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Insecticidal activity of fruit peel extracts of pomegranate (*Punica granatum*) against tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Abstract

Chemical constituents of Pomegranate*, Punica granatum* fruit peels, were extracted and tested for their toxicity against eggs and second instar larvae of tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) under laboratory conditions. The total methanol extract was the most effective against both eggs and larvae with LC_{50} values of 100.777 and 53.203 ppm, respectively. Methylene chloride fraction exhibits more ovicidal activity, with LC_{50} value of 253.084 ppm, while ethyl acetate fraction exhibits more larvicidal activity with LC_{50} value of: 83.508 ppm. Also, the volatile chemical constituents of each fraction were characterized and identified by GC/MS technique. The high potency of *P. granatum* fruit peels extracts against eggs and larvae of *T. absoluta* make it worthwhile as environmentally friendly alternatives of traditional insecticides.

Introduction

Tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is a serious invasive pest that originated from South America (Gebremariam, 2015) then spread to the Europe and North African Mediterranean Basin countries (Desneux *et al*., 2010). Due to its rapid spread, it has become a global economic threat to worldwide tomato production (Desneux *et al*., 2011). Since the first record of this pest in Egyptian tomato fields in 2009, it has become one of the economic pests of tomato and other Solanaceous plants (NAPPO, 2012). *T. absoluta* larvae infest tomato plants at any growth stage damaging all tomato plant parts, including leaves, stems, flowers, and fruits, resulting in crop growth interruption (Biondi *et al*., 2018) and significant adverse effects on its market value (Urbaneja *et al*., 2012 and Van Damme *et al*., 2014). In the absence of control strategies, yield losses can reach 80%–100% (Desneux *et al*., 2010). The indiscriminate use of chemical pesticides for controlling *T. absoluta* resulted in the rapid development of insect resistance, harmful pesticide residues in fruits and destruction of natural enemies (Nazarpour *et al*., 2016), as well as increasing production costs (Reis *et al*., 2005). Therefore, the scientific community was called upon to explore new secure alternatives to the widely spread synthetic insecticides. One of the most important alternatives is the natural insecticides derived from plant origin.

Pomegranate*, Punica granatum* is an important crop known by its delicious taste, nutritional and medicinal properties (Melgarejo *et al*., 2000; Melgarejo *et al*., 2004 and Mohammad, 2013). Also, the antimicrobial and anti-allergic (Al-Zoreky, 2009), anti-cancer (Malik *et al*., 2005), antibacterial, and insecticidal (Gandhi *et al*., 2010 and Ben Hamouda, 2014) properties of different tree parts extracts were reported.

The aim of the current study is to investigate the phytochemistry and the insecticidal activity of different extracts of *P. granatum* fruit peels against both eggs and the 2nd instar larvae of *T. absoluta*.

Materials and methods

1. Instruments:

GC/MS analysis was performed using GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST14 mass spectral database.

2. Plant material:

Fruits of pomegranate, P. granatum, were obtained from the local market, then they were washed. The peels were manually removed and dried at room temperature, then ground into a powder.

3.Extraction and isolation:

A kilogram of the powdered peels was macerated in methanol for seven days (cold extraction) then sieved through Whatman filter paper to remove peel particles. The remaining residue was re-macerated in the solvent for three days, and this process was repeated three times. The filtrate was concentrated to its 1/3 volume and then diluted with water and exhaustively extracted with petroleum ether, methylene chloride, and finally ethyl acetate using a separating funnel. All the extracts were dried over anhydrous sodium sulphate and evaporated to dryness. The volatile constituents of each fraction were characterized and identified by GC/MS technique.

4.The tested insect pest:

Larvae of T. absoluta were collected from tomato fields in Aga, Dakahlia governorate, Egypt. They are maintained in a net cage with an aluminium base (60cm x 60cm x 60cm) under laboratory conditions (25±2°C, 65±5% RH., and 14 hrs. photophase). Leaves of insecticides free tomato plants were used for feeding the larval stage, and a 10% sugary solution for feeding the adult stage.

5. Bioassay:

5.1. Ovicidal effect of pomegranate fruit peel extracts:

Twenty adult males with twenty females were released into a net cage containing insecticides free leaves of the upper third of a tomato plant and were allowed to deposit eggs. After 48 hrs., adults were removed. Thirty fresh eggs were transferred to uninfested tomato leaves in a Petri dish (15 cm in diameter), then sprayed with the tested extracts with 0.1% Tween-80 to be considered as a replicate. Each concentration was represented by three replicates in addition to a control, which was sprayed only with water and 0.1% Tween-80. All treatments were carried out under stable conditions of 25±2°C, 65±5% RH., and 14 hrs. photophase. Eggs hatching was observed daily for 7 days.

