APPLICATION OF TOMATO LEAVES EXTRACT AS PESTICIDE AGAINST APHIS GOSSYPII GLOVER (HEMIPTERA: APHIDIDAE).

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Abstract

This study aimed to evaluate the unused part of the tomato plant leaves, as a botanical pesticide. The cotton aphid, Aphis gossypii Glover (Hemiptera: Aphididae) is one of the most serious pest all over the world. They have a wide host range, and may transmit pathogenic virus. It attacks tomato leaves and sucks the plant sap from the leaves by its piercing and sucking mouth parts. Due to the problems of chemical pesticides to all organisms and environment, natural control replaced pesticides. Tomato extract contains many contents of phenolic and flavonoid compounds which were effective in control. Different little concentrations of tomato extract were applied in control of aphid and caused high mortality proportion. In the present study, the concentrations used were 250, 500, 750 and 1000 ppm and the mortality increased when the concentrations increased, which was 27.5 % with 250 ppm and reached 80% with 1000 ppm. Also, the chemical composition of extract of tomato (Lycopersicon spp.), was characterized by GC/ MS analysis which revealed the presence of 46 compounds, the major compound was Phytol (16.03%).

Introduction

Tomato (Lycopersicon spp.) is economically one of the most important vegetables (Polston and Anderson, 1999). Cotton aphid (Aphis gossypii Glov.) is one of the basic pest of tomato. It is a direct plant- sucking pest and it can cause serious problems on leaves, stems and fruits (Sharma and Joshi, 2010). It also causes direct damage by secreting honeydew that causes development of sooty-mould (Blackman and Eastop, 2000). Chemical control tactics have been the primary method for managing infestation, but this strategy has become less effective due to development of insecticide resistant population (Siebert et al., 2012). Pesticides produced from natural products have been recently attracting the attention of many scientists to avoid the problems caused by synthetic compounds. They are deeply interested in their chemical constituents and biological properties (Abou- Yousef et al., 2010). Tomato is a good source of phenolic compounds, pigments, antioxidants and other nutrients, these compounds prevent oxidative changes in cell by reducing the level of free radicals (Norma et al., 2015). The aim of this study was to determine the effect of tomato leaves extract on the cotton aphid, Aphis gossypii and made GC/ MS analysis to tomato leaves.
Material and methods:

Insects:
Tomato leaves carrying *A. gossypii* were collected from the unsprayed farm of Agriculture College, Mansoura University (Dakhlia, Egypt). The leaves were kept in jars at 27± 2 °C and 65± 5% RH. The colony was maintained for two generations before the beginning of the tests. Then, newly born nymphs of *A. gossypii* were placed, separately on tomato leaves in plastic Petri dishes (10 cm, in diameter). Each dish was covered with muslin for aeration and the tomato leaves were put on the bottom of the dish (Mahdi and Sahragard, 2012). Whenever leaves appeared discolored, they were replaced with fresh ones.

Preparation of Plant Sample and Extraction:
Leaves of tomato plant, 961 sorts, were left to dry at room temperature for one month then the dried leaves were grinded into fine powder. Powder was soaked in a mixture of hexane, acetone and ethanol solvents of equal proportion (1:1:1) in a flask for about one week. Finally, the flask was shake in a shaker and its contents were filtered. The solvents were evaporated under reduced pressure; the resulted crude extract was weighted and kept in deep freezer until use.

Preparing the Stock Solution of the Tested Plant Extract:
Convenient stock, concentrations of tomato extract, was prepared on basis of the tested plant weight and the volume of the distilled water (w/v) in the presence of tween 80(0.1%) as emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Four diluted concentrations for the plant extract were used to draw the LC-P lines. Four replicates were used for each concentration.

Method of application:
Spray method:
The adults of the aphid were used for application. Four concentrations were used as well as four replicates for each concentration. 10 individuals of aphids for each replicate were applied to estimate the mortality line. Different concentrations were sprayed directly on the aphids. The concentrations used were 250, 500, 750 and 1000 ppm. The percentage of mortality was recorded after one, three, five and seven days and the data were corrected relatively to control mortality (Abbott, 1925). LC₅₀ values were determined using Probit analysis statistical method of Finney, (1971).

