

Efficacy of some Entomopathogenic Fungal Extracts and their Chemical Constituents as Alternative Bio-Pesticides against Onion Thrips, *Thrips tabaci* L.

Amany S. M. Saad¹; M. I. Sergany²; M. E. Mostafa³ and Dina M. Fathy⁴

¹Plant Pathology Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt.

²Pesticides Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt.

³Plant Protection Research Institute, Agriculture Research Center (ARC), Dokki, Giza, 12618, Egypt.

⁴Economic Entomology Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt.

Corresponding author: dramany83@gmail.com and sergany@mans.mans.edu.eg



ABSTRACT

Three entomopathogenic fungal secondary metabolites of *Metarhizium anisopliae*, *Paceliomyces fumosoroseus* and *Trichoderma longibrachiatum* were extracted using two different organic solvent and thoroughly investigated as bio-insecticide against nymphs and adults of onion thrips, *Thrips tabaci* using spraying method technique. The toxicity index of LC₅₀ values revealed that *T. longibrachiatum* secondary metabolites extracts (chloroform and ethyl acetate) were the most effective against the two developmental stages (nymph stage and adult) of *T. tabaci* followed by *M. anisopliae* extracts and the least toxic one *P. fumosoroseus* extracts after 7-days of treatment. The chemical composition of the most promising extracts in each entomopathogenic fungi were analyzed using GC-MS technique. Thirty-five compounds belonging to six different classes of natural products were characterized.

Keywords: Entomopathogenic fungi, secondary metabolites, lufenuron, Bio-pesticides, onion, *Thrips tabaci*

INTRODUCTION

Entomopathogenic fungi are considered a safety and natural method for biological control of insects and arthropods such as leaf Hoppers, bugs, Aphid and thrips (Sparagano and Giangaspero 2011). Many species of fungal entomopathogen have been widely applied on pests and play an important role in biological control such as *Verticillium*, *Beauveria*, *Metarhizium*, *cladosporium* and *Paceliomyces* (Liu *et al.* 2017). It can act as an insect parasite and kill them without or with a very small risk on plant or environment, other microorganism and beneficial insects.

Recently researches reported that entomopathogenic fungi like *Paecilomyces fumosoroseus*, *Metarhizium* spp., *Lecanicillium lecanii*, *B. bassiana*, *Hirsutella thompsonii* and *Nomuraea rileyi* effective on Whiteflies, Thrips, Aphids, Beetles, Locusts, Grasshoppers, Hemiptera, spiders, Weevils, foliage- feeding caterpillars and citrus rust mite.

M. anisopliae have shown in many researches a promising action for arthropod vector Mnyone, *et al.* 2009 and Lwetoijera, *et al.* 2010, which reported that can infect and kill larval stages of soil insects.

P. fumosoroseus is observed as a species that successfully used for biocontrol of several pest insects, mainly whiteflies, this due to their produced a lethal metabolite for insects and parasitic on its cuticle (Zimmermann, 2008)

Trichoderma spp. is used as a biocontrol fungal agent of pathogens and reported on its entomopathogens, which can be used against insect pests (Srinivasan, 2008). These lethal action of fungal Entomopathogens due to invade their hosts by direct penetration of the host exoskeleton or cuticle, Penetration of the cuticle by both enzymes such as endoproteases and aminopeptidase N- acetylglucosaminidase which degradation the exoskeleton and other mechanical action of fungal Entomopathogens that produced toxins (Yashaswini and Sudarsanam, 2017).

So that the use of fungal Entomopathogens considered as promising applied method to reduce or prevent the use of chemical pesticides corresponds to the use of microbial metabolites.

Further studies on safety and toxicity use the microbial metabolites in the biological control strategy (Sinha, *et al.* 2016). Many reports have described for applications of microbial metabolites in modulate insect behavior (Strasser, *et al.* 2000) and now we are using metabolites for controlling pests especially *thrips tabaci*.

