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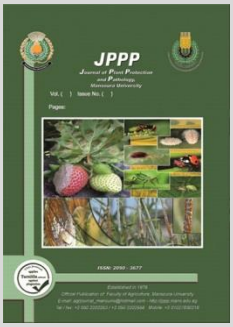
# Insecticidal Activity of Secondary Metabolites of Locally Isolated Fungal Strains against some Cotton Insect Pests

**Abdullah, R. R. H.***

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.




## ABSTRACT

Microbial pest control is one of the best alternatives to chemical insecticides. *Beauveria bassiana* and *Trichoderma harzianum* are important entomopathogens of many insect pests. Cotton leafworm (*Spodoptera littoralis*) and aphids (*Aphis gossypii*) are the most destructive pests in crop production such as cotton, cabbage, tomato, potato, and lettuce. The present study sought to isolate both fungal strains (*B. bassiana* and *T. harzianum)* and extract their secondary metabolites by ethyl acetate then evaluate the efficacy of crude extract against both insect pests (*S. littoralis* and *A. gossypii).* Also, Gas chromatography-mass spectrometry (GC-MS) analysis was conducted to both extracts of isolated fungi. The results indicated that both extracts of tested fungi have insecticidal activity against both tested insects. *B. bassiana* extract was more toxic against both insects than *T. harzianum.* The extract of *T. harzianum* was more toxic against *A. gossypii* more than *S. littoralis.* The $LC_{50}$ values of *B. bassiana* and *T. harzianum* against *S. littoralis* were 575 and 3238 ppm respectively but, they were 226 and 389 ppm against *A. gossypii* respectively. GC-MS analysis indicated the presence of some identified compounds as insecticides in both extracts of fungi such as: n-Hexadecanoic acid; Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid, methyl ester, (E,E)-; Tetradecanoic acid, 12-methyl-, methyl ester; 7,10-Octadecadienoic acid, methyl ester and trans-13-Octadecenoic acid. This study recommended that the ethyl acetate extract of both tested fungi can be used as an alternative of chemical pesticides to control *A. gossypii* but in the case of *S. littoralis* can be used the extract of *B. bassiana* only.

***Keywords:*** Fungal isolates, *Spodoptera littoralis*, *Aphis gossypii*, Ethyl acetate extracts, GC-MS analysis

## INTRODUCTION

The bioactive compounds of biological origin from entomopathogenic microbes are being bio-prospected as alternatives to chemical insecticides. The importance of these biopesticides is due to their biodegradability, specificity, eco-friendly nature and their usefulness as tools to manage insecticide resistance. Many researchers have interested in natural products isolated from microorganisms as alternatives to conventional chemical insecticides to use them in programs of pest control.The most important alternatives to chemical pesticides are the entomopathogen that are natural insect pathogens and having a potential effect on pest management. (Ravindran *et al.* 2018)

More than seven hundred species of fungi were investigated as biopesticides of insects and only a few species contribute to the regulation of their host populations (Roy & Pell 2010). The mode of action of entomopathogenic fungi as *Metarhizium anisopliae*, *Beauveria bassiana, Isaria fumosorosea, Nomuraea rileyi*, *Lecanicillium lecanii*, *Purpureocillium sp.* and *Cladosporium sp.*, involves the production of enzymes, toxic proteins, and bioactive metabolites to overcome the insect immune system and modify the host behavior (Isaka *et al.* 2005, Ortiz-Urquiza & Keyhani 2013). The metabolites, secreted by entomopathogenic fungi, are a rich source of bioactive compounds, including polyketides, non-ribosomal peptides, polyketide-peptide hybrid metabolites and terpenes (Fox & Howlett 2008). Several of secondary metabolites, secreted by entomopathogens, have been mentioned to have antifeedant and insecticidal properties (Bandani *et al.* 2000, Molnar *et al.* 2010).

*Aphis gossypii* Glover (Hemiptera: Aphididae) is a dangerous pest worldwide that causes damage to more than two hundred economically important crops, as cotton, okra, eggplant, cucumber, pepper, squash, and cantaloupes (Razmjou *et al.* 2012). This insect pest sucks the sap from the vascular tissues of plant, which produces many physiological disorders in the plant, also transmission more than seventy-five viruses as well as inhibiting the photosynthetic ability by producing a large amount of honeydew that provides a good medium for the growth of fungi (Blackman & Eastop 2000). The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a dangerous and highly polyphagous insect in Egypt that causes many damages not only for cotton plants but also for other field crops and vegetables. It is considered to be the main pest of economic importance in several countries because it attacks a multitude of host plants. (Lobna *et al.* 2013, Heidi *et al.* 2015). The aim of this study is to investigate the insecticidal activities of the extracted secondary metabolites by ethyl acetate from some indigenous fungal isolates on cotton aphid, (*Aphis. gossypii* ) and cotton leafworm, ( *Spodoptera littoralis*).

