

Insecticidal Activity and Synergistic Effect of *Gundelia tournefortii* L. (Asteraceae: Compositae) Extracts and Some Pure Constituents on *Drosophila melanogaster* Meigen (Drosophilidae: Diptera)

Ihab Husni Ghabeish*

ABSTRACT

Laboratory bioassay experiments were conducted using *Gundelia tournefortii* L. (Asteraceae: Compositae) plant as a source of active ingredients against the vinegar fly *Drosophila melanogaster* Meigen (Drosophilidae: Diptera) as a model insect. Extracts of all plant parts except roots were tested using seven solvents; acetone, benzene, chloroform, ethanol, ether, methanol and water. Seeds and flowers gave the highest percentages of extracted yield than leaves and stems. Chloroform seed-extract showed the highest toxicity to *D. melanogaster* larvae with the lowest LC₅₀ value (119.95 ppm), followed by the chloroform leaf and the ether leaf and seed-extracts. Mortality percentages to fly larvae due to both carbaryl and imidacloprid insecticides were increased from 10-20% to 40-70% with synergistic factors of 3-4 times after acetone and chloroform seed-extracts had been added. α -Terpinyl acetate and oleic acid of the *G. tournefortii* were responsible for 80% and 70% of larval mortalities with LC₅₀ of 22.98 and 23.15 ppm, respectively. Therefore, both chemicals can be further investigated as bioactive insecticides and could be incorporated into IPM programs of insect pests.

Keywords: Bioactive insecticide, *Drosophila melanogaster*, Eugenol, *Gundelia tournefortii*, Oleic acid, α -Terpinyl acetate.

INTRODUCTION

There has been a renewed interest in using plant extracts in pest control in recent years. One of the important plants found in Jordan nature is the tumbleweed, *Gundelia tournefortii* L. (Asteraceae: Compositae). It grows as a native of the semi-desert areas and mountains of many countries including Jordan, Palestine, Iraq, Syria, Turkey, Azerbaijan and Armenia

(Karis *et al.*, 2001; Coruh *et al.*, 2007; Cakilcioglu *et al.*, 2011; Matthaus and Ozcan, 2011). It is a perennial spiny herb of Irano-turanian origin (Halabi *et al.*, 2005; Karabulut *et al.*, 2006) and it is found as a wild herb growing during late winter and early spring. *G. tournefortii* stems, flowers, leaves and seeds could be used as a food for human (Coruh *et al.*, 2007; Sarper *et al.*, 2009), and for feeding camels (Kamalak *et al.*, 2005). It is also used in folk medicine in different parts of the world and is considered as a pivotal in human health care (Asadi-Samani *et al.*, 2013); liver diseases (Jamshidzadeh *et al.*, 2005), relieve pain and inflammation (Oryan *et al.*, 2011), antiparasitic effect for digestive system (Mosaddegh *et al.*, 2012), chest pain and heart stroke (Halabi *et al.*, 2005), and treat diabetes

* Associate Prof. of Biological Pest Control, Department of Plant Production and Protection, Faculty of Agricultural Technology, Al-Balqa' Applied University, Salt 19117/Jordan.

balappuniv@yahoo.com

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(Jarald *et al.*, 2008) and many other uses. In addition, *G. tournefortii* could accumulate phyto remediation of the soils polluted with metals (Chehregani *et al.*, 2009), and had high potential for accumulating arsenic and removing it from soils (Shahraki *et al.*, 2008).

The use of plant extracts that have insecticidal activity for insect pests control is one of the most effective and promising alternative control measures to synthetic chemicals, since it is economically, environmentally safe, less hazardous to humans and often less toxic to ecologically beneficial insects (Dyan *et al.*, 2009) as well as the development of resistance by insect pests (Stará and Kocourek, 2007). Furthermore, synthetic insecticides are not affordable to majority of peasant farmers (Ogunwolu and Ameh, 1999; Sarah and Ali, 2008).

