

## Assessment of Toxicological Effects of some New Plant Extracts on Cotton Aphid, *Aphis Gossypii*

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### Abstract

In contemporary ecological technologies, botanical pesticides are an alternative to synthetic chemical pesticides for pest management. These products pose no risk to the environment or to people's health. Plant extracts are preferred in contemporary organic agriculture due to a number of benefits. These items' variety is always growing, therefore it's important to understand how they work. This research was done to determine the toxicological effect of (Costunolide, Ottelione and Menthol) as plant extracts against *Aphis gossypii*. The result obtained that, the total mortality was 96.66%, 96.67% and 70% at the concentration of 5000, 1000 and 5000 from menthol, costunolide and ottelione, respectively. Generally with increasing the concentrations of plant extracts studied led to more mortality. Costunolide is the most effective compound that has sublethal concentration LC<sub>50</sub>, 81.9ppm followed by menthol with LC<sub>50</sub> 455.6 ppm then ottelione with LC<sub>50</sub> 1615.8 ppm. The most effective plant extract was Costunolide (Ti=100%), while the toxicity index being 17.97 and 4.15 % for Menthol and Ottelione, respectively. Now we have another option for controlling this pest by the strong efficiency demonstrated by this plant extracts.

**Keywords:** organic agriculture, *Aphis gossypii*, menthol, costunolide, ottelione.

### Introduction

Cotton aphid (*A. gossypii*) is a dangerous pest that threatens all crops. It feeds on leaves and shoots, extracting plant juices, causing chlorosis, foliage death, and hindering photosynthesis. Aphids excrete honeydew, promoting sooty mold growth, reducing crop quantity and quality. They are polyphagous

insect pests and vectors for various virus diseases like crinkle, mosaic, rosette, CTV, among others (Arif *et al.*, 2009; Sharma and Joshi (2010).

The usage of chemical pesticides frequently has unfavorable effects. In contemporary ecological technologies, botanical pesticides are an alternative for controlling pests. They don't pose a hazard to the environment or to people's health. The range

of these items is always expanding, necessitating an understanding of how they work (Isman, 2000 and Isman, 2006). Numerous natural chemicals found in plant extracts are beneficial for controlling numerous pests globally (Mateeva, 2000; Isman, 2006; Ramya and Jayakumararaj, 2009; Islam *et al.*, 2018 a and b).

Freshwater plant *Ottelia alismoides* partially floats, its leaves as it grows in the water. Every summer, Egypt's rice fields and irrigation canals bloom and prosper in the shallow waters surrounding ponds and rice fields (Guo and Zhang, 2020). From d-ribose, *ottelone* A and *ottelone* B have been synthesized in racemic and natural optical pure forms (Tsai *et al.*, 2021). Studies of the principal chemical elements revealed the discovery of two 4-methylene-2-cyclohexanones with diastereometric properties (Han *et al.*, 2020; Wagutu *et al.*, 2021).

*Costus speciosus* is a highly valued medicinal plant that is widely used for treating various ailments. This plant has numerous active ingredients and has been found to possess a wide range of pharmacological activities, such as antioxidant, anticancer, anti-inflammatory, antidiabetic, hypolipidemic, hepatoprotective, steroidogenic, adaptogenic, and antimicrobial effects. Active principles derived from plants are a valuable source of bioactive molecules that have the potential to be used in the development of novel drugs. Compared to synthetic alternatives, these products are considered relatively safe (El-Far *et al.*, 2018).

One promising class of compounds that can serve as natural pesticides is essential oils (EOs), which are secondary metabolites produced by plants and contain high levels of terpenoids, and aromatic compounds. Menthol mints, in particular, are rich in biologically active compounds, and their interactions with other components of agrobiocenoses, such as weeds or insect pests, have been observed for a long time. Recently, the use of menthol mints EOs as natural or botanical pesticides has been investigated. Peppermint essential oil is already exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as a pesticide formulation, either alone or in combination with other ingredients (USEPA, 2019).

The increasing interest in integrated pest management (IPM) is due to the issues associated with chemical pesticides, such as inducing pest resistance, residual toxicity in the

environment, and adverse effects on non-target organisms. Therefore, there is a need to identify new materials with specific modes of action to replace conventional insecticides. The current study aimed to evaluate the toxicological effects of plant extracts, specifically Costunolide, Ottelione, and Menthol, against 2<sup>nd</sup> instar cotton aphids (*A. gossypii*).

## Materials and Methods

**Experiment:** Botanical extracts were excessively evaluated against *A. gossypii* with different concentrations. A colony which obtained from cucumber field and multiplied in the laboratory on leaves of cucumber.