The species *T. tabaci* is different from the other species of the genus *Thrips*, continue to the order *Thysanoptera* and Family *Thripidae*. It is a very small and commonly known as the onion thrips, the potato thrips, the tobacco thrips or the cotton seedling thrips (Zhang, *et al.* 2008). It can damage crops of onions and other plants, and it can play act as a vector for plant viruses.

Onion thrips have a broad host range that includes grasses and broadleaves. They are pests of agricultural crops, home gardens, landscapes, and greenhouses. Primary vegetable hosts include onion, garlic, leek, cabbage, cauliflower, cotton, bean, tomato, cucumber, and asparagus. Common field crop hosts include alfalfa, small grains, and cotton. They may cause damage to bedding plants and some flowers (Nault and Shelton, 2010). Onions are most sensitive to thrips injury during the rapid bulb extension phase. Yield reduction due to reduced bulb size is the primary crop loss caused by onion thrips. Following harvest and during storage, thrips may continue to feed on onion bulbs, causing scars that reduce quality and visual appearance of bulbs (Coviello, *et al.* 2007).

The research work was done with the aim further scope the evaluation of various entomopathogenic fungi metabolites extracts against onion thrips under

laboratory condition and to identify their qualitative and quantitative composition using GC-MS technique.

MATERIALS AND METHODS

Thrips tabaci cultures rearing

Thrips were collected from infected onion plants and identified in the biological control of entomology Lab. Ten pest Thrips, *T. tabaci* (nymph stage and Adult) was reared on sterilized onion leaves upside down on moisten filter paper in Petri-dishes under controlled conditions at 24±2°C, and 24:72 h (L:D).

Entomopathogenic fungi

The entomopathogenic fungi as Follow: *Metarhizium anisopliae*, *Paceliomyces fumosoroseus* and *Trichoderma longibrachiatum* were obtained from the Assiut University Mycological Centre (AUMC), Egypt. The isolates were individually transferred in to Potato Dextrose Agar (PDA) medium plats and incubation at 26±2°C for 10 days in the dark. Then Purified by carried out using hyphal tip technique, according to the method reported in a previous study, Fathy and Saad (2017). The isolates were used throughout the present study.

Preparation of isolates culture filtrates

Flasks (1000 ml) containing 500 ml of potato dextrose broth (PDB), were inoculated with one disc (5 mm diameter) of a 7-day-old culture obtained from the fungal entomopathogens were prepared previously.

Flasks were incubated in the dark at 26±2 °C for 20 days until the secondary metabolite were exert, and then were filtrated through Whatman No.1 filter paper and the filtrates were centrifuged at 9660×g for 30 min. The supernatant was collected and stored at 5 °C for used in the next stage.

Extraction of the entomopathogenic fungi metabolites

Culture filtrates of the entomopathogenic fungi were concentrated to 10 % of its original volume using a rotary evaporator at 40 °C. Successive extraction was done using two different organic solvents of different polarities chloroform and ethyl acetate, respectively till complete exhaustion (using 0.3 volume of organic solvent per volume of filtrate). Each fraction was dried over anhydrous sodium sulphate (Na₂SO₄) and concentrated in a rotary evaporator to remove the organic solvents and to yield the crude extract.

Gas chromatography–Mass spectrometry (GC-MS) analysis

The analysis of chloroform and ethyl acetate extracts of entomopathogenic fungi under study were performed using an Agilent 6890 gas chromatography equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5MS (30 m×0.32 mm×0.25 µm film thickness). The column oven temperature was initially held at 40 °C and then increased by 8 °C /min to 280 °C. The injector and detector temperatures were 250 and 280 °C, respectively. The carrier gas was helium, at a ratio of 1 ml/min, pulsed splitless mode. The solvent delay was 3 min and the injection size was 1.0 µl. EI mass spectra were collected at 70 eV

ionization voltages over the range of m/z 50–500. The electron multiplier voltage was 1250 V. The ion source and quadrupole temperatures were set at 230 and 150 °C, respectively. The compounds were identified using WILEY and NIST 05 mass spectral database.