Chemical analysis of *G. tournefortii* showed the presence of many flavonoids, alkaloids, glycosides, tannins, phenols, saponins, carbohydrates and free amine groups constituents (Halabi *et al.*, 2005; Al-Younis and Argushy, 2009; Bagci *et al.*, 2010; Khanzadeh *et al.*, 2012). Some of these are known to have insecticidal activity (Dadang *et al.*, 1996; Shola-Hezekiah, 2008; Bamoniri and Mazoochi, 2009; Chang *et al.*, 2009; Huang *et al.*, 2009; Farag *et al.*, 2011; Pérez-Gutiérrez *et al.*, 2011; Dell'Agli *et al.*, 2012; Murugesan *et al.*, 2012; Abay *et al.*, 2013; Chu *et al.*, 2013; Liu *et al.*, 2013; Willis *et al.*, 2013).

To the best of our knowledge, the use of *G. tournefortii* extracts and constituents as insecticides against insect pests is still lack. Therefore, the

insecticidal and synergistic activity of the extracts and some of the pure constituents from different plant parts were bioassayed using *Drosophila melanogaster* Meigen (Drosophilidae: Diptera) as insect model.

2. MATERIALS AND METHODS

2.1. Preparation and extraction of plant

The plant materials of *G. tournefortii* parts (leaves, flowers, stems and seeds) were collected from Al-Salt Mountains from February to April, 2012 and brought to the laboratory, and hereafter washed with distilled water to remove dust and other contaminants. The cleaned materials were shade-dried for 6-7 days at room temperature ($25\pm 2^\circ\text{C}$) until all moisture content was evaporated. The dried materials were milled in an electric grinder. Ten grams of the ground powders were subjected to extraction in Soxhlet apparatus using 150 ml of each of the solvents: acetone, benzene, chloroform, ethanol, ether, methanol and water (Table 1). The extraction proceeded up to 3-4 days at room temperature with shaking. The solvents were evaporated under reduced pressure in a rotary evaporator at 56°C and the yield was weighed. The yield of each extraction is calculated using the equation: Yield (%) = [dry weight of solvent extract / dry weight of plant part \times 100]. The yields were dissolved in an analytical grade of Dimethyl sulfoxide (DMSO) (w/v; 1gm/5ml) and kept in dark until being used.

Table 1. Origin of the analytical grade chemicals used in the *Gundelia tournefortii* L. extraction and insecticides used in studying the synergistic effect.

Chemical Name	PubChem CID*	Abbrev.	Role	Origin
Acetone	180	Act.	Solvent	Scharlau chemie- Spain
Benzene	241	Benz.	Solvent	GPR (BDH limited Poole- England)
Chloroform	6212	Chlor.	Solvent	Scharlau chemie- Spain
Ethanol	702	Ethn.	Solvent	AZ chem.- Canada
Ether	8254	Ethr.	Solvent	Tedia- USA
Methanol	887	Methn.	Solvent	Tedia- USA
Dimethyl sulfoxid	679	(DMSO)	Buffer	Scharlau chemie- Spain
Carbaryl	6129	-	Insecticide	Wakojunyaku Co.- Japan
Parathion	991	-	Insecticide	Wakojunyaku Co.- Japan
Imidacloprid	86418	-	Insecticide	Wakojunyaku Co.- Japan
Permethrin	40326	-	Insecticide	Wakojunyaku Co.- Japan

*Source: PubChem Compound: www.ncbi.nlm.nih.gov/pccompound

2.2. Rearing *Drosophila melanogaster* for bioassay test

About 100 *D. melanogaster* adults were obtained from an available colony at the Biotechnology Laboratory, and were introduced into a new culture bottle to breed. The content of the *Drosophila* artificial diet used per one liter is: 700 ml distilled water, 48 gm sucrose, 18 gm bacteriological agar, 18 gm yeast, 4 ml propionic acid and 54 gm wheat cream.

2.3. Extracts and constituents treatments for insecticidal activity

Volumes of 5, 10 and 15 µl of each plant-part extract were mixed with 11 ml of artificial diet. Each extract concentration (90.8, 181.6 and 272.4 ppm) was replicated four times. Also, the control diet was treated with DMSO in four replicates to serve as a negative control. Ten 2nd-instar larvae were transferred to each Petri-dish containing the treatment extract or the chemical to be tested in the diet. Larval mortalities after 72-hr of exposure were recorded. This extract-bioassay is considered a preliminary study to find out the

promising plant part(s) and the promising solvent(s) for further studies. Based on the results of the preliminary study, a literature search was performed on the most important *G. tournefortii* constituents expected to be dissolved in the solvent(s) showing the highest mortality. These candidate constituents were obtained from the Faculty of Medicine/Damascus University and were bio-assayed in its pure forms.