Chemical analysis:
GC/MS analyses were conducted in Central Agricultural Pesticide Laboratory. They used an Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 ms (30m x 0.32mm x 0.25 μm film thicknesses). Sample was injected under the following conditions. Helium was used as carrier gas at approximately 1.0 ml/ min., pulsed split less mode. The solvent delay was 3 min. and the injection size was 1.0 μl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 230 °C. The electron multiplier voltage (EM voltage) was maintained 1650 v about auto tune. The instrument was manually tuned using perfluoro tributyl amine (PFTBA). The GC temperature program was started at 60 °C (2 min.) then evaluated to 300 °C at rate if 5 °C/ min. the injector temperature was set at 280 °C, respectively. Wiley and Wiley NIST mass spectral data base was used in the identification of the separated peaks.

Results and discussion:
(1) Toxicity Effect:
Efficiency of tomato leaves extract on *Aphis gossypii* adults:
The data in Table (1) demonstrated that, although the extract concentrations were low, the mortality rate of the adults of *Aphis gossypii* was high and when the concentrations increased, the total mortality increased. These results were in agreement with Hansson *et al.* (2012) which proved the effectiveness of ethyl ester oil on *Myzus persicae*. 
Table 1: Corrected mortality % of the adults of cotton aphid, *Aphis gossypii* treated with tomato leaves extract under laboratory conditions 27±2°C and 65±5% RH.

| Treatment                  | Conc. (ppm) | One day | Three days | Five days | Seven days | Total Mortality % |
|----------------------------|-------------|---------|------------|-----------|------------|-------------------|
| Tomato leaves extract      | 250         | 5       | 7.5        | 7.5       | 7.5        | 27.5              |
|                            | 500         | 15      | 5          | 12.5      | 22.5       | 55                |
|                            | 750         | 7.5     | 7.5        | 20        | 37.5       | 72.5              |
|                            | 1000        | 15      | 20         | 20        | 25         | 80                |

However, Table (2) and Fig. (1) demonstrated that, LC$_{50}$ was 439.89 ppm and LC$_{90}$ was 1483.36. The probability was 0.999. *Esther et al.* (2008) proved a significant effect of tomato extract against lycopene level. *Vinelina et al.* (2014), also, proved effectiveness of plant extracts on the cotton leaf worm, *Aphis gossypii*.

Table 2: Efficiency of tomato leaves extract against Aphis gossypii.

| Treatment                  | Conc. (ppm) | Corrected mortality% | LC$_{50}$ | LC$_{90}$ | Slope±S.D. | LC$_{90}$/LC$_{50}$ | R       | P     |
|----------------------------|-------------|----------------------|-----------|-----------|-------------|---------------------|---------|-------|
| Tomato leaves extract      | 250         | 27.5                 | 439.89    | 1483.36   | 2.43        | 3.37                | 0.950   | 0.999 |
|                            | 500         | 55                   |           |           |             |                     |         |       |
|                            | 750         | 72.5                 |           |           |             |                     |         |       |
|                            | 1000        | 80                   |           |           |             |                     |         |       |