Screening Test of Entomopathogenic Fungi Extracts against *Thrips tabaci*

Ten Thrips, *T. tabaci* insects (nymph stage and Adult) 48 h were placed on sterilized onion leaves in Petri-dishes as mentioned before. *Thrips* pest were treated by spraying the prepared organic solvents extracts using hand sprayer at five different diluted concentrations, Organic solvents extract Chloroform and ethyl acetate were dissolved in 0.5 ml dimethyl sulfoxide (DMSO) and added to the distilled water to obtain final tested concentrations, while, the control treatment containing (0.5 ml/l) DMSO mixed with distilled water. Another chemical control treatment was applied by spraying diluted aqueous dispersions concentrations of the commercially recommended insecticide lufenuron (Match 50% EC) as a standard control to be compared with the entomopathogenic fungal extracts treatments, three replicates for each treatment were used. The experiments were conducted twice. The mortality was recorded under a binocular after seven days. The symptomatic development of dead *Thrips* was noted under microscope.

Statistical analysis

The average of mortality percentages was corrected using Abbott's formula (Abbott, 1925) and statistically analyzed according to Finney (1971) to determine LC₅₀, LC₉₀ and slope values. Toxicity index was calculated using Sun's equation for different fractions or isolated compounds by comparing these materials with the most effective one (Sun, 1950).

RESULTS AND DISCUSSION

The current study focus on utilization of entomopathogenic fungi secondary metabolites as bio-insecticides, searching for new eco-friendly leads could offer an alternative and complementary tool for use in integrated pest management programs. This approach makes the bio-control strategy more target specific and environmental friendly.

Entomopathogenic fungi considered to be a rich source of secondary metabolites (Gibson *et al.*, 2014). The insecticidal activity of any entomopathogenic fungi extract depends upon their chemical contents (Mostafa *et al.*, 2017).

The toxicity effects of three different entomopathogenic fungi secondary metabolites extracts as well as a standard recommended insecticide lufenuron were designed under the laboratory conditions to determine the susceptibility of two developmental stages (nymph stage and adult) of *T. tabaci* using spraying method technique.

The results obtained in table (1) showed the efficiency of the tested entomopathogenic fungi secondary metabolites extracts against the nymphs of *T. tabaci* after 7 days of treatment. The standard lufenuron

was the most effective at LC₅₀ level followed by *T. longibrachiatum* ethyl acetate, *T. longibrachiatum* chloroform, *M. anisopliae* chloroform, *M. anisopliae* ethyl acetate, *P. fuosoroseus* ethyl acetate and the least minor toxic *P. fuosoroseus* chloroform extracts. The LC₅₀ values of these tested extracts were 47.20, 103.54, 118.81, 221.42, 424.80, 561.86 and 607.47 ppm, respectively.

The slopes of the toxicity lines were calculated to be fluctuated and increased from 1.492 in *M. anisopliae* ethyl acetate to 2.949 in *P. fuosoroseus* ethyl acetate extracts. The other tested lines came between these two extracts.

The effectiveness of the tested entomopathogenic fungi as well as the standard lufenuron were also examined against the adults of *T. tabaci* after 7-days of treatments, table (1). It could be shown that the LC₅₀ values were 78.70, 194.75, 230.61, 341.07, 446.41, 987.37 and 1076.94 ppm for lufenuron, *T. longibrachiatum* chloroform, *T. longibrachiatum* ethyl acetate, *M. anisopliae* chloroform, *M. anisopliae* ethyl

acetate, *P. fuosoroseus* ethyl acetate and *P. fuosoroseus* chloroform extracts, respectively. The slopes of the toxicity lines were calculated to be fluctuated and increased from 1.048 in *T. longibrachiatum* ethyl acetate to 3.220 in lufenuron. The other extracts lines came between these two lines.

On the basis of the toxicity index, it could be also observed that the entomopathogenic fungi *T. longibrachiatum* secondary metabolites extracts (chloroform and ethyl acetate) were the most potent against the two developmental stages (nymph stage and adult) of *T. tabaci* followed by *M. anisopliae* extracts and the least effective one *P. fuosoroseus* extracts after 7-days of treatment.