2.4. Extracts treatments for synergistic effect to insecticides

In the media, different solvents seed-extracts were mixed with analytical grades of carbaryl (carbamate group), parathion (organophosphate group), imidacloprid (neo-nicotinoid group) and permethrin (pyrethroid group) insecticides. Both the extract and the insecticide are at sublethal concentrations resulted in relatively low larval mortalities to investigate its synergistic effect to these representative insecticides. Insecticides were also mixed alone with the media to serve as a control. Mortalities were recorded for each insecticide alone and

for each insecticide mixed with each of the solvent extract. Synergistic factor (SF) of each treatment was calculated using the equation; $SF = \text{mortality of insecticide with extract} \div \text{mortality of insecticide alone}$. Sublethal concentrations of the insecticides and of the plant extracts were determined using serial dilutions of LC_{50} s against *D. melanogaster* larvae. Concentration caused mortality up to 20% after 72-hr of exposure was considered sublethal to fly larvae; and found to be 0.2 of the LC_{50} for the insecticides and also for the solvents' seed-extracts.

2.5. Plant-constituents treatments for insecticidal activity

Volumes of 0.5, 1.0 and 2.0 μl of each of the 7 pure plant-constituents: germacrene d, myristic acid, oleic acid, palmitic acid, α -terpinyl acetate, zingiberene and eugenol were each mixed with 11 ml of the larval media per Petri-dish. Ten *Drosophila* larvae in the 2nd instar were transferred to each dish for feeding after 12 hr of starvation. Mortalities of larvae for each constituent

treatment at each concentration was recorded after 72-hr of exposure. LC_{50} s were calculated (Abbott, 1925) and compared among the 7 constituents and with the control (only media).

2.6. Literature survey of *G. tournefortii* constituents

The expected biologically active constituents of *G. tournefortii* having insecticidal activity and their solubility in different solvents were collected from the literature and shown in Table 2.

2.7. Data analysis

Each treatment was replicated four times in all conducted experiments. Mortality percentages of larvae were corrected using Abbott's Formula (Abbott, 1925). Data were analyzed based on completely randomized design, one-way Analysis of Variance (ANOVA) using SAS. LC_{50} s were calculated using the probit analysis (Finney, 1971). Means of the synergistic factors were separated by LSD (Steel and Torrie, 1980).

Table 2. Biologically active constituents of *Gundelia tournefortii* L. against insects and their solubility.

Chem. Group	Chem. name	Formula- PubChem CID****	% Content	Effective solvent ***	Insecticidal activity (Ref.)
Fatty acid *	Myristic acid	$C_{14}H_{28}O_2$ - 11005	0.1645%	Chlor., Ethn., Ethr.	Abay <i>et al.</i> , 2013
	Palmitic acid	$C_{16}H_{32}O_2$ - 985	9.8881%	Chlor., Ethn., Ethr.	Abay <i>et al.</i> , 2013
	Palmitoleic acid	$C_{16}H_{30}O_2$ - 445638	0.3426%	Chlor., Ethr.	-
	Margaric acid	$C_{17}H_{34}O_2$ - 10465	0.1618%	Chlor., Ethn., Ethr.	-
	Stearic acid	$C_{18}H_{36}O_2$ - 5281	3.3501%	Chlor., Ethn., Ethr.	Pérez-Gutiérrez <i>et al.</i> , 2011
	Elaidic acid	$C_{18}H_{34}O_2$ - 637517	0.1513%	Chlor., Ethr.	-
	Oleic acid	$C_{18}H_{34}O_2$ - 445639	27.991%	Chlor., Ethr.	Farag <i>et al.</i> , 2011
	Vaccenic acid	$C_{18}H_{34}O_2$ - 5282761	1.6011%	Chlor., Ethr.	-

