Original article (Orijinal araştırma)

Toxic and in vitro anti-acetylcholinesterase and anti-carboxylesterase effects of various plant extracts on Aphis gossypii Glover, 1877 (Hemiptera: Aphididae)

Bazı bitki ekstratlarının Aphis gossypii Glover, 1877 (Hemiptera: Aphididae) üzerine toksik in vitro anti-asetilkolinesteraz ve anti-karboksil esteraz enzim etkisi

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Abstract

The significance of discovering new active substances that are environment friendly when compared to pesticides, agriculturally sustainable, plant-based, and in the status of GRAS (generally regarded as safe) has been increasing every day. For this purpose, leaves of Daphne odora L., Dieffenbachia amoena L., Eucalyptus camaldulensis L., Ficus carica L., Lantana camara L., Matricaria chamomilla L., Mentha pulegium L. and Nerium oleander L. were collected from Adana in 2018. Toxic and in vitro anti-acetylcholinesterase (AChE) and anti-carboxylesterase (CE) activities of aqueous leaf extracts of these species on the important polyphagous pest, Aphis gossypii Glover, 1877 (Hemiptera: Aphididae), were determined after 24 and 72 h. The fastest and greatest toxic effect was obtained with 20% F. carica extract giving 75.6% mortality. This was followed by N. oleander with 71.6% and D. odora with 62.1% mortality. Aphis gossypii in vitro anti-AChE and anti-CE activities were highest at 10% concentration of the plant extracts and inhibition levels were 51.8-82.5% with F. carica extract, 40.9-54.9% with D. odora extract and 40.2-82.5% with E. camaldulensis extract. In conclusion, D. odora, E. camaldulensis, F. carica and N. oleander extracts gave promising results for future studies on the discovery of potential xenobiotics against A. gossypii and for pest control.

Keywords: Anti-acetylcholinesterase, anti-carboxylesterase, Aphis gossypii, plant extract

Öz


Anahtar sözcüklər: Anti-asetilkolinesteraz, anti-karboksil esteraz, Aphis gossypii, bitki ekstraktı

1 Part of this work was presented as a poster presentation at the Third International Mediterranean Science and Engineering Congress (24-26 October 2018, Adana, Turkey) and published as an abstract.
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Received (Alınış): 02.01.2019 Accepted (Kabul edilİŞ): 02.07.2019 Published Online (Çevrimiçi Yayın Tarihi): 30.07.2019
Introduction

Cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is one of the main pests among aphids that cause economic losses to various agricultural crops. *Aphis gossypii* is a polyphagous species with a wide range of hosts; the pest causes severe damage to cotton and Cucurbitaceae plants in Turkey and many other countries around the world (Ozgur & Sekeroglu, 1986; Tomizawa & Casida, 2005). Today, the chemical control of *A. gossypii* is an important issue in agriculture as this pest can severely decrease yield and quality in cultivated cotton. Although there are various natural enemies of *A. gossypii* in cotton cultivation, chemical control is the most preferred method by producers (Godfrey et al., 1997).

As a result of the increase in insecticde use throughout the world in the last century, different resistance levels of 586 insect pest species against 325 active substances have been reported (Sparks & Nauen, 2015). Widespread and inappropriate use of insecticides affects environment, human health and non-target organisms. Insecticides used against insects and arthropods have a broad-spectrum impact; they cause oxygen deficiency, paralysis as a result of inactivity, and eventually cause death with inhibition or reduction of respiration due to neuro-inhibition in the nervous system (Scharf et al., 2003). Resistance mechanism in organisms included enhanced metabolic enzyme activities and a decrease in the level of sensitivity towards xenobiotics as a result of mutations in target proteins (Nauen, 2007). In the process of xenobiotic detoxification, enzymes have a multigene family that is transcribed in living organisms such as several esterases, oxidases and glutathione S transferase. (Field et al., 1999; Bass & Field, 2011).

