insecticide and ginger oil of the same concentrations caused mortality rates of 53.30, 63.00 and 90.00 % (for the third instar), and 33.30, 46.60 and 63.00% (for the fifth instar), respectively. A study was conducted to determine the efficacy of 13 medicinal PEs against *G. mellonella* larvae in the laboratory where larval mortality was greatest (93.33%) with extract prepared with *P. psyllium* husk (Lalita and Yadav, 2018). The effects from ethanolic extracts of many plants on the growth of GWM were investigated. *Abrus precatorius*, *Laurus nobilis*, *Petroselinum sativum*, and *Plantago psyllium extracts* were insecticidal against the moth, killing 100 or 95% of the wax moths (Zaitoun, 2007). The results of the current study agree with the mentioned studies.

CONCLUSION

The toxicity of *Urtica dioica* ethanolic extracts increases as concentrations rise, the 3rd instar larvae were more sensitive to the plant extract than the 5th instar larvae, the effect of time periods had statistically the same effect at the first time of exposure (24 hours) meaning that the effect of plant extract was lethal after the first period especially with the 3rd larval instar and finally, the influence of plant extract resulted in the establishment of deadly morphological deformations in both instars of larvae.

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Conflicts of Interest/ Competing interest

All authors declare that they have no conflicts of interest.

Data availability statement:

All data sets collected and analyzed during the current study are available from the corresponding author on reasonable request.

References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*, *18*(2), 265–267.

Abdulmajeed, A. M. and Al-Chalabi, B. M. (2011). The cumulative effects of Tavri Shrub (*Daphne Mucronata*) and Stinging Nettle (*Urtica dioica*) extracts on some biological aspects of Mosquito *Culex Molestus* Forskal', *M.S.c thesis submited to the Council of the college of education, University of*

Al-Joary, Y. I. M. A., Al-Obaidi, RG, and Al-Ejaidy, S. (2021). 'Study of the repellent activity of some medicinal herbs powder against adults of *Tribolium confusum* duv.(tenebrionidae: coleoptera).' *Plant Archives*, *21*(1), 445–450.

Al-Sinjari, S.H.S. (2017). The Effect of the Different Concentration and Proportion of the Actara Insecticide and Ginger Oil Mixtures on the Mortality Rate of Larvae of Greater Wax Moth Larvae, *Galleria mellonella* L. *Science Journal of the University of Zakho*, *5*(1), 32–36.

Banat, F., Pal, P., Jwaied, N., and Al-Rabadi, A. (2013). Extraction of olive oil from olive cake using the soxhlet apparatus. *American Journal of Oil and Chemical Technologies*, *1*(4), 1–8.

Beyene, T. and Woldatsadik, M. (2019). Laboratory evaluation of the effectiveness of some botanical extracts against the larvae of the greater wax moth, *Galleria mellonella* (L.).

Birah, A., Chilana, P., Shukla, U. K., and Gupta, G. P. (2008). Mass rearing of greater wax moth (*Galleria mellonella* L.) on artificial diet. *Indian Journal of Entomology*, *70*(4), 389–392.

Bisht, S.,, Bhandari, S. and Bisht, N. S. (2012). *Urtica dioica* (L): an undervalued and economically important plant. *Agric Sci Res J*, *2*(5), 250–252.

Coile, N. C. (1999). *Urtica chamaedryoides* Pursh: a stinging nettle, or fireweed, and some related species. *Fla. Department of Agriculture and Consumer Services, Division of Plant Industry.*

Dadang, E.D. F. and Prijono, D. (2009). Effectiveness of two botanical insecticide formulations for two major cabbage insect pests in field application. *J. ISSAAS*, *15*(1), 42–51.

Ellis, J. D., Graham, J.R., and Mortensen, A. (2013). Standard methods for wax moth research. *Journal of Apicultural Research*, *52*(1), 1–17.

Fawzy, A. M., Al-Ahmadi, S., S. and Al-Hazmi, H. M. (2017). Influence of some natural substances to control the Greater Wax Moth *Galleria mellonella* l.(Lepidoptera: Pyralidae). *Journal of Plant Protection and Pathology*, *8*(8), 407–413.

Lalita, Y.K. and Yadav, S. (2018). Effectiveness of different plant extracts against larvae of *Galleria mellonella* in laboratory. *Archives of Agriculture and Environmental Science*, *3*(1), 64–67.

Lynch, R. E.; Wiseman, BR; and Plaisted, D. (1999). Evaluation of transgenic sweet corn hybrids expressing Cry 1 a toxin for resistance to earworm and fall armyworm (Lepidoptera: Noctuidae). Environmental Entomology, 92: 246-252,

Marii, B., Radman, S., Romić, M., Perkovi, J., Major, N., Urlić, B., Pali, I., Ban, D., Zorić, Z. and Ban, S. G. (2021). Stinging Nettle (*Urtica dioica* L.) as an Aqueous Plant-Based Extract Fertiliser in Green Bean (*Phaseolus vulgaris* L.) Sustainable Agriculture. *Sustainability*, *13*(7), 4042.

Ori, D., Franciškoviċ, M., Bekvalac, K., Svirev, E., Beara, I., Lesjak, M. and Mimica-Duki, N. (2014). Quantitative determination of plant phenolics in *Urtica dioica* extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. *Food Chemistry*, *143*, 48–53.

Owayss, A. A. and Abd-Elgayed, A. A. (2007). Potential efficacy of certain volatile oils and chemicals against the wax moth, *Galleria mellonella* L.(lepidoptera: pyralidae). *Bull. Ent. Soc. Egypt*, *Econ. Ser*, *33*, 67–75.

Perveen, A.; Sarwar, G. and Hussain, I. (2008). Plant Biodiversity phytosociological attributes. Pak. J. Bot., 40(1): 17-24,

Pinto Junior, A. R., Carvalho, R. I. N. de, Netto, S. P., Weber, S. H., Souza, E. de and Furiatti, R. S. (2010). Bioatividade de óleos essenciais de sassafrás y eucalipto em cascudinho. *Ciência Rural*, *40*(3), 637–643.

Popescu, C., Pruteanu, A., Voicea, I., Ivancu, B., Gageanu, G., Popa, L., and Vladut, V. (2014). Study on the biochemical characterization and some preparations from nettle and wormwood in order to capitalise them as bioinsecticide/biofertilizers in organic agriculture. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, *44*(2), 175–185.

Souza, T. F., Fevero, S., and Conte, C. de O. (2010). Bioatividade de óleos essenciais de espécies de eucalipto para o controle de Spodoptera frugiperda (JE Smith, 1797) (Lepidoptera: Noctuidae). *Revista Brasileira de Agroecologia*, *5*(2), 157–164.

Zaitoun, S. T. (2007). The effect of different mediterranean plant extracts on the development of the great wax moth *Galleria mellonella* L.(Lepidoptera: Pyralidae) and their toxicity to worker honeybees *Apis mellifera* L.(Hymenoptera: Apidae) under laboratory conditions. *Journal of Food Agriculture and Environment*, *5*(2), 289.