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THE EFFECT OF URTICA DIOICA EXTRACTS ON THIRD LARVAL INSTARS OF TRIBOLIUM CONFUSUM (DU VAL) USING TWO METHODS OF EXTRACTION

Mehvan M. Ali Rasheed [a,*], Badia'a Mahmoud Al-Chalabi [a], Sofyan H. Sedo Al-Sinjari [a]

[a] Dept. of Biology, Faculty of Science, University of Zakho, Zakho, Kurdistan Region, Iraq – (Mehvan.ali@stud.uoz.edu.krd; badeyamahmoud50@gmail.com; sofyan.sedo@uoz.edu.krd)

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ABSTRACT:

The effect of ethanolic extracts of *Urtica dioica* on mortality of the third Larval instar of *Tribolium confusum,* was studied using the Soxhlet and solvent extraction methods. Experiments at 30 ± 1°C and 70 ± 5% relative humidity. In laboratory conditions. The mortality data were recorded after 24, 48, and 72 hours. The mortality rate increased by an increase in concentrations (0.5, 1, 1.5, and 2.5 mg/ml), the highest mortality rate was 100% at 1.5 mg/ml, while the lowest percentage of mortality 73.33% was observed after 24 hrs of exposure at 0.5 mg/ml dose by Soxhlet extraction method, whereas the highest mortality rates 100% at 2.5 mg/ml concentration, while the lowest percentage of mortality 90% was observed after 24 hrs of exposure at 0.5 mg/ml dose by Solvent extraction methods. The results show that the plant extracts tested at different concentrations were toxic to the larvae of *T. confusum*, and both methods of extraction had nearly similar effects on larvae.

KEYWORDS: *Urtica dioica*, *Tribolium confusum*, Extraction methods, mortality rate.

1. INTRODUCTION

The confused flour beetle (CFB) (*Tribolium confusum* Du Val) of the family Tenebrionidae (order Coleoptera), is among the greatest common and harmful pests of stored products, such as flour mills, cereals, and other products worldwide (AL-Jamil and AL-Sinjari, 2019). Verheggen *et al.* (2007) state that their adults produce benzo-quinones and volatile hydrocarbons as defensive compounds that act as repellents on conspecific larvae and adults. Contamination of the human food supply is one of the most important dangers that people face (Al Abadi *et al*., 2008). These pests can cause post-harvest losses of 9% or more in developed countries and 20% or more in developing countries (Phillips and Throne, 2010). Currently, methyl bromide and phosphine are the most often used fumigants for suppressing grain-infesting insects. However, their usage is restricted due to the detrimental impact they have on the environment, where the use of natural compounds as fumigants is an alternative technique for preventing insect infestations of stored products (Lee *et al*., 2004). 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 plant-natural insecticides are employed directly by drying, grinding, and blending their powders with grains, or they are utilized indirectly by extracting dangerous chemicals in various methods and combining them with alcohol (Derbalah and Ahmed, 2011). 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). Farmers use a variety of plant botanicals as insecticides, including Neem, garlic, onions, stinging nettle, Mexican sunflower, Chilies, Hot pepper, Tobacco, Pyrethrum, and Chilies (Kareru *et al*., 2013). Citronella, eucalyptus, cinnamon, turmeric, ginger, and galangal might be used to protect stored products (Lee *et al*., 2004), contact insecticides (Ngamo *et al*., 2007), repellent effects (García *et al*., 2005), and also reduce ovicidal activities (Raja and William, 2008).

Very few studies are available in the Kurdistan region of Iraq about the comparative effects using some plant extracts of stinging nettle (*Urtica dioica*) to control (*Tribolium confusum*). Therefore, the purpose of this study to investigating the effective compounds of *U. dioica* extract on 3rd instar larvae of CFB and compare the effect of two different methods of extractions.

2. MATERIALS AND METHODS

2.1 Insect rearing

The infected flour by *T. confusum* was obtained from Kurdistan shaking store for flour/Sumel region/Duhok/Iraq, then placed inside a container of 3L capacity and added to it 2kg of sterilized flour (stored in the freezer for one week). After one month, when the growth rate of CFB was increased they were placed in 10 wide-mouth plastic jars of 1L volume, 100 adult insects were placed, after covering the nozzles of the bottles with sterile gauze and fixing them with rubber bands, they were kept in an incubator in below conditions (Saleem *et al*., 2013). The beetles were fed on flour containing 5% yeast. In an incubator, the plastic containers were kept in the dark. (Esmaili *et al*., 2013). Rearing was controlled under incubator circumstances (Binder) (at 30±1C° and 70±5% R.H) (Al-Sinjari and Al-Attar, 2015). This experiment utilized the 3^{rd} instar larvae of *T. confusum*.