Acetylcholineesterase (AChE) and carboxylesterases (CE) are in phase I metabolic enzyme group and they can metabolize various internal and external substrates in pests; this metabolic enzyme group is made of broad-spectrum enzymes that are capable of metabolizing chemical insecticides such as organophosphate, carbamate or pyrethroid (Hollingworth & Dong, 2008). Increase or decrease in the amount of these enzymes leads to the loss of efficiency in insecticides; thus, agents with new and different action mechanisms should be developed in insect control. In the last decade, the demand for biodegradable substances, which are considered as an alternative to synthetic pesticides and could be used in integrated control programs, has significantly increased. Plant-based pesticides are the center of attention since they are eco-friendly and conform with integrated control approaches due to GRAS (generally regarded as safe) status in terms of environmental and human health. Plant-based secondary metabolites (e.g., alkaloids, carotenoids, fats, gums, phenols, resin acids, sterols, suberins, tannins and terpenes) have actives role in ensuring self-protection against microbial pathogens and invertebrate pests (Gottlieb, 1990; Wink & Schimmer, 1999). Before the discovery of modern pesticides, plant-based nicotine and pyrethrin extracts were commonly used as insecticides in agriculture. Pyrethroids, which are derived from the leaves and flowers of chrysanthemum species, are important toxins which may cause death and paralysis of the nervous system. This toxin was specifically developed in order to obtain the most successful commercial pesticide (Raffa & Priester, 1985; Gershenzon & Croteau, 1991). Given that the structure of these compounds is quite complex when compared to artificial pesticides, either development of resistance is delayed or resistance in organisms becomes completely impossible (Völlinger, 1987). In laboratory studies, although exposed to neem oil for 42 generations, resistance development was not observed in a species such as *Plutella xylostella* (L.), which normally develops resistance to all synthetic pesticides within a short period of time. This was due to the complex mechanism of action of the plant metabolite components (Völlinger, 1987; Schmutterer, 1988). There is an increasing need for new active substances that are less harmful or completely harmless, economically feasible, highly efficient, ecologically sustainable and that can be used instead of synthetic chemicals used in pest control.

For this purpose, toxic and anti-AChE and anti-CE effects of aqueous extracts of eight plants on *A. gossypii*, a polyphagous agricultural pest, are investigated and analyzed in order to determine ecological bioactive substances affective under detoxification mechanisms.
Materials and Methods

The test insect, *A. gossypii*, was collected from the cotton cultivation by field surveys in 2017 and cultured under greenhouse conditions at 22°C, 65±5% RH and 16:8 h L:D photoperiod. Previously labeled plant materials used in the experiment were *Daphne odora* L. (Malvales: Daphne), *Dieffenbachia amoena* L. (Alismatales: Thymelaeaceae), *Eucalyptus camaldulensis* L. (Myrtales: Myrtaceae), *Ficus carica* L. (Rosales: Moraceae), *Lantana camara* L. (Lamiales: Verbenaceae), *Matricaria chamomilla* L. (Asterales: Asteraceae), *Mentha pulegium* L. (Lamiales: Lamiaceae) and *Nerium oleander* L. (Gentianales: Apocynoideae); plants were obtained from campus area of Adana Biological Control Research Institute (37°00′38.0″ N, 35°20′23.7″ E). The treatment with the leaf extracts were compared to the insecticide, with malathion active ingredient (25% w/w malathion), treatments in order to determine toxicity and inhibition effect on *A. gossypii*.

Preparation of plant extracts

The leaves of the plants were separated and dried at 40°C for 72 h in an incubator (Nüve incubator). The dried leaves were then removed from the incubator and crushed in a mill (IKA, homogenisator). Following this step, they were separated and weighed as 200 g/L of sterile purified water. They were allowed to infuse on a magnetic mixer in Erlenmeyer flask (250 ml) for 24 h. The prepared aqueous solutions were filtered by coarse filter paper and stored in the refrigerator in light-proof bottles until used for the experiments.

Toxicity of plant extracts on *Aphis gossypii* individuals

Firstly, the bioassay experiment was established for the correct concentration of malathion. Leaf samples taken from cotton plants were cut into 4 cm diameter discs. The leaves were dipped in the insecticide solutions for 10 s, dried and then placed in Petri dishes containing 1.5% agar. Three replicates of about six different rates, excluding a control, were tested. The field collected populations were tested against 1-750 ppm for malathion insecticides. Distilled water was used as the control. About 30 adult aphids were transferred to each Petri dish. After the Petri dishes were covered with Parafilm, they were placed in a controlled environment at 22±1°C, 70% RH and 16:8 h L:D photoperiod. Mortality was assessed after 72 h. Rate-response regressions were computed using Polo-Plus computer program (LeOra Software, Berkeley, CA, USA). And it was calculated LC$_{50}$ (lethal concentration to kill 50%) of the test population.