* **Corresponding author.**
**E-mail address: redakenany@yahoo.com**
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## MATERIALS AND METHODS

**Fungal isolates:**

*Beauveria bassiana* and *Trichoderma harzianum* were isolated from soil and insect samples which were collected from Dakahliah governorates, Egypt. These fungi were identified firstly by their colony color and spores shape(Fig. 1), then confirmed by sequencing of ITS region as amplified from genomic DNA using the primer pairs ITS1 / ITS4 (5'-TCCGTAGGGTGAACCTGCGG-3') / (5' TC CT C C G CTTATTGATATGC-3') as the method described by White *et al.* (1990).

**Fig. 1. Colony shape and color of *B. bassiana* and *T. harzianum* after 10 days of incubation on PDA medium**

**Extraction of metabolites of fungal isolates by ethyl acetate:**

Fungal conidia (30 ml, $1\times10^6$ conidia $ml^{-1}$) of both fungal isolates were inoculated into shake cultures in a 1 liter flask containing 300 ml of potato dextrose broth (PDB) medium and incubated at 25 ± 1°C for 7 days for the production of the seed inoculum. The seed inoculum was added to fresh broth medium (4 Littre total) and the mixture was incubated at 25 ± 1°C for 21 days. After incubation period, the metabolites were extracted as described method by Chen *et al*. (2018) using ethyl acetate (EthOAc) from the cell-free culture supernatant. Aliquots of the fungal culture were mixed with a half volume of ethyl acetate (1 : 0.5) and mixed vigorously then put the mixtures in separating funnel and leave them 2 hr. The organic phase from the above extraction mixture was collected and filtered over anhydrous sodium sulphate ($Na_2SO_4$) to remove water from the solvent. The extracts were subjected to dryness using a rotary evaporator to remove any traces of solvents and to obtain a crude extract Yield of crude extract was weighed as shown in Table 1

**Table 1. Yield of metabolites derived from ethyl acetate extracts of both fungal isolates**

| Fungal strains | Yield of metabolites (mg)/ 4L culture broth medium |
|---|---|
| *Trichoderma harzianum* (Tr) | 298 |
| *Beauveria bassiana* (Bb) | 257 |

**Bioassay experiments:**

The crude extract of both fungal isolates was weighed and dissolved in 20 ml ethyl acetate as stock solution. Six concentrations from each extract (250, 500, 1000, 2000, 3000, 4000 ppm) were prepared by diluting in distilled water containing 0.1% Tween 80 to use them in bioassay experiments. The 3rd instar larvae of the cotton leafworm, *Spodoptera littoralis* and the adult individuals of the cotton aphids, *Aphis gossypii* were used to evaluate the efficiency of ethyl acetate extracts of both fungal isolates as the following:

***S. littoralis*:**

The efficiency of ethyl acetate extracts of the tested fungal isolates was evaluated against cotton leafworm, *S. littoralis* by leaf-dip bioassay method using castor oil leaves and six concentrations of each extract as described by Tabashnik *et al.* (1991). Castor oil leaves were first washed with distilled water then dipped in the prepared concentrations to 30 second and then air-dried. Then leaves were placed individually into plastic cups. Twenty individuals of the 3rd instar larvae of *S. littoralis* were placed in each prepared plastic cups. Each cup was tightly covered with a piece of muslin by means of a rubber band. Five replicates were used for each concentration. Larval mortality was recorded after 24, 48 and 72 hrs post treatment. The mortality percentages were corrected by Abbott's formula (Abbott 1925). Median lethal concentration ($LC_{50}$), slope values and 95 % fiducially limits were estimated by Finney's probit analysis method (Finney 1971). Also, the toxicity index was calculated according to Sun's equation (1950).

***A. gossypii*:**

Naturally infested cotton leaves by *A. gossypii* were obtained from untreated cotton leaves by pesticides. Twenty adult individuals of *A. gossypii* were counted on an infested leaf and placed in foam plates. Each plate was treated by prepared concentrations (250, 500, 1000 ppm) by spraying bioassay method (approximately 3 ml/plate). Each treatment was replicated five times. Mortality was recorded after 24 and 48 hrs. Mortality percentages were corrected by Abbott's formula (Abbott 1925). Median lethal concentration ($LC_{50}$), slope values and 95 % fiducially limits were estimated by Finney's probit analysis method (Finney 1971). Also, the toxicity index was calculated according to Sun's equation (1950).

**Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

An Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a water GCT Premier mass spectrometer (Waters Corporation, Milford, MA, USA) was used to carry out GC-MS analysis of the ethyl acetate extracts obtained from tested fungi cultures. A Clarus 680 30 m x 0.25 mm ID x 250 µm silica column was used. An elite-5MS (5% biphenyl 95% dimethylpolysiloxane) was used to fill this column. Helium gas was used as a carrier gas to separate the chemical constituents at a flow rate of 1 ml/min. The crud extracts (1 µL) were injected into the GC-MS device at 260 °C during the running time of the column. The temperature ramp was the following: 60 °C (2 min); followed by 300 °C at the rate of 10 °C $min^{-1}$; and finally 300 °C, where it was held for 6 min. The conditions of the mass detector were 240 °C; the ion source temperature was 240 °C; and ionization mode electron impact at 70 eV, the scan time was 0.2 s and the scan interval was 0.1 s. Fragments from 40 to 600 Da have been collected. The NIST 05 L mass spectral library was used to identify the compounds by comparison of their mass spectra.

## RESULTS AND DISCUSSION

**Insecticidal activity of secondary metabolites of isolated fungal strains:**

In recent years, many researchers have interested in natural products extracted from plants or microorganisms as alternatives to conventional chemical insecticides to use them in integrated pest management programs (IPM). Wang *et al.* (2007) mentioned that the entomopathogenic fungi able to secrete a wide range of bioactive compounds known as secondary metabolic compounds as well as the fungal metabolites have been shown to possess the potential insecticidal activity and may be used as biopesticides. Pampapathy *et al.* (2011) reported that metabolites in culture filtrates and mycelial extracts of *B. bassiana* significantly reduced the survival of *A. gossypii* and the number of aphids settling on the treated leaf discs. Similarly, McGee (2002) mentioned that methanol extracts of four fungal isolates that are isolated from the cotton leaves reduced the larval growth rates of *Helicoverpa armigera* (Hubner) and *Helicoverpa punctigera* (Wallengren). The crude extracts of *B. bassiana* caused significant mortality of *Spodoptera littoralis* Boisduval larvae when applied either on alfalfa leaf disks or to the artificial diet (Quesada-Moraga *et al.* 2006).

In the present study the ethyl acetate extract of liquid culture from *B. bassiana* showed high insecticidal activity against cotton leafworm (*S. littoralis*) and cotton aphids (*A. gossypii*). Initial kill of the *S. littoralis* after 24 h reached to 86.7% mortality percentage at 1000 ppm concentrate but in case of *A. gossypii* the initial kill after 24h was 100% mortality percentage at the same concentrate as shown in Tables 2 and 4. The $LC_{50}$ value of *B. bassiana* extract was 575 ppm in case of *S. littoralis* but it was 226 ppm in case of *A. gossypii* as shown in Tables 3 and 5. Generally, the toxicity of *B. bassiana* extract on *A. gossypii* was more than their toxicity on *S. littoralis* also, the toxicity increased directly with the increasing of concentration.

Ahmed and El-katatny (2007) reported that *Trichoderma harzianum* can be used as a biopesticide against different insect pests, in addition, its ability to produce some effective antimicrobial agents for controlling plant diseases. Also, they mentioned that spore suspension and metabolites of *T. harzianum* caused a high larvicidal activity against cotton leafworm, *Spodoptera littoralis* where the mortality percentage reached 80% after treatment by $1x10^8$ spores/ml of this fungus. Kaleil *et al.* (2016) studied the impact of the entomopathogenic fungus, *Trichoderma hamatum* on the cotton aphid (*Aphis gossypii*). They found that *T. hamatum* was more toxic to *A. gossypii* also; they mentioned that the mortality percentage was increased with increasing the concentration of fungus spore suspension. Binod *et al.* (2007) reported that the filtrate of *T. harzianum* culture able to negatively affect the growth and metamorphosis of *Helicoverpa armigera* larvae. It is also a potent anti-feeding agent as it decreases the feeding rate and bodyweight of the larva. Also, this fungus filtrate decreases the successful pupation process and leads to a 70% mortality of larvae. Vijaykumar *et al.* (2009) found a high mortality percentage of the cotton pests (*Helicoverpa, Earias* and *Pectinophora spp*) after treated by *T. harzianum* culture filtrate.