2.2 Botanical insecticide

Nettle (*U. dioica* L.) is a Urticaceae family member that can be found in North America and Europe, and it is a perennial herb with stinging trichomes. (Bisht *et al*., 2012). The plant trichomes are naturally built to defend themselves from insects (Kregiel *et al*., 2018). Which contains formic acid, histamine,

* Corresponding author

acetylcholine, and serotonin (Austin, 2008; Bourgeois *et al*., 2016).

2.3 Plant collection

Urtica dioica was collected from Derishke and Qumurey village/ Kani Massi sub-district/ Kurdistan Region/ Iraq in May 2021. After collection, the plants were washed (for removing the dust) with tap water, the plants were dried in shadow and the entire plant (except the root) was ground into fine powder by an electric mill cutter (Silver Crest 3000W), then the obtained powder was kept in closed plastic bags and placed at room temperature until the time of use (Abdulmajeed and Al-Chalabi, 2017).

2.4 Methods for the Plant Extraction

The extraction of bioactive chemicals from *U. dioica* has been developed utilizing both traditional procedures and various innovative techniques, including maceration, reflux, Soxhlet, hydro distillation, ultrasound- and microwave-assisted extraction (UAE), and microwave-assisted extraction (MAE). When using these methods to extract bioactive chemicals, ethanol was the most productive organic solvent used, leading to the greatest extraction yield. Additionally, the yield of isolated chemicals increased when extraction time was increased. The Soxhlet method provided the highest extraction yield among traditional techniques (Bandar *et al*., 2013).
Two methods of extraction were used in this study: Soxhlet and solvent extraction methods.

2.4.1 Soxhlet apparatus: The plant extract was prepared according to Banat *et al.* (2013) with slight variation. For one cycle of the Soxhlet apparatus, 20 gm of plant powder (*U. dioica*) was placed in the Soxhlet continuous extraction apparatus. The oil was extracted from it with 300 ml of ethanol 70% (60-80C° boiling point). The yielded solution was later filtered using filter papers and stored in a refrigerator until separated by a vacuum rotary evaporator.

2.4.2 Solvent extraction method: The plant extract was prepared according to Bashir and Al-Habib, (2021) with slight modification, where 50 gm of *U. dioica* powder had been mixed with 200 ml of ethanol 70% and put in a flask of 1000 ml capacity, and stored in ultra-low temperature freezer (-10 to -15 C°) for 24 hrs. Then, all solids were eliminated by a filtration process that requires just a vacuum pump, a Buchner funnel, two filter papers, and a vacuum flask. After the filtration (Whatman No.1 filter paper), a solution of ethanol and extract remained. Both methods of extraction need to be separated from the ethanol, for separating a rotary evaporator was used at 45 C°, 80 rpm, and 0 bar. The resultant extract or oil was fully ethanol-free (Scientific, 2018). The extracts were put in glass Petri plates and dried in an oven until the weight of the extracts was set before being stored in a refrigerator until needed.

2.5 Bioassay

Four concentrations were prepared from stock solution (0.5, 1, 1.5, 2.5 mg/ml) using dechlorinated tap water, and were used for spraying the 3rd instar larvae of *T. confusum*. Thirty larvae for each concentration are placed in sterile Petri dishes (100 X 15mm) divided into three sections (ten for each section) and enclosed by a Petri dish to avoid insects escaping. During the bio-test, each day the number of dead larvae was recorded. The larval mortalities were calculated and compared with the control (sprayed with dechlorinated water) of both methods. For each test, three replications were set up. Treated larvae in all groups were incubated at 30 ± 1 C° and R.H of 70% ± 5 (Al-Sinjari and Al-Attar, 2015). The Results were calculated after 24, 48, and 72 hours. The percentage of larval mortality was determined using the Abbott formula (Abbott, 1925).

$$M\% = \frac{M\%\ in\ certain\ dose - control\ M\%}{(100) - \%\ control\ mortality} * 100 \qquad (1)$$

Where M = Mortality

2.6 Statistical Analysis

The data were analysed using the analysis of variance technique in the form of Two-way ANOVA, using the Minitab software package 17. Subsequently, Tukey's test was used to compare means.

2.7 Ethical approval

Ethical approval was obtained from the Protocol Review Committee of the Biological Sciences Committee (BSCZ) of the University of Zakho (ID: "BSCZ/25/7/2021").