Fresh cotton leaf samples taken from cotton plants were cut into 4 cm diameter discs. These were dipped in aqueous plant extracts at 10 and 20% concentrations and 175 ppm insecticide (malathion) for 30 s for four replicates. Then, the cotton leaves left to dry on the metal grid and placed with the lower surface facing upwards in plastic Petri dishes (4 cm diameter with ventilation pore) containing 1.5% agar solution. Cotton leaves dipped in pure water were included as a control. Thirty *A. gossypii* apterous individuals were transferred to the extract treated leaves in each Petri dish, and alive and dead individuals were counted after 24 to 72 h after. During counting process, aphids were gently touched with a fine-tipped art brush to determine their vitality. According to the following equation the Henderson-Tilton formula (Henderson & Tilton, 1955) was used to calculate the toxic effect level of each treatment.

$$\text{Corrected } \% = \left(1 - \frac{n(\text{Control before treatment}) \times n(\text{treated after treatment})}{n(\text{Control after treatment}) \times n(\text{treated before treatment})}\right) \times 100$$

Inhibitory effect of plant extracts on carboxylesterase and acetylcholinesterase

Enzyme activity was determined by the method of Nauen et al., (2002). Twenty aphids were homogenized in 100 μl sodium phosphate buffer (0.1 M, pH 7.5), then centrifuged at 10,000 g for 4 min at 4°C and the supernatant used as the enzyme source. Supernatant was diluted 10 times and 25 μl diluted
supernatant was used in an enzyme analysis. The substrate solution was prepared with 0.06 mg/ml fast blue RR salt and 500 μl 100 mM α-napthyl acetate sodium phosphate (0.2 M, pH 6.0). Plant extracts were used for enzyme analyses by diluting them in sodium phosphate (0.2 M, pH 6.0) buffer to 1, 3 and 10%. Two hundred μl substrate solution, 25 μl enzyme and 25 μl plant extract were used for each reaction for three replicates. Pure water was used as a control to replace the enzymes that were individually prepared for each extract. Enzyme activity was read for 10 min with a microplate spectrophotometer at 23°C at 450 nm. Mean levels of CE activity were based on protein content and α- naphthol standard curves.

AChE was determined with the method developed by Stumpf & Nauen (2001). Fifty A. gossypii individuals were homogenized with a plastic crusher in phosphate buffer (0.1 M, pH: 7.5) with 500 μl 0.1% Triton X-100 in an Eppendorf tube. The homogenate was used as supernatant enzyme source after centrifugation at 10,000 g, at 4°C for 5 min. One hundred μl (0.5 mM) acetylcholine iodide, 100 μl 5.5-dithiobis (2-nitrobenzoic acid) and 70 μl enzyme solution and 30 μl plant extract were added to the microplate wells in order to measure AChE activity. Plant extracts were used for enzyme analyses by diluting them in sodium phosphate buffer (0.2 M, pH 6.0) at 1, 3 and 10%. AChE activity was measured at 23°C for 10 min at 412 nm in the kinetic microplate reader for three replicates. Control cells were read without homogenate.

All protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Comparative activity levels were calculated as percentages according to the following equation. The control that did not contain any extract and inhibition effect.

\[
\text{Inhibition activity percentage (\%)} = 100 - \left\{ \frac{\text{Control sample activity} - \text{Inhibition sample activity}}{\text{Control sample activity}} \times 100 \right\}
\]

Statistical analysis

One-way ANOVA and Duncan’s multiple range test have been done by IBM SPSS Statistics 23.

Results and Discussion

When the toxic effects of aqueous extracts treatments on A. gossypii were observed, it was determined that the fastest and greatest toxic effect following the insecticide was with F. carica extract with 33.7% (Day 1) and 75.7% mortality (Day 3) at 20% (Table 1). The lowest mortality rate was observed with M. chamomilla extract at 10 and 20% with 1.3 and 3.9% mortality, respectively. After 24 h, the highest toxic effect following F. carica was with M. pulegium extract giving 29.9% mortality at 20% and D. odora extract giving 22.8% mortality at 20%. After 72 h, the most effective plant extracts following F. carica were N. oleander giving 71.6% mortality at 20% and D. odora giving 62.2% mortality at 20%. The malathion LC50 value was computed as 152 ppm with a confidence interval (50.2-191). Mortality with the insecticide used as a control was 85.1% after 72 h (Table 1). There are several published studies on the toxic and repellent effects of plant extracts on A. gossypii. Dadel & Saleh (2017) reported 79% mortality of A. gossypii 48 h after the treatment with N. oleander chloroform extract. In the same study, 71.6% mortality was reported 72 h after the treatment with N. oleander aqueous extract. Singh et al. (2012) reported that E. globulus plant extract had a repellent effect of 96% against A. gossypii; and in the current study it was found that there was 54% insecticidal effect of E. camaldulensis on aphids after 72 h.