**Plant extract:** Three plants extract Duck lettuce, Crepe ginger, and menthol were used in this study as following:

**Duck lettuce as Ottelione:** *Ottelia alismoides*, (Family: Hydrocharitaceae), 350 g of entire *O. alismoides* plants were harvested from River Nile, Delta, Egypt canals. Plants were air dried at ambient temperature and kept until extraction at -20 °C. A preliminary thin-layer chromatography (TLC) procedure was used to produce pure OTTE from an initial *O. alismoides* extract that had been produced through extraction with petroleum ether (Ayyad *et al.*, 1998). By using <sup>1</sup>H-NMR and <sup>13</sup>C-NMR, the structure of pure ottelione was confirmed.

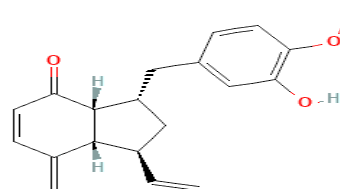


Figure 1: Chemical structure of ottelione (C<sub>20</sub>H<sub>22</sub>O<sub>3</sub>) (Ayyad *et al.*, 1998).

**Crepe ginger as Costunolide:** *Costus speciosus* (Family: Costaceae): The used concentrations of plant extract were 100, 500, 750 and 1000 ppm during the experiment.

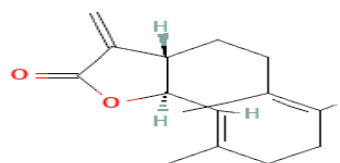


Figure 2: Chemical structure of costunolide (C<sub>15</sub>H<sub>20</sub>O<sub>2</sub>) (Park *et al.*, 2022)

**Menthol (99%):** *Mentha aquatica* L. (Family:

Lamiaceae): Menthol is a crystalline material was brought from El-Gomhoria company-Mansoura. The used concentrations of plant extract were 500, 1000, 3000 and 5000 ppm during the experiment.

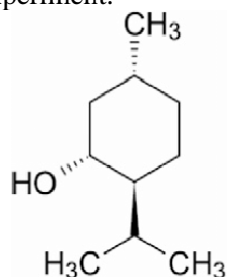


Figure 3: Chemical structure of menthol ( $C_{10}H_{20}O$ ) (PubChem, 2021).

To prepare the stock solution of the tested plant extracts, we used a convenient concentration based on the weight of the plant and the volume of distilled water (w/v), along with tween 80 (0.1%) as an emulsifier. The stock concentrations were prepared regularly, stored in glass stoppered bottles, and kept refrigerated. We used four diluted concentrations for each plant extract, with three replicates for each, to generate the LC-P lines.

For the bioassay tests in this study, we selected the 2<sup>nd</sup> larval instar of *A. gossypii*. In a preliminary experiment, we determined the sublethal concentration levels of the tested compounds against the 2<sup>nd</sup> instar larvae.

Circular discs were prepared from fresh and clean leaves of castor bean. Each of these discs was treated with one of the four concentrations by dipping them for 15 seconds, followed by air drying for 5 minutes, and then weighed. Newly moulted 2<sup>nd</sup> instar larvae were placed individually inside glass jars containing wet filter paper to prevent the leaf discs from drying out. The larvae were starved for 3 hours and then forced to feed on the treated leaf discs for 24 hours using a no-choice method. After 24 hours, the larvae were replaced with fresh untreated leaves for the remainder of the larval stage. The control group of 2<sup>nd</sup> instar larvae was provided with untreated leaves throughout their larval stage. Three replicates were conducted for each concentration in both the treatment and control groups, with each replicate consisting of 10 larvae. The replicates were kept separately in glass jars for observing and determining nutritional parameters as described, and the average mortality percentage was corrected using **Abbot's formula (1925)**. The corrected

mortality percentage of each compound was then statistically computed according to **Finney (1971)**. The toxicity index of the tested compounds was determined using the method described by **Sun (1950)**.

Toxicity Index

$$= \frac{LC_{50} \text{ of the most toxic compound}}{LC_{50} \text{ of the tested compound}} \times 100$$

The statistical analysis involved the estimation of concentration probit lines (LC-P lines), as well as the determination of the  $LC_{50}$  and  $LC_{90}$  slope values for the tested compounds.

## Results

*Efficiency of some plant extracts on the cotton aphid (A. gossypii):*

1. *Efficiency of menthol on adult stage mortality of cotton aphid (A. gossypii):*

Data in **Table (1)** and **Figure (1)** indicate the effect of menthol against adult stage mortality of cotton aphid (*A. gossypii*). After one day of treatment with concentrations 500, 1000, 3000 and 5000 ppm, mortalities percentages were 3.33, 10, 13.33 and 20% respectively. After three day of treatment, were 20, 20, 33.33 and 33.33%. After five days of treatment with the same concentrations, were 23.33, 30, 26.67 and 33.33% and after seven days of treatment, were 3.33, 16.67, 16.67, 10%.