5.2. Larvicidal effect of pomegranate fruit peel extracts:

Tomato leaves were sprayed with different concentrations of the tested extracts, then left to dry before being put in plastic Petri dishes (15 cm in diameter) lined with filter papers to get rid of excessive humidity. Ten second instar larvae of *T. absoluta* were carefully transferred from their mines by using a zero brush to the treated tomato leaves, then the lid of the Petri dishes bearing ventilation holes was sealed. Each treatment was replicated three times, in addition to three replicates of control only sprayed with water containing 0.1% Tween 80. All treatments were carried out under laboratory conditions at 25± 2oC, 65 ± 5 % RH., and 14 hrs. photoperiod. Mortality percentages were daily recorded along 5 days after treatments.

6. Statistical analysis:

Mortality percentages of both eggs and the 2nd instar larvae of *T. absoluta* were determined and corrected by using Abbott's formula (Abbott, 1925). The LC50, LC90 and slope values of each treatment were estimated according to Finney (1971). Also, toxicity indexes of different tested extracts of pomegranate fruit peels were evaluated according to Sun's equation (Sun, 1950) by comparing them with the most effective one.

Results and discussion

1. Chemical constituents of *Punica granatum* fruit peel extracts:

Both methylene chloride and ethyl acetate extracts of pomegranate fruit peels were analysed by the GC/MS technique (Table 1). Methylene chloride fraction contained 27 compounds, and ethyl acetate fraction contained 14 compounds. The most abundant constituents of methylene chloride fraction were cis-Vaccenic acid (25.48%), Methyl oleate (15.74%) and Hexadecanoic acid (14.55%). While the major components of ethyl acetate fraction were Pyrogallic acid (19.95%), 3-methyl-2,5-furandione (16.25%) and Hexadecanoic acid (10.72%).

2. Ovicidal activity of fruit peel extracts of *Punica granatum* against *Tuta absoluta* eggs:

It's clear from the data shown in Table (2) that the total methanol extract possessed the highest ovicidal properties at the LC50 and LC90 levels, followed by methylene chloride fraction, then, ethyl acetate fraction. The total methanol extract suppressed eggs hatchability with LC_{50} value: 100.777 ppm, LC_{90} value: 5728.063 ppm, and toxicity index at LC_{50} of 100% followed by methylene chloride fraction with LC_{50}: 253.084 ppm, $LC_{90:}$ 29217.406 ppm and toxicity index at LC_{50} of 39.82%, then, ethyl acetate fraction with LC_{50}: 491.035 ppm, $LC_{90:}$ 47981.051 ppm, and toxicity index at LC_{50} of 20.523%.

3. Larvicidal activity of fruit peel extracts of *Punica granatum* against *Punica granatum* 2nd instar larvae:

Data in Table (3) revealed that the total methanol extract was the most efficient extract at the LC_{50} and LC_{90} levels, followed by ethyl acetate fraction, then, methylene chloride fraction. The total methanol extract suppressed *T. absoluta* 2nd instar larvae with LC_{50} value: 53.203ppm, LC_{90} value: 2648.314ppm, and toxicity index at LC_{50} of 100% followed by ethyl acetate fraction with LC_{50}: 83.508 ppm, $LC_{90:}$ 5572.01 ppm and toxicity index at LC_{50} of 63.71%, then, methylene chloride with LC_{50}: 196.479 ppm, $LC_{90:}$ 40002.287ppm, and toxicity index at LC_{50} of 27.078 %. Stop feeding is the obvious symptoms shortly happened after ingestion of the treated leaves. Overtime, this led to developmental disability and finally death.

Table (1): The GC/MS analysis of different fractions of *Punica granatum* fruit peels.