In the present study, the ethyl acetate extract of *T. herzianum* had no or low effect on *S. littoralis* until 48h at all concentrations but the mortality percentage reached 76.7% after 72h of treatment at 4000 ppm. On the other hand, in the case of *A. gossypii*, the mortality percentage was 96.7% after 24h of treatment as shown in Tables 2 and 4. The $LC_{50}$ value of *T. herzianum* extract was 3238 ppm in the case of *S. littoralis* but it was 389 ppm with *A. gossypii* as shown in Tables 3 and 5. The presented data in this research illustrated that the insecticidal activity of *B. bassiana* extract was more than *T. harzianum* extract also, the toxicity of both fungal extracts on *A. gossypii* was more than *S. littoralis.*

**Table 2. Mortality % of treated 3rd instar larvae of *S. littoralis* by ethyl acetate extracts of tested fungi**

| Conc. Ppm | Mortality % of *S. littoralis* corrected by Abbot's formula | | | | | |
|---|---|---|---|---|---|---|
| | After 1 day | | After 2 day | | After 3day | |
| | Tr | Bb | Tr | Bb | Tr | Bb |
| 250 | 0 | 0 | 0 | 0 | 0 | 0 |
| 500 | 0 | 0 | 0 | 3.3 | 0 | 26.7 |
| 1000 | 0 | 86.7 | 0 | 100 | 0 | 100 |
| 2000 | 0 | 100 | 0 | 100 | 16.7 | 100 |
| 3000 | 0 | 100 | 6.7 | 100 | 30 | 100 |
| 4000 | 0 | 100 | 23.3 | 100 | 76.7 | 100 |

**Tr: *T. harzianum* Bb: *B. bassiana***

**Table 3. Toxicity of fungal ethyl acetate extracts on 3rd instar larvae of *S. littoralis* after 72 h.**

| Ethyl acetate extracts | $LC_{50}$ ppm | Confidence limits 95% | | Slope ± SE | Toxicity index % |
|---|---|---|---|---|---|
| | | Lower (ppm) | Upper (ppm) | | |
| Bb | 575 | 517 | 671 | 10±2.5 | 100 |
| Tr | 3238 | 2882 | 3753 | 5.6±1.15 | 17.75 |

**Tr: *T. harzianum* Bb: *B. bassiana***

**Toxicity Index (%) = ($LC_{50}$ of the most effective fungal extract / $LC_{50}$ of the tested fungal extract)×100.**

**Table 4. Mortality % of treated *A. gossypii* adults by ethyl acetate extracts of tested fungi**

| Conc. Ppm | Mortality % of *A. gossypii* corrected by Abbot's formula | | | |
|---|---|---|---|---|
| | After 24 hrs | | After 48 hrs | |
| | Tr | Bb | Tr | Bb |
| 250 | 26.3 | 57.8 | 37.3 | 74.3 |
| 500 | 61.9 | 86.2 | 78.6 | 97.3 |
| 1000 | 96.7 | 100 | 100 | 100 |

**Tr: *T. harzianum* Bb: *B. bassiana***

**Table 5. Toxicity of fungal ethyl acetate extracts on *A. gossypii* adults after 24 h.**

| Ethyl acetate extracts | $LC_{50}$ Ppm | Confidence limits 95% | | Slope ± SE | Toxicity index % |
|---|---|---|---|---|---|
| | | Lower (ppm) | Upper (ppm) | | |
| Bb | 226 | 180 | 262 | 3.5 ± 0.5 | 100 |
| Tr | 389 | 346 | 430 | 3.5 ± 0.3 | 58 |

**Tr: *T. herzianum* Bb: *B. bassiana***

**Toxicity Index (%) = ($LC_{50}$ of the most effective fungal extract / $LC_{50}$ of the tested fungal extract) ×100.**

**Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

The chemical constituents identified from *B. bassiana* and *T. herzianum* ethyl acetate extracts were listed in Tables 6 and 7. Peaks area of each chemical compound to both fungal strains were illustrated in the GC-MS chromatogram as shown in Fig. 2 and 3. In case of *B. bassiana* extract, the detected constituents were 25 compounds (Table 6). The major compounds were Hexadecanoic acid, methyl ester (14.96%), n-Hexadecanoic acid (21.14%), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (11.98%) and Tetradecanoic acid, 12-

methyl-, methyl ester (12.25%). On the other hand, the detected constituents in *T. herzianum* extract were 19 compounds (Table 7). The major compounds were Hexadecanoic acid, methyl ester (10.82%), n-Hexadecanoic acid (13.01%), 7,10-Octadecadienoic acid, methyl ester (18.19%) and trans-13-Octadecenoic acid (15.32). Mass spectrum of the major compounds was shown in Figures 4, 5, 6, 7, 8 and 9.