**Fig. 1:** LC-P line for tomato leaves extract of *Aphis gossypii*.

Chemical Analysis:-

GC/MS analysis detected forty-six compounds in tomato leaves extract. The main defined components are twenty-seven and listed in Table (3) and Fig. (2) and arranged according to their retention times and their percentage composition. These compounds comprise 89.08% of the total composition. Phytol was the most abundant compound (16.03%), followed by hexadecanoic acid, ethyl ester (6.14%), ethyl 9, 12, 15- octadecatrienoate, alpha- linolenate (6.06%), 3,7- dimethyl-2, 6- octadienal (5.03%), Docosane (4.97%), 2- Pentanone, 4- hydroxy- 4- methyl (4.73%), Tetracosane (4.66%), Z-Citral (4.10%), 2- Pentadecanone, 6, 10, 14- trimethyl (3.91%), beta- Myrcene (2.99%), Pentanediol (2.91%), Behenyl alcohol (2.81%), Dihydroactinidiolide (2.57%), Loliolide (2.33%), Bis (2- ethylhexyl) phthalate (2.21%), Hexadecanoic acid (2.20%), Behenyl alcohol (1.91%), Hexadecanoic acid, methyl ester (1.79), 2,7- Anhydro-7- trideoxy-1 (1.73%), Octacosane (1.63%), 3- Buten-2- one (1.35%), Benzene, 1- fluoro-2 (2- methoxyethyl)-, (z) (1.35%), Propanoic acid, 3- amino-3- (4- fluorophenyl)-, ethyl ester (1.25%), 2-(1,3- Benzodioxol-5- ylmethyl)- 1H- isoindole-1, 3 (2H)- Dione (1.23%), 2, 7-Anhydro-4, 5, 7- trideoxy- 7 [(diphenoxyphosphoryl) amino]- 1, 3- di- o-methyl- beta (1.11%), Octadecanoic acid, ethyl ester (1.08%), 3- Chloroorfomianilide (1.00 %). Similar results were obtained by *Juliana et al.* (2016) who recognized phytol as bioactive compound of tomato extract. Also, *Hansson et al.* (2012) proved the presence of ethyl ester in tomato extract. *Tikunov et al.* (2005), also, recognized hexadecanoic acid, ethyl ester in tomato plant extract (*Lycopersicon sculentum*) genotype. Also, the obtained results proved the presence of phenolic and flavonoid in the extract and these results were in agreement with *Norma et al.* (2015).
**Fig. 2:** GC/MS analysis of tomato extract

**Table 3:** Main components of tomato leaves extract identified by GC/MS

| No. | Retention time (min.) | Components                                      | Compound percentage % |
|-----|-----------------------|-------------------------------------------------|-----------------------|
| 1   | 3.46                  | Pentanediol                                     | 2.91                  |
| 2   | 3.83                  | 2- Pentanone, 4- hydroxy- 4- methyl.             | 4.73                  |
| 3   | 7.17                  | beta- Myrcene.                                  | 2.99                  |
| 4   | 14.16                 | Z-Citral.                                       | 4.10                  |
| 5   | 14.96                 | 3,7- dimethyl-2, 6- octadienal.                 | 5.03                  |
| 6   | 21.67                 | Dihydroactinidiolide.                           | 2.57                  |
| 7   | 21.98                 | Chloroformanilide.                              | 1.00                  |
| 8   | 24.12                 | Benzene, 1- fluoro-2 (2- methoxyethyl)-, (z)    | 1.35                  |
| 9   | 25.25                 | 3- Buten-2- one                                 | 1.35                  |
| 10  | 27.03                 | Loliolide                                       | 2.33                  |
| 11  | 27.46                 | Propanoic acid, 3- amino-3- (4- fluorophenyl)-, ethyl ester | 1.25                  |
| 12  | 28.54                 | Pentadecanone, 6, 10, 14- trimethyl             | 3.91                  |
| 13  | 30.14                 | Hexadecanoic acid methyl ester                 | 1.79                  |
| 14  | 30.93                 | Hexadecanoic acid.                              | 2.20                  |
| 15  | 31.47                 | hexadecanoic acid ethyl ester.                 | 6.14                  |
| 16  | 33.71                 | Phytol                                          | 16.03                 |
| 17  | 34.69                 | ethyl 9, 12, 15- octadecatrienoate, alpha- linolenate | 6.06                  |
| 18  | 35.12                 | Octadecanoic acid, ethyl ester                 | 1.08                  |
| 19  | 36.71                 | Behenyl alcohol                                 | 1.91                  |
| 20  | 40.01                 | Behenyl alcohol                                 | 2.81                  |
| 21  | 40.93                 | Bis (2- ethylhexyl) phthalate                   | 2.21                  |
| 22  | 43.13                 | Docosane                                        | 4.97                  |
| 23  | 45.41                 | 2,7- Anhydro-7- trideoxy-1                      | 1.73                  |
| 24  | 45.69                 | 1, 3- di- o-methyl- beta                        | 1.11                  |
| 25  | 48.06                 | Octacosane                                      | 1.63                  |
| 26  | 48.55                 | Tetracosane                                     | 4.66                  |
| 27  | 51.05                 | 2-(1,3- Benzodioxol-5- ylmethyl)- 1H- isoindole-1, 3 (2H)- Dione | 1.23                  |
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