3. RESULT AND DISCUSSION

3.1 The effect of *U. dioica* on the 3rd instar larvae using the Soxhlet method

The goal of this experiment was to evaluate the insecticidal activity of *U. dioica* extracts against *T. confusum* third-instar larvae. **Table (3.1)** shows the effectiveness of plant extracts on *T. confusum* mortality exposed to various doses across various periods by the Soxhlet extraction method.
The percentage of mortality increased as the dose of plant extract increased. The highest mortality rate was 100% at 1.5 mg/ml, while the lowest percentage of mortality 73.33% was observed after 24 hrs of exposure at 0.5 mg/ml dose.

Table 3-1: The effect of *U. dioica* extract on the 3rd instar larvae of *T. confusum* at different exposure periods by the Soxhlet method.

Doses mg/ml	% Mortality		
	24 hrs	**48 hrs**	**72 hrs**
Control	16.66 d	16.66 d	16.66 d
0.5	73.33 c	80 c	83.33 bc
1	96.66 ab	96.66 ab	96.66 ab
1.5	100 a	100 a	100 a
2.5	100 a	100 a	100 a
	Times	**Dose**	**Times*Doses**
P value	0.611	< 0.0001	0.879
F value	379.44	0.50	0.50
At a p-value of 0.05, the means that do not share a letter in any of the columns are statistically significant.			

It was detected that there was a significant effect of doses on mortality ($p < 0.001$), but the effect of time and interactions was found to be not significantly affected ($p > 0.05$) mortality. The significantly lower value of mortality was recorded under control compared to another dose. Generally, the mortality increased with increasing doses. No significant interaction was found between time and dose, meaning that the effect of dose was the same for three periods of time.

3.2 The effect of *U. dioica* on the 3rd instar larvae using solvent extraction method

Table (3.2) displays the effect of *U. dioica* with different doses on the mortality percentage of 3rd instar larvae of *T. confusum*. The percentage of mortality increased as the dose of plant extract increased. Where the highest mortality rate was 1 of 00% at 2.5 mg/ml concentration, while the lowest percentage of mortality was 90% observed after 24 hrs of exposure at 0.5 mg/ml dose.

Table 3-2: The effect of *U. dioica* extract on the 3rd instar larvae of *T. confusum* at different exposure periods by Extraction method.

Doses mg/ml	% Mortality		
	24 hrs	48 hrs	72 hrs
Control	16.66 b	16.66 b	20 b
0.5	90 a	90 a	93.33 a
1	90 a	90 a	93.33 a
1.5	93.33 a	96.66 a	96.66 a
2.5	100 a	100 a	100 a
	Times	Doses	Times*Doses
P value	0.285	< 0.0001	0.993
F value	497.68	1.30	0.22

The effect of dosages on mortality was found to be significant ($P < 0.001$), however, the effect of time and interactions with dose on mortality was not significant ($p > 0.05$). The significantly lower value of mortality was recorded under control compared to other doses. By raising the dosage, the mortality rate was increased. Interactions between time and dose were determined to be non-significant, indicating that the dose had the same effect over three time periods.

3.3 The comparative effect of *U. dioica* on the 3rd instar larvae using Soxhlet and solvent extraction methods

Figure (3.1) shows the comparative effect of treatment with different concentrations and two methods of extractions on the mortality percentage of 3rd instar larvae of *T. confusum*.

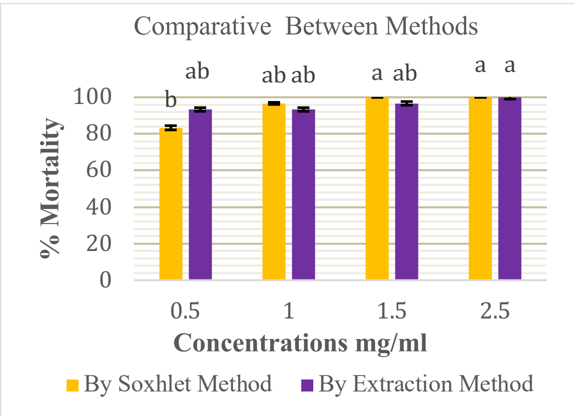

Figure 3-1: The comparative effect of *U. dioica* extract on the 3rd instar larvae of *T. confusum* after 72 hrs exposure by using Soxhlet and Extraction methods.

The results revealed that there were no significant differences between both used methods in terms of their effect on the mortality of insects ($P > 0.05$ and $F = 0.13$). In general, increasing doses led to a rise in mortality. Regarding interactions, no significant interaction was seen between the method and dose.