The major expected compounds in ethyl acetate extracts of both fungi were mentioned in several previous studies as larvicidal, nematicidal, pesticide, Insectifuge agents. (Kumar *et al.* 2010, Krishnamoorth & Subramaniam 2014, Sivakimar *et al*. 2011, Tulika & Mala 2017, Yokeswari *et al*. 2018)

**Table 6. GC-MS analysis of ethyl acetate extract of *B. bassiana* broth culture**

| No. | Rt (min) | Compound | MF | MW | Peak Area (%) | Biological activity |
|---|---|---|---|---|---|---|
| 1 | 12.367 | 4-Hydroxy-10-methyl-3,4,7,8,9,10-hexahydro-2H-oxecin-2-one # | C10H16O3 | 184 | 1.20 | ---------- |
| 2 | 13.264 | 2-Carboxymethyl-3-n-hexylmaleic acid anhydride | C12H16O5 | 240 | 0.97 | Antimicrobial |
| 3 | 14.740 | 1-Hexadecanol | C16H34O | 242 | 1.29 | Antimicrobial |
| 4 | 14.912 | 4,5-Diethyl-2,3-dimethyl-2,3-dihydrofuran # | C10H18O | 154 | 1.32 | ---------- |
| 5 | 15.730 | 4,7,7-Trimethyl-3,9-dioxatricyclo[6.1.0.0(2,4)]nonan-5-one | C10H14O3 | 182 | 1.77 | ---------- |
| 6 | 18.854 | Methyl 11-(3-pentyl-2-oxiranyl)undecanoate, trans- | C19H36O3 | 312 | 1.84 | ---------- |
| 7 | 20.483 | Hexadecanoic acid, methyl ester | C17H34O2 | 270 | 14.96 | Larvicidal activity |
| 8 | 20.710 | 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione | C17H24O3 | 276 | 1.29 | Antimicrobial |
| 9 | 21.608 | n-Hexadecanoic acid | C16H32O2 | 256 | 21.14 | Pesticide & Nematicide |
| 10 | 22.069 | cis-10-Heptadecenoic acid, methyl ester | C18H34O2 | 282 | 1.50 | Anticancer |
| 11 | 22.992 | cis-10-Heptadecenoic acid | C17H32O2 | 268 | 0.23 | Anticancer |
| 12 | 23.145 | 10,18-Bisnorabieta-8,11,13-triene | C18H26 | 242 | 1.97 | ---------- |
| 13 | 23.410 | 1-Hexadecanol, 2-methyl- | C17H36O | 256 | 1.71 | Antimicrobial |
| 14 | 23.594 | 9,12-Octadecadienoic acid, methyl ester, (E,E)- | C19H34O2 | 294 | 11.98 | Nematicide & Insectifuge |
| 15 | 23.693 | 11-Octadecenoic acid, methyl ester | C19H36O2 | 296 | 1.94 | Antimicrobial |
| 16 | 24.123 | Tetradecanoic acid, 12-methyl-, methyl ester | C16H32O2 | 256 | 12.25 | Pesticide, Insectifuge & Nematicide |
| 17 | 24.652 | Oleic Acid | C18H34O2 | 282 | 2.86 | Antibacterial |
| 18 | 25.162 | Octadecanoic acid | C18H36O2 | 284 | 1.50 | Antimicrobial |
| 19 | 25.359 | Erucic acid | C22H42O2 | 338 | 0.88 | Antimicrobial |
| 20 | 25.451 | Ethanol, 2-(octadecyloxy)- | C20H42O2 | 314 | 1.07 | Antimicrobial |
| 21 | 27.191 | Heptacosane | C27H56 | 380 | 1.94 | Antibacterial |
| 22 | 27.425 | Z-5-Methyl-6-heneicosen-11-one | C22H42O | 322 | 1.10 | Antimicrobial |
| 23 | 29.995 | 17-Pentatriacontene | C35H70 | 490 | 1.25 | Antibacterial |
| 24 | 31.108 | Diisooctyl phthalate | C24H38O4 | 390 | 2.01 | Antimicrobial |
| 25 | 31.421 | Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester | C19H21NOS | 311 | 1.03 | Antimicrobial |