Chem. Group	Chem. name	Formula- PubChem CID****	% Content	Effective solvent ***	Insecticidal activity (Ref.)
	Rumenic acid	C ₁₈ H ₃₂ O ₂ – 5280644	0.1727%	Ethn., Ethr.	-
	Linoleic acid	C ₁₈ H ₃₂ O ₂ – 5280450	54.592%	Ethn., Ethr.	Farag <i>et al.</i> , 2011
	Linolenic acid	C ₁₈ H ₃₀ O ₂ – 5280934	1.0271%	Chlor., Ethr.	Willis <i>et al.</i> , 2013
	Gadoleic acid	C ₂₀ H ₃₈ O ₂ – 5282767	0.2653%	Acetone	-
	Behenic acid	C ₂₂ H ₄₄ O ₂ – 8215	0.2931%	Chlor., Ethr.	-
	Heptadecanoic	C ₁₇ H ₃₄ O ₂ – 10465	-	Chlor., Ethr.	-
Sterol *	Brassicasterol	C ₂₈ H ₄₆ O – 5281327	3.70%	Chlor.	-
	Campesterol	C ₂₈ H ₄₈ O – 173183	4.04%	Chlor.	-
	Stigmasterol	C ₂₉ H ₄₈ O – 5280794	11.7%	Chlor.	Huang <i>et al.</i> , 2009
	Δ ⁷ -Campesterol	C ₂₇ H ₄₄ O – 71314484	0.22%	Chlor.	-
	β-Sitosterol	C ₂₉ H ₅₀ O – 222284	35.3%	Chlor.	Chu <i>et al.</i> , 2013
	Δ ⁵ -Avenasterol	C ₂₉ H ₄₈ O – 5281326	11.6%	Chlor.	-
Steroids **	α-Terpinyl acetate	C ₁₂ H ₂₀ O ₂ – 111037	36.2%	Methn., Chlor.	Liu <i>et al.</i> , 2013
	Methyl eugenol	C ₁₁ H ₁₄ O ₂ – 7127	12.6%	Act., Chlor.	Chang <i>et al.</i> , 2009
	Eugenol	C ₁₀ H ₁₂ O ₂ – 3314	6.70%	Act., Chlor.	Shola-Hezekiah, 2008
	β-Caryophellene	C ₁₅ H ₂₄ O – 5281515	5.94%	Ethn.	Murugesan <i>et al.</i> , 2012
	Zingiberene	C ₁₅ H ₂₄ – 92776	5.84%	Ethn.	Dadang <i>et al.</i> , 1996
Terpene	Thymol	C ₁₀ H ₁₄ O – 6989	-	Ethn.	Dell'Agli <i>et al.</i> , 2012
	Germacrene d	C ₁₅ H ₂₄ – 5317570	21.6%	Ethn., Ethr.	Bamoniri and Mazoochi, 2009
Peroxide	Scopoletin	C ₁₀ H ₈ O ₄ – 5280460	-	Act., Methn., Ethr.	-
	Isoscooletin	C ₁₀ H ₈ O ₄ – 69894	-	Act., Methn., Ethr.	-
Glucoside	Esculin	C ₁₅ H ₁₆ O ₉ – 5281417	-	Methn.	-

*Fatty acids and sterols% of seed oil. Source: Khanzadeh *et al.* (2012), **Steroids% of volatile oil of the aerial parts. Source: Halabi *et al.* (2005)

The effective solvent: Source: www.matreya.com/Product_info.aspx?productid=1010, *Source: PubChem Compound: www.ncbi.nlm.nih.gov/pccompound

3. RESULTS

Methanol and water extracts were provided the highest

percentages of yield for all plant parts. The lowest yield of methanol extracts was from leaves (6.29%) and the highest

yield was from seeds (11.8%). In case of water extracts; the lowest yield was from leaves (2.99%) and the highest was from seeds (13.4%). On the contrary, ether extracts were provided the lowest percentages of yield for all plant parts

ranged 0.10-1.44%. Moreover, seed extracts gave the highest yields of all solvents used, followed by the flower extracts (Table 3).

Table 3. The percentage yields of *Gundelia tournefortii* L. extracts.