Compound	RT(min.)	Area%	M. F.	Mol. Wt
Chemical constituents of methylene chloride fraction of *Punica granatum* fruit peel				
2(3H)-Furanone, dihydro-3-methylene-= Tulipalin A	4.34	0.51	$C_5H_6O_2$	**98**
5-Hydroxymethylfurfural	10.43	1.03	$C_6H_6O_3$	126
Citric acid, trimethyl ester	15.78	0.38	$C_9H_{14}O_7$	234
2-Carboxymethyl-3-hexylmaleic anhydride	16.33	0.38	$C_{12}H_{16}O_5$	240
1-eicosanol = Arachidic alcohol	24.02	0.54	$C_{20}H_{42}O$	298
9-Hexadecenoic acid, methyl ester,(Z)-	25.92	0.22	$C_{17}H_{32}O_2$	268
Hexadecanoic acid, methyl ester = Methyl palmitate	26.45	9.48	$C_{17}H_{34}O_2$	270
Oleic Acid.	26.83	2.09	$C_{18}H_{34}O_2$	282
Hexadecanoic acid = Palmitic acid	27.50	14.55	$C_{16}H_{32}O_2$	256
Pentadecanoic acid	28.36	0.25	$C_{15}H_{30}O_2$	242
Hexadexanoic acid 2,3-dihydroxypropyl ester	28.68	0.24	$C_{19}H_{38}O_4$	330
9,12-Octadecadienoic acid (Z,Z)-,methyl ester =Methyl linoleate	29.48	7.61	$C_{19}H_{34}O_2$	294
9-Octadecenoic acid (Z)-, methyl ester = Methyl oleate	29.69	15.74	$C_{19}H_{36}O_2$	296
Methyl octadecanoate. Methyl stearate	30.20	0.79	$C_{19}H_{38}O_2$	298
cis-Vaccenic acid	30.72	25.48	$C_{18}H_{34}O_2$	282
trans-13-Octadecenoic acid	31.62	0.43	$C_{18}H_{34}O_2$	282
9,12-Octadecadienoic acid (Z,Z)- =Linoleic acid.	31.96	0.48	$C_{18}H_{32}O_2$	280
Linoleic acid ethyl ester	32.99	0.24	$C_{20}H_{36}O_2$	308
cis-13-Eicosenoic acid = Paullinic acid	33.14	0.23	$C_{20}H_{38}O_2$	310
Cyclopentanetridecanoic acid= Dihydrochaulmoogric acid	33.66	0.67	$C_{19}H3_6O_2$	296
Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis-	35.28	0.22	$C_{19}H_{36}O_3$	312
Docosanoic acid, methyl ester	36.85	0.38	$C_{23}H_{46}O_2$	354
2-hydroxy-3-[(9e)-9-octadec Enoyloxy]propyl (9e)-9-octadecenoate	38.35	0.14	$C_{39}H_{72}O_5$	620
Docosanoic acid, methyl ester	39.83	0.31	$C_{23}H_{46}O_2$	354
(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one = Santalcamphor	41.11	3.94	$C_{15}H_{24}O_2$	236
.psi.,.psi.-Carotene,1,1',2,2'-tetrahydro-1,1'-dimethoxy- = 3,3',4,4'-Tetrahydrospirilloxanthin	43.48	0.69	$C_{42}H_{64}O_2$	600
Vitamin E	43.88	1.76	$C_{29}H_{50}O_2$	430
Chemical constituents of ethyl acetate fraction of *P. granatum* fruit peel				
3-methyl-2,5-furandione	4.14	16.25	$C_5H_4O_3$	112
1,2,3-Benzenetriol = Pyrogallic acid	15.68	19.95	$C_6H_6O_3$	126
Hexadecanoic acid, methyl ester = Methyl palmitate	26.43	2.54	$C_{17}H_{34}O_2$	270
Hexadecanoic acid	27.29	10.72	$C_{16}H_{32}O_2$	256
á-Sitosterol	28.88	6.75	$C_{29}H_{50}O$	414
9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl Ester, (z,z,z)-	29.46	2.67	$C_{21}H_{36}O_4$	352
11-Octadecenoic acid, methyl ester	29.64	5.28	$C_{19}H_{36}O_2$	296
Linoleic acid ethyl ester	29.76	1.23	$C_{20}H_{36}O_2$	308
Ethyl iso-allocholate	30.15	3.03	$C_{26}H_{44}O_5$	436
1,6-Didehydro-3,15-dimethoxyerythrinan-16-ol	30.31	1.19	$C_{18}H_{23}NO_3$	301
2-hydroxy-3-[(9e)-9-octadec Enoyloxy]propyl (9e)-9-octadecenoate	30.94	1.43	$C_{39}H_{72}O_5$	620
Cholestan-3-ol, 2-methylene-, (3á,5à)-	31.22	1.68	$C_{28}H_{48}O$	400
psi.,.psi.-Carotene,1,1',2,2'-tetrahydro-1,1'-dimethoxy-	43.48	1.66	$C_{42}H_{64}O_2$	600
4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	43.96	4.28	$C_{18}H_{16}O_7$	344

RT: Retention time, M.F.: Molecular formula, Mol. wt: Molecular weight

Table (2): Insecticidal activity of fruit peels extracts of *Punica granatum* against *Tuta absoluta* eggs after 7 days of treatment under laboratory conditions.