The most active extract from each entomopathogenic fungi were analyzed to identify its qualitative and quantitative compositions using Gas chromatography- Mass spectrometry. GC-MS has been the most popular and useful analytical tool for the isolation and detection of compounds (Mostafa *et al.*, 2018)

Table 1. Susceptibility of nymphs and adults of *Thrips tabaci* to some entomopathogenic fungal extracts using insect-spray method under laboratory conditions after 7 days of treatment.

Insect spray method under laboratory conditions after 7 days of treatment.											
Tested Extracts		Nymphs					Adults				
		LC ₅₀ (ppm)	LC ₉₀ (ppm)	Slope ± SE	X ²	Toxicity index*	LC ₅₀ (ppm)	LC ₉₀ (ppm)	Slope ± SE	X ²	Toxicity index*
		and confidence	and confidence				and confidence	and confidence			
		limits at 95%	limits at 95%				limits at 95%	limits at 95%			
<i>Paceliomyces fuosoroseus</i>	Chloroform	607.47	1731.97	2.817± 0.62	3.03	7.77	1076.94	5202.50	1.873± 0.52	0.29	7.31
		456.09	1174.96				722.99	2265.93			
		811.48	4151.43				2688.43	75308.83			
	Ethyl acetate	561.86	1528.49	2.949± 0.67	3.54	8.40	987.37	4966.77	1.827± 0.49	0.01	7.97
		417.98	1032.70				665.92	2203.75			
		765.55	3775.26				2244.03	58065.03			
<i>Metarhizium anisopliae</i>	Chloroform	221.42	1111.03	1.830± 0.27	5.97	21.32	341.07	3993.09	1.200± 0.34	0.40	23.07
		156.46	694.68				195.58	1154.70			
		313.12	2396.32				1204.86	252465.02			
	Ethyl acetate	424.80	3072.13	1.492± 0.37	0.25	11.11	446.41	1347.48	2.671± 0.49	3.38	17.63
		257.77	1128.02				326.81	944.54			
		1178.89	46119.20				589.47	2583.29			
<i>Trichoderma longibrachiatum</i>	Chloroform	118.81	602.29	1.818± 0.38	0.09	39.73	194.75	1604.73	1.399± 0.34	0.74	40.41
		79.93	342.07				122.70	667.99			
		176.98	2035.54				375.58	16539.74			
	Ethyl acetate	103.54	490.41	1.897± 0.40	0.03	45.59	230.61	3854.27	1.048± 0.33	0.05	34.13
		69.44	289.41				126.63	1001.86			
		150.31	1514.02				766.43	881594.10			
Match 50% EC lufenuron		47.20	163.50	2.376± 0.40	0.001	100.00	78.70	196.80	3.220± 0.62	0.001	100.00
		34.60	107.70				62.50	144.70			
		64.20	371.60				98.60	359.70			

*Toxicity index = LC₅₀ of the most effective compound/ LC₅₀ of the tested compound × 100

The most toxic extract *T. longibrachiatum* ethyl acetate was analyzed by GC-MS and revealed the presence of 19 peaks corresponding to 19 compounds (Table 2 and Fig.1). These compounds were identified by comparing their mass spectra with those of their analogous reported by NIST library. The identified compounds belong to six different classes 13 acetogenins (fat derivatives) (55.77%), one shikimates (1.85%), one sesquiterpene (0.32%), one diterpene (0.62%), one alkaloid (1.07%) and two phthalate derivatives (6.48%).

Among various identified compounds total four major compounds were in significant quantity, 1-

Octadecene (20) (40.08%), Dibutyl phthalate (17) (5.57%), 1-Heptacosanol (23) (4.80%) and E-15-Heptadecenal (6) (3.05%).