**Table 7. GC-MS analysis of ethyl acetate extract of *T. harzianum* broth culture**

| No. | Rt (min) | Compound | MF | MW | Peak area (%) | Biological Activity |
|---|---|---|---|---|---|---|
| 1 | 13.646 | 2,4-Di-tert-butylphenol | C14H22O | 206 | 1.14 | Antifungal Antioxidant |
| 2 | 14.759 | 1-Hexadecanol | C16H34O | 242 | 0.91 | Antimicrobial |
| 3 | 14.857 | 1-Hexadecanol, 2-methyl- | C17H36O | 256 | 0.54 | Antimicrobial |
| 4 | 18.103 | 1-hexdecen | C16H32 | 242 | 1.05 | Antibacterial |
| 5 | 20.489 | Hexadecanoic acid, methyl ester | C17H34O2 | 270 | 10.82 | Pesticide |
| 6 | 20.680 | 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione | C17H24O3 | 276 | 0.56 | Antimicrobial |
| 7 | 21.258 | n-Hexadecanoic acid | C16H32O2 | 256 | 13.01 | Pesticide |
| 8 | 21.393 | Phthalic acid, butyl octyl ester | C20H30O4 | 334 | 1.21 | Antibacterial |
| 9 | 21.657 | 10-Heneicosene (c,t) | C21H42 | 294 | 1.55 | ---------- |
| 10 | 23.164 | Androst-5,7-dien-3-ol-17-one | C19H26O2 | 286 | 0.72 | ---------- |
| 11 | 23.404 | cis-13-Eicosenoic acid | C20H38O2 | 310 | 0.56 | Anti-inflammatory |
| 12 | 23.594 | 7,10-Octadecadienoic acid, methyl ester | C19H34O2 | 294 | 18.19 | Nematicide & Insectifuge |
| 13 | 23.779 | trans-13-Octadecenoic acid | C18H34O2 | 282 | 15.32 | Insectifuge |
| 14 | 25.346 | 1-Hexadecanol, 2-methyl- | C17H36O | 265 | 1.31 | Antimicrobial |
| 15 | 25.439 | Ethanol, 2-(octadecyloxy)- | C20H42O2 | 314 | 0.97 | Antimicrobial |
| 16 | 27.173 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366 | 4.65 | Antimicrobial |
| 19 | 31.102 | Diisooctyl phthalate | C24H38O4 | 390 | 4.15 | Antibacterial |