In similar studies, it was reported that different concentrations of Acalypha indica L., Cassia angustifolia M. Vahl., Cascabela thevetia (L.) Lippold, Ocimum basilicum L. and Schinus molle L. aqueous extracts had different repellent effects on A. gossypii (Bayhan et al., 2006; Singh et al., 2012; Pinto et al., 2013; Birgücü et al., 2015).
In studies conducted with other plants, it was observed that plant oils obtained from plants such as *Azadirachta indica* A. Juss., *Achillea millefolium* L. and *Cannabis sativa* L., and aqueous extracts of *Lycopersicum esculentum* and *Nicotiana tabacum* had insecticidal effects on *A. gossypii* (Özger et al., 2013; Yankova et al., 2014; Ghosh, 2015; Dadel & Saleh 2017; Ghada et al., 2017).

Table 1. Mean percentage toxic effects of aqueous plant extracts on *Aphis gossypii*

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Mortality (%±SE)</th>
<th>10%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
<td>24 h</td>
</tr>
<tr>
<td><em>Daphne odora</em></td>
<td>3.9±1.7</td>
<td>b</td>
<td>43.2±3.3</td>
</tr>
<tr>
<td><em>Dieffenbachia amoena</em></td>
<td>1.3±4.3</td>
<td>b</td>
<td>27.1±1.3</td>
</tr>
<tr>
<td><em>Eucalyptus camaldulensis</em></td>
<td>3.9±1.0</td>
<td>b</td>
<td>52.7±1.4</td>
</tr>
<tr>
<td><em>Ficus carica</em></td>
<td>14.3±3.4</td>
<td>b</td>
<td>64.9±3.1</td>
</tr>
<tr>
<td><em>Lantana camara</em></td>
<td>2.6±1.3</td>
<td>b</td>
<td>48.7±2.0</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em></td>
<td>1.3±2.8</td>
<td>b</td>
<td>35.1±5.7</td>
</tr>
<tr>
<td><em>Mentha pulegium</em></td>
<td>16.9±4.1</td>
<td>ab</td>
<td>47.3±2.5</td>
</tr>
<tr>
<td><em>Nerium oleander</em></td>
<td>5.2±1.0</td>
<td>b</td>
<td>48.6±3.6</td>
</tr>
<tr>
<td><em>Malathion</em></td>
<td>48.1±2.7</td>
<td>a</td>
<td>85.1±1.4</td>
</tr>
</tbody>
</table>

Means follow by the same letter are not significantly different according to Duncan’s multiple range test (p < 0.05).

Analysis of the inhibitory effect of AChE and CE activities of the aqueous extracts indicated that the most effective extract was *F. carica* (all concentrations) on both enzymes (Table 2). *Ficus carica* extract had high inhibitory effect on AChE (51.9% inhibition) and CE (82.5% inhibition) at 10%. The lowest inhibitory effect at 10% was with *D. amoena* extract on AChE (20.9% inhibition) and with *L. camara* extract on CE (28.7% inhibition) (Table 2). The most effective plant extracts following with *F. carica* were *D. odora* (41.0% inhibition) and *E. camaldulensis* (40.3% inhibition) on AChE, and *E. camaldulensis* (82.5% inhibition) and *M. pulegium* (79.5% inhibition) on CE.