Number	Plant part	Extract type	% Yield*
1	Leaf	Acetone	1.00%
		Benzene	0.20%
		Chloroform	0.71%
		Ethanol	2.16%
		Ether	0.10%
		Methanol	6.29%
		Water	2.99%
2	Stem	Acetone	1.00%
		Benzene	0.20%
		Chloroform	0.54%
		Ethanol	3.17%
		Ether	0.10%
		Methanol	9.03%
		Water	3.80%
3	Flower head	Acetone	2.00%
		Benzene	1.20%
		Chloroform	2.08%
		Ethanol	3.77%
		Ether	0.30%
		Methanol	9.80%
		Water	3.91%
4	Seed	Acetone	2.74%
		Benzene	4.14%
		Chloroform	3.26%
		Ethanol	6.30%
		Ether	1.44%
		Methanol	11.8%
		Water	13.4%

*Yield (%) = [dry weight of solvent extract / dry weight of plant part × 100]

Chloroform seed-extract showed the lowest value of

LC₅₀; representing the highest toxicity to *D. melanogaster*

larvae among all extracts tested, followed by the ether seed and leaf. On the other hand, acetone, benzene and water extracts from all plant parts showed neglected mortalities not

exceeding 20% to fly larvae even at the highest concentration used (Table 4).

Table 4. Mortality of larvae of *Drosophila melanogaster* due to 72-hr exposure to different concentrations of *Gundelia tournefortii* L. plant extracts.

Plant part	Solvent	% Mean larval mortality			LC ₅₀ (ppm)*	Slope
		Concentration (ppm)				
		90.8	181.6	272.4		
Leaf	Acetone	10	10	20	-	-
	Benzene	0	0	10	-	-
	Chloroform	40	60	80	134.74	12.47
	Ethanol	30	30	40	-	-
	Ether	40	60	90	130.9	18.97
	Methanol	30	40	70	197.6	12.09
	Water	20	20	20	-	-
Stem	Acetone	10	0	10	-	-
	Benzene	0	10	10	-	-
	Chloroform	30	50	60	202.4	7.00
	Ethanol	30	30	30	-	-
	Ether	30	40	70	197.6	12.10
	Methanol	30	30	70	210.51	14.35
	Water	10	20	10	-	-
Flower	Acetone	10	10	10	-	-
	Benzene	10	10	10	-	-
	Chloroform	40	40	70	181.6	6.96
	Ethanol	40	40	40	-	-
	Ether	30	40	80	181.61	18.09
	Methanol	40	40	70	181.6	6.95
	Water	10	10	20	-	-
Seed	Acetone	10	20	20	-	-
	Benzene	10	10	10	-	-
	Chloroform	40	70	90	119.95	19.47
	Ethanol	40	30	40	-	-
	Ether	40	60	90	130.91	18.98
	Methanol	30	50	80	168.39	18.17
	Water	10	20	20	-	-

The mortalities of *Drosophila* larvae due to carbaryl and imidacloprid were increased from 10-20%

(insecticide alone) to 40-70%, representing 3 to 4 folds (synergistic factor=3-4) after acetone and chloroform

seed-extracts had been added. Slight increase in mortality was noticed for the same insecticides when mixed with the benzene seed-extract (synergistic factor=2). The situation was opposite for the parathion

and permethrin insecticides while no or neglected synergism (synergistic factors ranged 1-2) was found for all extracts added (Table 5).

Table 5. The change in the 72-hr. larval toxicity of synthetic insecticides when mixed with seven solvents seed-extracts of *Gundelia tournefortii* L.