Eight naturally products were also identified from GC/MS spectra of *M. anisopliae* chloroform extract belonging to three classes four acetogenins (fat derivatives) (14.9%), two sesquiterpenes (25.47%) and two phthalate derivatives (32.45%) (Table 2). The main constituents were phthalic acid, isobutyl 3-methylbut-3-enyl ester (11) (29.40%), 4a,cis-7-epoxy-cis-9a-perhydro-trans-5-hydroxy-4,4,trans-7,9a tetramethyl-5H-benzocycloheptene (16) (24.15%), 2-methoxy-6-(pent-3-en-2-yl)naphthalene (9)

(6.54%), 1-Octadecene (20) (6.01 %) and phthalic acid, di(2-propylpentyl) ester (30) (3.05%).

A sample from ethyl acetate extract of *Paceliomyces fumosoroseus* were analyzed using GC/MS technique, which resulted in characterization of 14 compounds belonging to three classes nine acetogenins (fat derivatives) (39.35%), one diterpene (0.94%) and four phthalate drevitaves (45.95%) (Table 2). The most

abundant compounds characterized from *P. fumosoroseus* ethyl acetate extract were Decyl isobutyl phthalate (12) (28.56%), 1-Octadecene (20) (14.85 %), Diethyl phthalate (1) (9.51%), 2,4-dioctylphenol (19) (7.86%), (3R/S)-1-methylidene-1,2,3, 4-tetrahydro-3- methyl- 9H-xanthen-9-one (10) (6.76%) and Bis (2-ethylhexyl) phthalate (31) (3.96%).

Table 2. Chemical constituents of three investigated fungi culture filtrates

No.	Compound	*R _t (min.)	Molecular weight	Molecular Formula	<i>Trichoderma</i> <i>longibrachiatum</i> Area %	<i>Metarhizium</i> <i>anisopliae</i> Area %	<i>Paceliomyces</i> <i>fumosoroseus</i> Area %
1	Diethyl Phthalate (1)	21.82	222	C ₁₂ H ₁₄ O ₄			9.51
2	n-Heptadecane (2)	25.05	240	C ₁₇ H ₃₆			1.79
3	Norphytane (3)	25.21	268	C ₁₉ H ₄₀			0.94
4	α-Hexylcinnamic aldehyde (4)	26.57	216	C ₁₅ H ₂₀ O	0.73		
5	4-methoxy-6-(2-oxopropyl)-2H-pyran-2-one (5)	26.60	182	C ₉ H ₁₀ O ₄		1.57	
6	E-15-Heptadecenal (6)	27.46	252	C ₁₇ H ₃₂ O	3.05		
7	i-Propyl 12-methyl-tridecanoate (7)	28.16	270	C ₁₇ H ₃₄ O ₂	0.64		