Table 2. Inhibition effects of aqueous plant extracts on acetylcholinesterase and carboxylesterase from *Aphis gossypii*

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Acetylcholinesterase</th>
<th>Mean inhibition (%±SE)</th>
<th>Carboxylesterase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>3%</td>
<td>10%</td>
</tr>
<tr>
<td><em>Daphne odora</em></td>
<td>33.0±1.1</td>
<td>e</td>
<td>37.6±4.1</td>
</tr>
<tr>
<td><em>Dieffenbachia amoena</em></td>
<td>12.8±2.5</td>
<td>b</td>
<td>18.1±3.1</td>
</tr>
<tr>
<td><em>Eucalyptus camaldulensis</em></td>
<td>29.1±1.8</td>
<td>de</td>
<td>39.2±2.0</td>
</tr>
<tr>
<td><em>Ficus carica</em></td>
<td>38.8±3.4</td>
<td>f</td>
<td>40.6±2.0</td>
</tr>
<tr>
<td><em>Lantana camara</em></td>
<td>7.6±1.3</td>
<td>a</td>
<td>17.6±1.2</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em></td>
<td>22.3±2.5</td>
<td>c</td>
<td>23.6±0.1</td>
</tr>
<tr>
<td><em>Mentha pulegium</em></td>
<td>30.6±2.7</td>
<td>e</td>
<td>36.7±1.3</td>
</tr>
<tr>
<td><em>Nerium oleander</em></td>
<td>25.3±2.7</td>
<td>cd</td>
<td>28.7±2.3</td>
</tr>
<tr>
<td>M/min/mg protein</td>
<td>13.28±0.77</td>
<td></td>
<td>9.34±0.51</td>
</tr>
</tbody>
</table>

Means follow by the same letter are not significantly different according to Duncan’s multiple range test (p < 0.05).

There are only a few in vitro studies on *A. gossypii* anti-AChE and anti-CE; however, several studies about inhibition of different enzymes have been reported. In other studies, it was reported that *Artemisia annua* L. extract and 4% azadirachtin of *Periplaneta americana* L. (40%) had an inhibitory effect on AChE (Zibae et al., 2010), and 80% neem oil had an inhibitory effect on AChE (Singh & Singh, 2005; Shafeek et al., 2004). It was reported that 25 g/L distilled water extracts of *Artemisia absinthium* L., *Punica granatum* L. and *Thymus vulgaris* L. significantly inhibited AChE activity (Korayem et al., 1993). Senthil et al. (2008)
observed that LC$_{50}$ azadirachtin concentration significantly inhibited AChE activity when compared to the control. In this study, analysis of both toxic and enzyme inhibitory effects of tested plant extracts showed that there are anti-AChE and anti-CE activities of *F. carica*, *E. camaldulensis* and *M. pulegium* plants in parallel with their toxic effects.

AChE and CE inhibition is an important target for insecticides and for several plant metabolites in insects (Houghton et al., 2006). Esterase enzymes are especially important and responsible for detoxification which hydrolyzes ester bonds in synthetic chemicals (Hemingway & Karunaratne, 1998). AChE inhibitors are used effectively in the fields of pharmacology and pesticides for controlling insects, other arthropods and some vertebrates. Different degrees of toxic or enzyme inhibition effects of plant extracts can result from substances such as flavonoids, terpenes phenols, alkaloids, sterols, waxes, fats, tannins, sugars, gums, suberins, resin acids and carotenoids, and concentration levels of the components in an organism (Wink & Schimmer, 1999). Thus, this study was undertaken to determine the potential plant extract activities and to provide data for future studies. It was reported that there may be increases in insecticidal and enzyme inhibition periods in parallel with the increase in plant extract concentrations (Junqing et al., 2011; Hansson et al., 2012). In the literature, it is mentioned that plant metabolite alkaloids and terpenes can have significant insecticide effects; however, they are not economic or safe in addition to the fact that they are difficult to isolate (Rattan, 2010). Thus, in this study it was determined that an efficiency of up to 80% with aqueous plant extracts can be obtained more easily, so is promising in terms of potential use as insecticides. Considering the negative effect of synthetic pesticides against non-target organisms, it can be concluded that plant metabolites are better products with GRAS status (Scott et al., 2003). Furthermore, plant metabolites could affect more than one target area in insect metabolism with little or no resistance development. In conclusion, it was determined that aqueous *D. odora* *E. camaldulensis*, *F. carica* and *M. pulegium* leaf extracts have significant bioinsecticide effect and in vitro anti-AChE and anti-CE activities on *A. gossypii*. It was also determined that these plant extracts can be used as bioinsecticides for *A. gossypii* control. Especially in organic agriculture and integrated farming practices, alternative methods have increasingly gained significance for pest control. Further detailed studies about the extension of encapsulation, shelf life and expiration date of these metabolites could enable the use of these pesticides widely and more practically.

References


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