Insecticide	Extract	% Mean larval mortality		Mean synergistic factor(SF)* ± SE
		Insecticide+extract	Insecticide alone	
Carbaryl (carbamate)	Acetone	40	10	4 a ± 0.58
	Benzene	20	10	2 c ± 0.00
	Chloroform	70	20	3.5 ab ± 0.29
	Ethanol	20	20	1 d ± 0.00
	Ether	10	10	1 d ± 0.00
	Methanol	20	20	1 d ± 0.00
	Water	10	10	1 d ± 0.00
Parathion (organophosphate)	Acetone	20	20	1 d ± 0.00
	Benzene	20	20	1 d ± 0.00
	Chloroform	30	20	1.5 cd ± 0.29
	Ethanol	20	20	1 d ± 0.00
	Ether	20	20	1 d ± 0.00
	Methanol	20	10	2 c ± 0.58
	Water	10	10	1 d ± 0.00
Imidacloprid (neo-nicotinoid)	Acetone	60	20	3 b ± 0.58
	Benzene	20	10	2 c ± 0.00
	Chloroform	40	10	4 a ± 0.58
	Ethanol	10	10	1 d ± 0.00
	Ether	10	10	1 d ± 0.00
	Methanol	20	20	1 d ± 0.00
	Water	20	20	1 d ± 0.00
Permethrin (pyrethroid)	Acetone	10	10	1 d ± 0.00
	Benzene	20	10	2 c ± 0.00
	Chloroform	20	20	1 d ± 0.00
	Ethanol	20	20	1 d ± 0.00
	Ether	20	20	1 d ± 0.00
	Methanol	30	20	1.5 cd ± 0.29
	Water	20	20	1 d ± 0.00

*SFs with different letters are significantly differing at 5% probability level by LSD. *SF = mortality of insecticide with extract ÷ mortality of insecticide alone.

Further bioassay for the pure selected constituents showed that oleic acid and α -terpinyl acetate were responsible for 70% and 80% of the larval mortality at the higher concentration of constituents with LC₅₀ of

23.15 and 22.98 ppm, respectively. Moderate toxicity was obtained for germacrene d, myristic acid and palmitic acid, while low toxicity was found for zingiberene and eugenol (Table 6).

Table 6. Mortality of larvae of *Drosophila melanogaster* due to 72-hr exposure to some promising *Gundelia tournefortii* L. pure constituents.

<i>G. tournefortii</i> constituent	% Mean larval mortality			LC ₅₀ (ppm)*	Slope
	Concentration (ppm)				
	10	20	40		
Germacrene D	20	30	40	-	-
Myristic acid	20	20	40	-	-
Oleic acid	30	50	70	23.15	11.64
Palmitic acid	20	20	40	-	-
α -Terpinyl acetate	30	40	80	22.98	19.53
Zingiberene	10	20	20	-	-
Eugenol	10	10	20	-	-

4. DISCUSSION

Certain plant families, particularly Annonaceae, Asteraceae, Labiateae, Meliaceae, Rutaceae and Piperaceae were viewed as exceptionally promising sources of plant-based insecticides (Jacobson, 1989; Schmutter, 1990, Isman, 1995). Therefore, *G. tournefortii* belongs to Asteraceae was chosen to be the target of this study, since no previous literature found dealing with *G. tournefortii* as a source of insecticidal ingredients. Chloroform and ether extracts caused the highest mortalities to *D. melanogaster* larvae. In their pure form, mainly oleic acid and α -terpinyl acetate were responsible for the insecticidal activity of the *G. tournefortii*. The high mortalities resulted from oleic acid and α -terpinyl acetate confirm the results of high mortalities of chloroform and ether extracts to fly larvae; since both constituents are highly soluble in these two solvents. Liu *et al.* (2013) found that α -terpinyl acetate

exhibited stronger toxicity against the booklice insect *Liposcelis bostrychophila* Badonnel (Liposcelidae: Psocoptera) than the other oil constituents of *Artemisia rupestris* L. (Family: Asteraceae). Moreover, α -terpinyl acetate was also regarded as an active termiticidal compound of hinoki wood *Chamaecyparis obtusa* Siebold and Zucc. (Family: Cupressaceae) (Ohtani *et al.*, 1997). In addition to, oleic acid of *Melia azedarach* L. (Family: Meliaceae) oil recorded having higher insecticidal activity against larvae of *Spodoptera littoralis* (Boisd.) (Noctuidae: Lepidoptera) than the crude extract (Farang *et al.*, 2011) and also against larvae of *Spodoptera frugiperda* Smith with 51.5% mortality (Pérez-Gutiérrez *et al.*, 2011). Ramsewak *et al.* (2001) found that oleic acid was considered as an insecticide against the fourth instar larvae of *Aedes aegyptii* L. (Culicidae: Diptera). However, high oleic acid content was found in seed's oil of *G. tournefortii* ranged 20-40%