The mortality of the larvae via both procedures may be attributable to the presence of chemical compounds such as formic acid (Coile, 1999). Lectin and iron in great quantity, histamine, and serotonin (Austin, 2008; Bourgeois *et al.*, 2016; Kregiel *et al.*, 2018). These compounds might disturb the digestive system of larvae especially the midgut and gastric caecum (Ndione *et al.*, 2007). Furthermore, extremely hazardous plant extracts are rich in non-polar saturated fatty acids that rapidly permeate the cuticle layers and subsequently have a harmful effect. (Zhou *et al.*, 2005). In addition to the persistence of extracts on the outside surface of the insect's body, which covers the spiracles and so impedes the insect's ability to breathe, the extracts also close the insect's spiracles (Fathi and Shakarami, 2014). James *et al.* (2015) Researchers examined the relationship between parasitic, predatory, and helpful insects with *U. dioica*. The research concluded that stinging nettle may serve as an essential home for helpful insects and play a role in encouraging biological management to protect crops.

These results are consistent with prior studies indicating that several plant extracts are poisonous to *T. confusum* larvae. Numerous studies have shown that some plant extracts have potent larvicidal actions against insect pests (Liu *et al.*, 2012; Mahfuz and Khanam, 2007; Zaka *et al.*, 2019). The results of the current research were similar to the study of sixteen aromatic plant extracts that were assessed for their contact toxicity on *T. confusum* larvae. *Mantisalca duriaei* methanolic and ethyl acetate extracts and *Rhaponticum acaule* petroleum ether, chloroformic, and methanolic extracts substantially inhibited larval growth. Although only *R. acaule* has a very toxic impact on larvae for all extracts, larval mortality was the greatest. Using petroleum ether and methanol extracts of *R. acaule*, it attained 83% and 77%, respectively (Boussaada et al., 2008). The results of the current study are relatively in accordance with those reported by Ali and Mohammed (2013) who investigated the lethality of the larval stage of *T. confusum* using methanol extracts of six indigenous plants from the Kurdistan area. The most effective of the six test plant extracts was *Zingiber officinale* with a 100% death rate at 2 hours, followed by *Anethum graveolens* at 56.67% after 4.5 hours, which is the least poisonous to larvae.

In addition, the comparable study investigated the repellence of ethanolic extracts of *E. glauca* against *T. confusum* larvae. Effective repellents have been discovered in all plant extracts, with the *E. gluaca* activating at a concentration of 7.5% to 100% repellency (Mohammed, 2013).

A study was accepted to assess the toxicity of lambda-cyhalothrin and the essential oils of cardamom seeds from *Elettaria cardamomum* L., either separately or in combination, to the larval stages of the *T. confusum*. The outcomes demonstrated that the oil extract was substantially more effective than the insecticide lambda. Larvae exposed to cardamom oil at concentrations of 0.1, 0.3, 0.5, and 1 µl /larva experienced mortality rates of 60, 76.67, 90, and 96.67 percent, respectively (Al-Sinjari and Al-Attar, 2015). The effect of cardamom seeds extracts was the same as the effect of Nettle extracts used in this research. This is consistent with the results of Saidana *et al.* (2007), who discovered that petroleum ether extracts from a variety of plant species were very efficient against CFB larvae and adults, with the larvae being more susceptible than the adults. Another study by AL-Jamil and AL-Sinjari (2019) showed the death rate of *T. confusum* increased as the ratio of castor extract to insecticide lambda increased, and the toxicity varied depending on the surface type.

Also, Khoshnoud and Khayamy (2008) addressing the insecticidal activities of an ethanolic extract of *Verbascum cheiranthifolium* against two insect pest species of stored products demonstrated that insect mortality rose as the dose rate increased. AL-Attar (2006) indicates that Actara has strong insecticidal action and would be very successful in controlling *T. confusum*. Fathi and Shakarami (2014) analysis of essential oils from five species of Eucalyptus on *T. confusum* larvae for fumigant toxicity. The results suggested that *E. viminalis* essential oils were the most harmful to CFB larvae. In another experiment, Khanam *et al.* (1990) stated that seed coat extracts of *Aphanamixis polystachea* had a lethal effect on the growth and development of *T. confusum.*

4. CONCLUSION

The toxicity of *Urtica dioica* extracts increases as concentrations rise for both methods of extractions, but there were no significant differences between both methods, meaning that both methods had a similar effect on insect's larvae. The effect of periods of time also had statistically the same effect at the first time of exposure, meaning that the effect of plant extract was lethal after the first period.

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