8	4-hydroxy-4-methyl-1,2,3,4-tetrahydro anthracene-1-one (8)	28.46	226	C ₁₅ H ₁₄ O ₂			1.08
9	2-methoxy-6-(pent-3-en-2-yl)naphthalene (9)	28.68	226	C ₁₆ H ₁₈ O		6.54	
10	(3R/S)-1-methylidene-1,2,3,4-tetrahydro-3-methyl-9H-xanthen-9-one (10)	28.70	324	C ₁₅ H ₁₄ O ₂			6.76
11	phthalic acid, isobutyl 3-methylbut-3-enyl ester (11)	29.10	290	C ₁₇ H ₂₂ O ₄		29.40	
12	Decyl isobutyl phthalate (12)	29.12	362	C ₂₂ H ₃₂ O ₄			28.56
13	Benzyl salicylate (13)	29.14	228	C ₁₄ H ₁₂ O ₃	1.85		
14	Methyl palmitate (14)	30.17	270	C ₁₇ H ₃₄ O ₂	0.82		
15	Widdrol hydroxyether (15)	30.83	238	C ₁₅ H ₂₆ O ₂	0.32		
16	4a,cis-7-epoxy-cis-9a-perhydro-trans-5-hydroxy-4,4,trans-7,9a-tetramethyl-5H-benzocycloheptene (16)	30.86	238	C ₁₅ H ₂₆ O ₂		24.15	
17	Dibutyl phthalate (17)	30.92	278	C ₁₆ H ₂₂ O ₄	5.57		3.92
18	Cycloeicosane (18)	31.46	280	C ₂₀ H ₄₀			1.58
19	2,4-Dioctylphenol (19)	32.63	318	C ₂₂ H ₃₈ O			7.86
20	1-Octadecene (20)	33.19	252	C ₁₈ H ₃₆	40.08	6.01	14.58
21	Heliannuol H (21)	34.58	248	C ₁₅ H ₂₀ O ₃		1.32	
22	1-Docosene (22)	35.12	308	C ₂₂ H ₄₄	1.56	0.78	1.87
23	1-Heptacosanol (23)	35.13	396	C ₂₇ H ₅₆ O	4.80		
24	Eicosyl acetate (24)	35.41	458	C ₂₂ H ₄₄ O ₂			2.11
25	1-Nonadecene (25)	36.89	266	C ₁₉ H ₃₈	1.16		
26	Dehydroabietic acid, methyl ester (26)	37.75	314	C ₂₁ H ₃₀ O ₂	0.62		
27	(Z)-9-Octadecenamide (27)	37.99	281	C ₁₈ H ₃₅ NO	1.07		
28	Cyclotetracosane (28)	38.48	336	C ₂₄ H ₄₈			1.72
29	Dioctyl adipate (29)	38.58	370	C ₂₂ H ₄₂ O ₄	0.82		
30	phthalic acid, di(2-propylpentyl) ester (30)	40.92	390	C ₂₄ H ₃₈ O ₄		3.05	
31	Bis(2-ethylhexyl) phthalate (31)	40.94	390	C ₂₄ H ₃₈ O ₄	0.91		3.96
32	(Z) 9-tricosene (32)	41.58	322	C ₂₃ H ₄₆	0.49		
33	Glutaric acid, 6-methylhept-2-yl octadecyl ester (33)	43.10	496	C ₃₁ H ₆₀ O ₄	0.65		
34	6a,12a-Dehydro-β-toxicarol (34)	43.61	408	C ₂₃ H ₂₀ O ₇	0.44		
35	Succinic acid, docosyl ethyl ester (35)	44.00	454	C ₂₈ H ₅₄ O ₄	0.53		