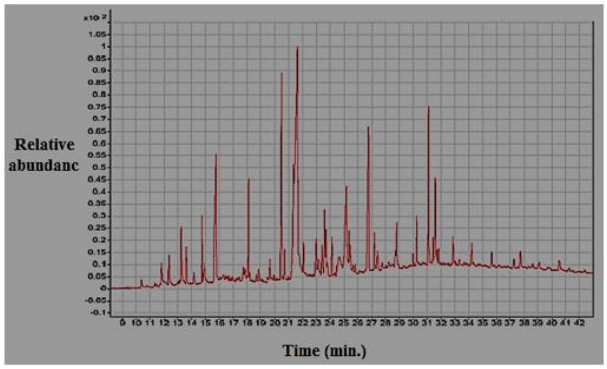


**Figure 2. GC-MS chromatogram of the ethyl acetate extract of *B. bassiana***

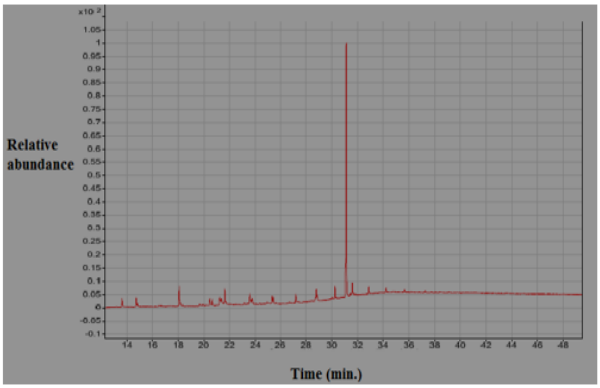


**Figure 3. GC-MS chromatogram of the ethyl acetate extract of *T. harzianum***

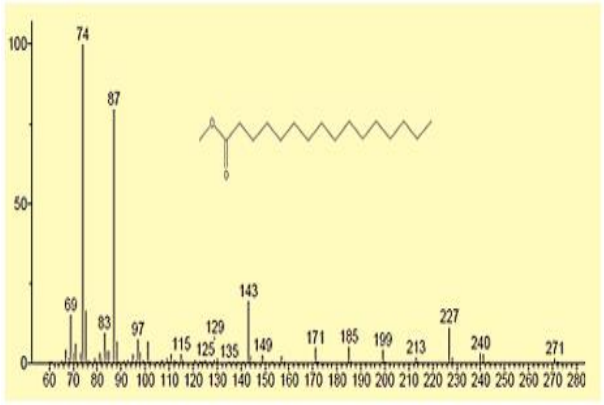

**Figure 4. Mass spectrum of Hexadecanoic acid, methyl ester**

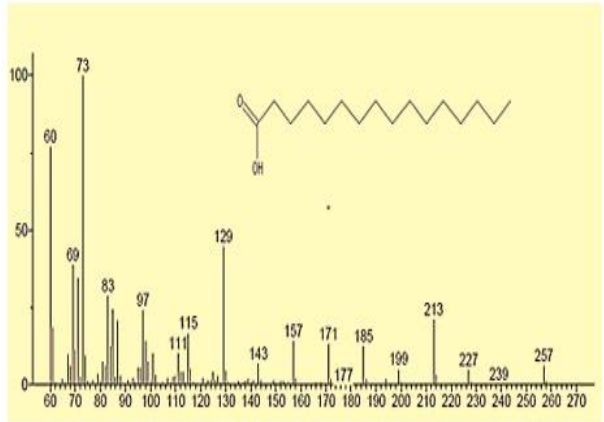

**Figure 5. Mass spectrum of n-Hexadecanoic acid**

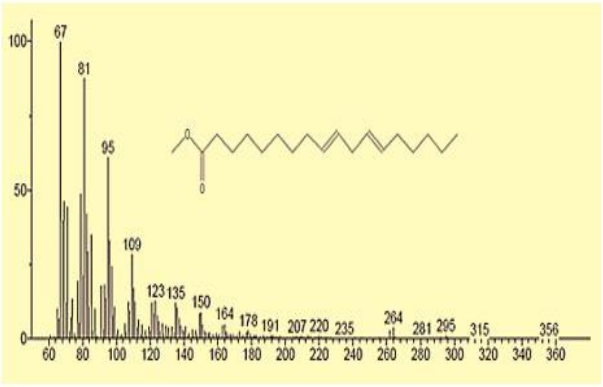

**Figure 6. Mass spectrum of 9,12-Octadecadienoic acid, methyl ester, (E,E)-**

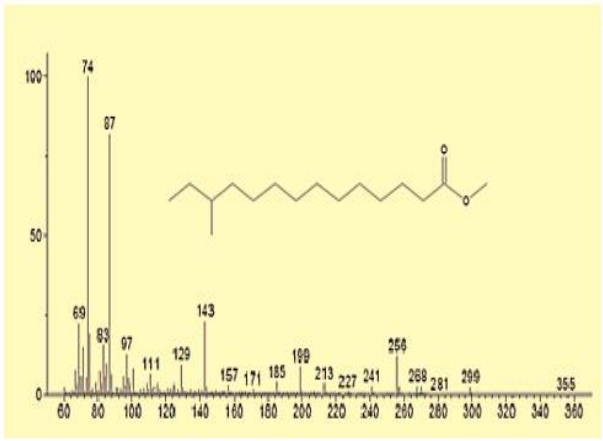

**Figure 7. Mass spectrum of Tetradecanoic acid, 12-methyl-, methyl ester**

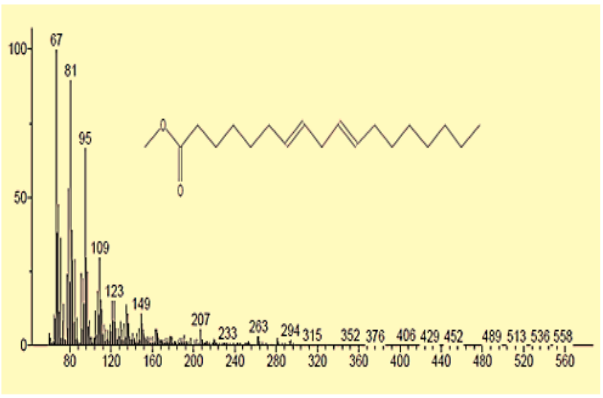

**Figure 8. Mass spectrum of 7,10-Octadecadienoic acid, methyl ester**

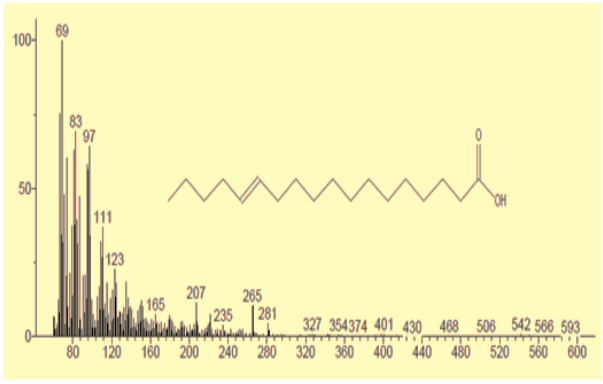

**Figure 9. Mass spectrum of trans-13-Octadecenoic acid**

## CONCLUSIONS

Based on obtained results it could be concluded that using the extract of entomopathogen metabolites is one of the best bio-pesticides. The ethyl acetate extract of *Beauveria bassiana* culture had high an insecticidal activity against cotton leafworm and cotton aphids followed by *Trichoderma harzianum*. Using the secondary metabolites of entomopathogens may be the best compared with using directly the organism especially at unsuitable weather as the high-temperature degree and the drought and presence of chemical pesticides.

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## النشاط الإبادي لنواتج التمثيل الغذائي الثانوية للفطريات المعزولة محليا ضد بعض الافات الحشرية التي تصيب القطن