Extract	Eggs						
	LC50 (ppm) and confidence limits at 95%		LC90 (ppm) and confidence limits at 95%		Slope ± SE	*X2*	Toxicity index
Methanol (Total extract)	100.777		5728.063		0.730± 0.073	5.642	100
	62.309	159.717	2711.525	16298.666			
Methylene chloride fraction	253.084		29217.406		0.621± 0.069	2.628	39.82
	149.705	441.422	10540.859	133102.067			
Ethyl acetate fraction	491.035		47981.051		0.644± 0.071	1.372	20.523
	293.204	883.73	16762.009	229839.386			

Table (3): Insecticidal activity of fruit peels extracts of *Punica granatum* against *Tuta absoluta* 2nd instar larvae under laboratory conditions.

Extract	*Tuta absoluta* 2nd instar larvae						
	LC50 (ppm) and confidence limits at 95%		LC90 (ppm) and confidence limits at 95%		Slope ± SE	*X2*	Toxicity index
Methanol (Total extract)	53.203		2648.314		0.755 ± 0.13	1.601	100
	20.767	116.755	893.924	18462.416			
Methylene chloride fraction	196.479		40002.287		0.555 ±0.115	0.26	27.078
	67.995	604.804	6641.386	2089745.495			
Ethyl acetate fraction	83.508		5572.01		0.703 ±0.124	0.026	63.71
	32.59	192.729	1646.805	53455.217			

All previous data emphasized the high insecticidal activity of the total methanol extract against both eggs and larvae of *T. absoluta*. But, when the methanolic extract was fractionated with methylene chloride and ethyl acetate fraction, their toxicity was varied against eggs and/ or larvae. This can be explained by the fact that the insecticidal potential of any natural extract is an obvious reflection of its chemical constituents. As such, the high insecticidal activity of methanol extract may be due to the rich content of different chemical compounds, which may have a synergistic effect when found together. Also, the methylene chloride fraction showed high insecticidal activity against eggs more than against larvae. This may be due to the presence of some components such as Tulipalin A (0.51%) which has been previously found to possess high insecticidal activity against *Thrips palmi* and other agricultural pest species (Datta *et al*., 1999), 5-hydroxymethyl furfural (1.03%) which previously revealed high Insecticidal activity against adults and larvae of *Drosophila melanogaster* (Miyazawa *et al*., 2003). Also, many unsaturated and saturated fatty acids, especially oleic (Don-Pedro, 1990 and Tare and Sharma, 1991), linoleic (Yousef *et al*., 2013; Salinas-Sánchez *et al*., 2021 and Aguilar-Marcelino *et al*., 2022) and palmitic (Mahesh-Babu *et al*., 2016) have been reported to have ovicidal (Malek and Wilkins, 1994) and larvicidal activity (Perumalsamy *et al*. 2015). Also, this fraction contained methyl oleate (15.74%) which showed high acaricidal effect on *Tetranychus cinnabarinus* and inhibitory effects on the growth of the eggs (Du *et al*., 2012).

Ethyl acetate fraction revealed larvicidal more than ovicidal activity, this might be as a result of presence of methyl palmitate (2.54%) which was reported previously as strong acaricide (Wang *et al*., 2009) beside other high polar components which previously reported such as major phenolic compounds (Gallic acid and ellagic acids in addition to punicalagin as a major ellagitannin) (Middha *et al.,* 2013). Also, quercetin and vanillic acid were reported in *P. granatum* fruit peels (Mansour *et al*., 2013).

Different fractions of *P. granatum* fruit peels extract revealed ovicidal and larvicidal activity against *T. absoluta*. Methanol extract was the most efficient against both eggs and the 2nd instar larvae. Methylene chloride fraction showed more ovicidal activity, while ethyl acetate fraction showed more larvicidal activity. Also, the major volatile components of each fraction were characterized and identified by GC/MS technique in order to understand their activity to some extent. All previous data emphasized that *P. granatum* fruit peels extracts are promising factors that can be used as eco- friendly insecticides.

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