*R_t, retention time (min).

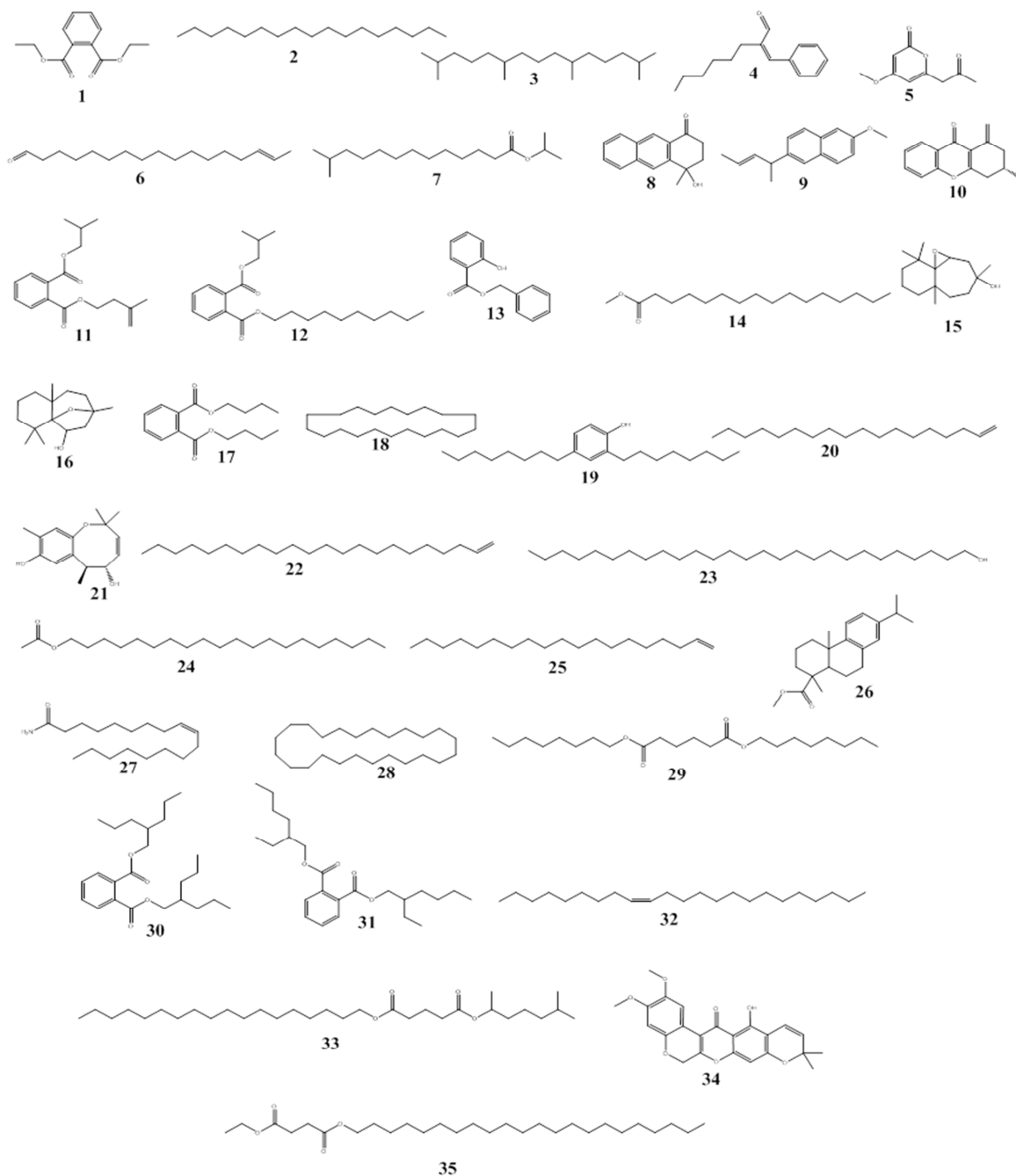


Fig. 1. Structure of chemical constituents of three investigated fungi culture filtrate

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فاعلية بعض المستخلصات الفطريات الممرضة للحشرات ومكوناتها الكيميائية كمبيدات حيوية بديلة ضد تريبس البصل ،

Thrips tabaci

أماني سعد محمد سعد¹ ، محمد إبراهيم السرجاني²، محمد الحسيني مصطفى³ و دينا مندوه فتحي⁴

¹قسم أمراض النبات- كلية الزراعة - جامعة المنصورة- مصر.

²قسم المبيدات - كلية الزراعة - جامعة المنصورة- مصر.

³معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقي بالجيزة- مصر.

⁴قسم الحشرات الاقتصادية - كلية الزراعة - جامعة المنصورة- مصر.

تم استخلاص نواتج الأيض الثانوية للفطريات الممرضة للحشرات من *Paecilomyces* و *Metarhizium anisopliae* و *Trichoderma longibrachiatum* باستخدام اثنين من المذيبات العضوية المختلفة وهي الكلوروفورم والإيثيل أسيتات وتقييمها كمبيد حشري حيوي ضد الحوريات والحشرات البالغة من تريبس البصل، *Thrips tabaci* باستخدام طريقة الرش. كشف مؤشر السمية لقيم LC50 أن مستخلصات الأيض الثانوية *T. longibrachiatum* (الكلوروفورم والإيثيل أسيتات) كانت الأكثر فعالية ضد المرحلتين التطوريتين (مرحلة الحورية والحشرة الكاملة) ل *T. tabaci* متبوعة بمستخلصات *M. anisopliae* وأقلها سمية *P. fumosoroseus*. بعد 7 أيام من المعاملة. تم تحليل التركيب الكيميائي لمستخلصات كل من الفطريات الممرضة باستخدام تقنية GC-MS. تم توصيف خمسة وثلاثين مركبًا تنتمي إلى ست فئات مختلفة من المنتجات الطبيعية.