**رضا راضي حسن عبدالله**

**معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة – مصر**

تعتبر المكافحة الميكروبية للحشرات من أفضل البدائل للمبيدات الحشرية الكيميائية المخلقة. كما تعتبر فطريات *Beauveria bassiana* , *Trichoderma harzianum* هي من أهم مسببات الأمراض للعديد من الأفات الحشرية. وكذلك تعتبر حشرة دودة ورق القطن *Spodoptera littoralis* وحشرة من القطن *Aphis gossypii* هي أكثر الأفات الحشرية التي تسبب خسائر في انتاجية العديد من المحاصيل مثل القطن و الكرنب و الطماطم و البطاطس والخس وغيرها. سعت هذه الدراسة إلى عزل الفطريات *B. bassiana* و *T. harzianum* واستخلاص نواتج التمثيل الغذائي الثانوية لها باستخدام خلات الايثيل. وأيضا تقييم كفاءة هذه المستخلصات ضد العمر اليرقي الثالث لدودة ورق القطن والطور الكامل لمن القطن . في هذه الدراسة أيضا تم عمل فصل وتعريف للمركبات الموجودة في كلا الخلاصتين للفطريات المعزولة باستخدام جهاز GC-MS . أشارت النتائج إلى أن كلا المستخلصين كان لهما نشاط ابادي على الحشرات المختبرة إلا أن خلاصة فطر *B. bassiana* كانت أعلي تأثيرا من خلاصة فطر *T. harzianum* تجاه كلا الحشرتين. وكذلك كان تأثير خلاصة فطر *T. harzianum* أكثر سمية على المن مقارنة بدودة ورق القطن. والجدير بالذكر أن قيمة التركيز القاتل لنصف الحشرات المختبرة ($LC_{50}$) المعاملة بخلاصة فطر *B. bassiana* كانت 575 و 226 جزء في المليون ضد كل من دودة ورق القطن ومن القطن على التوالي . بينما كانت 3238 و 389 جزء في المليون في حالة خلاصة *T. harzianum* على التوالي. كما أشارت النتائج المتحصل عليها من تحليل GC-MS الى وجود العديد من المركبات المعروفة بكفاءتها في مكافحة الآفات الحشرية في كلا المستخلصين للفطريات المعزولة وهي مثل : n-Hexadecanoic acid; Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid, methyl ester, (E,E)-; Tetradecanoic acid, 12-methyl-, methyl ester; 7,10-Octadecadienoic acid, methyl ester and trans-13-Octadecenoic acid. وأخيرا توصي هذه الدراسة بإمكانية استخدام خلاصة الايثيل أسيتات لكل من الفطريات المعزولة كبدائل للمبيدات الكيميائية في مكافحة حشرة من القطن. ولكن في حالة حشرة دودة ورق القطن فإنه يفضل استخدام خلاصة فطر *B. bassiana* فقط.