(Abdul and Hamed, 2012). This bumper presence of such fatty acid in *G. tournefortii* may justify the high mortality caused by chloroform and ether extracts to the fly larvae. Acetone and benzene extracts showed neglected mortalities to larvae. These solvents expected to dissolve gadoleic acid, methyl eugenol, eugenol, scopoletin and isoscapoletin, and all were known in the literature having no or neglected insecticidal toxicity (Chang *et al.*, 2009; Kegley *et al.*, 2010; Duke, 2013). Moderate mortality (40%) was obtained by both myristic acid and germacrene d, such results were matching those of Abay *et al.* (2013), who indicated a moderate toxicity of myristic acid extracted from *Polytrichastrum formosum* (Hedw.) (Family: Polytrichaceae) against *Sitophilus granaries* L. (Curculionidae: Coleoptera). Moreover, Gershenzon and Dudareva (2007) found that floral terpenes, mainly germacrene d fulfill many ecological roles in plants interacting with their environment, including defense against insects. The intermediate level (21.6%) of germacrene d in *G. tournefortii* estimated by Kilic (2013) might stand behind its moderate insecticidal effect, but the results of the present study revealed a moderate toxicity (40%

mortality) of germacrene d to fly larvae when used in its pure form, when compared with the other constituents (oleic acid and α -terpinyl acetate). Chloroform and acetone extracts showed significant synergistic effect for two different insecticides (carbaryl and imidacloprid) against larvae. These two solvents; mainly the acetone expected to dissolve eugenol. Bessette *et al.* (2009) considered eugenol as a strong synergist to pyrethrum insecticide against cockroaches rather than having an insecticidal toxicity as cited by Chang *et al.* (2009); and this agrees with the results of neglected insecticidal toxicity of eugenol revealed in this study.

In conclusion, *G. tournefortii* constituents; oleic acid and α -terpinyl acetate are promising active ingredients as insecticides of plant origin. Also, eugenol is a promising synergist for carbamate and neo-nicotinoid insecticides. These constituents could be incorporated in IPM programs of insect pests after further field studies.

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فاعلية مستخلصات نبات العكوب وبعض مركباته النقية كمبيدات حشرية وكمواد منشطة للمبيدات على ذبابة الخل

ايهاب حسني غبيش*

ملخص

اجريت تجارب حيوية مخبرية على مستخلصات نبات العكوب كمصدر للمواد الفاعلة ضد ذبابة الخل كنموذج. تم استخدام مستخلصات جميع أجزاء النبات ما عدا الجذور بوساطة سبعة مذيبات: الاسيتون والبنزين والكلوروفورم والايثانول والايثر والميثانول والماء. أعطت مستخلصات البذور والازهار ناتج زيت أعلى من الاوراق والسيقان. وأظهر مستخلص البذور بالكلوروفورم السمية الأعلى ليرقات ذبابة الخل بتركيز قاتل نصفى هو الاقل (119.95 جزءاً في المليون) تلاه مستخلص الاوراق بالكلوروفورم ثم تلاه مستخلصي الاوراق والبذور بالايثر. وازدادت نسب الوفيات لمبيدي الكارباميل والايמידاكلوبريد ضد يرقات الذبابة من (10-20%) الى (40-70%) بعامل تنشيط 3-4 أضعاف بعد إضافة مستخلصي البذور بالاسيتون والكلوروفورم. وأظهرت مادتي الالفا تيرينيل اسيتيت وحامض الاوليك مسؤوليتهما عن 80% الى 70% من وفيات اليرقات بتركيز قاتل نصفى 22.98 و 23.15 جزء في المليون، على التوالي. لذا يمكن استخدام هاتين المادتين كمبيدات حشرية حيوية نشطة في برامج مكافحة المتكاملة للافات بعد إجراء المزيد من الدراسات عليهما.

الكلمات الدالة: مبيد حيوي فاعل، ذبابة الخل، يوجينول، العكوب، حامض أوليك، ألفا تيرينيل أسيتيت.

* استاذ مكافحة الحيوية المشارك، قسم انتاج ووقاية النبات، كلية الزراعة التكنولوجية، جامعة البلقاء التطبيقية، السلط 19117- الاردن
balappuniv@yahoo.com

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