**Table (1)** and **Figure (1)** demonstrate that, when adult stage of cotton aphid (*A. gossypii*) treated with menthol, the total mortality was 50, 76.67, 90 and 96.66% at concentrations 500, 1000, 3000 and 5000 ppm respectively.

2. *Efficiency of costunolide on adult stage mortality of cotton aphid (A. gossypii):*

Data in **Table (1)** and **Figure (1)** indicate the effect of costunolide against adult stage mortality of cotton aphid (*A. gossypii*). After one day of treatment with concentrations 100, 500, 750 and 1000 ppm, mortality was 6.67, 10, 16.67 and 20% respectively. After three day of treatment, mortality was 13.33, 20, 30 and 46.67%. After five days of treatment with the same concentrations, mortality was

33.33, 30, 33.33 and 26.67%. After seven days of treatment after seven days, mortality was 3.33, 20, 10, 3.33%.

**Table (1)** demonstrate that, when adult stage mortality of cotton aphid (*A. gossypii*) treated with costunolide, the total mortality was 56.67, 80, 90 and 96.67% at concentrations 100, 500, 750 and 1000 ppm respectively.

3. Efficiency of ottelione on adult stage mortality of cotton aphid (*A. gossypii*):

Data in **Table (1)** and **Fig. (1)** indicate the effect of ottelione against adult stage mortality of cotton aphid (*A. gossypii*). After

one day of treatment with concentrations at 500, 1000, 3000 and 5000 ppm, mortality was 0, 3.33, 10 and 13.33% respectively. After three day of treatment, mortality was 6.67, 16.67, 20 and 26.67%. After five days of treatment with the same concentrations, mortality was 10, 13.33, 20 and 20%. After seven days of treatment after seven days, mortality was 3.33, 6.67, 6.67 and 10%.

**Table (1)** and **Figure (1)** demonstrate that, when adult stage mortality of cotton aphid (*A. gossypii*) treated with ottelione, the total mortality was 20, 40, 56.67 and 70% at concentrations 500, 1000, 3000 and 5000 ppm respectively.

Table (1): Susceptibility of adult stages of cotton aphid (*A. gossypii*) to tested plant extracts.

| Treatments  | Conc. (ppm) | Mortality after treatments % |            |           |            | Total Mortality % |
|-------------|-------------|------------------------------|------------|-----------|------------|-------------------|
|             |             | One day                      | Three days | Five days | Seven days |                   |
| Menthol     | 500         | 3.33                         | 20         | 23.33     | 3.33       | 50                |
|             | 1000        | 10                           | 20         | 30        | 16.67      | 76.67             |
|             | 3000        | 13.33                        | 33.33      | 26.67     | 16.67      | 90                |
|             | 5000        | 20                           | 33.33      | 33.33     | 10         | 96.66             |
| Costunolide | 100         | 6.67                         | 13.33      | 33.33     | 3.33       | 56.67             |
|             | 500         | 10                           | 20         | 30        | 20         | 80                |
|             | 750         | 16.76                        | 30         | 33.33     | 10         | 90                |
|             | 1000        | 20                           | 46.67      | 26.67     | 3.33       | 96.67             |
| Ottelione   | 500         | ----                         | 6.67       | 10        | 3.33       | 20                |
|             | 1000        | 3.33                         | 16.67      | 13.33     | 6.67       | 40                |
|             | 3000        | 10                           | 20         | 20        | 6.67       | 56.67             |
|             | 5000        | 13.33                        | 26.67      | 20        | 10         | 70                |

**Table (2)** and **Figure 2** illustrate the most efficient compound against the adult stage of cotton aphid (*A. gossypii*). Costunolide is the most effective compound that has sublethal concentration LC<sub>50</sub>, 81.9 ppm followed by menthol 455.6 ppm then ottelione 1615.8 ppm. Also, LC<sub>90</sub> of costunolide was 727.7 ppm followed by menthol 1125.9 ppm then ottelione 9220.9 ppm.

In the same **table**, data showed that menthol displayed higher slop (1.70) over other plant extract which recorded 1.70 and 1.26 for costunolide and ottelione, respectively.

The most effective plant extract was costunolide (Ti=100%), while the toxicity index being 17.97 and 4.15 % for menthol and ottelione, respectively.

Table (2): Efficacy of some plant extracts against the adult stage of cotton aphid (*A. gossypii*):

| Treatment   | Conc. (ppm) | Corrected mortality% | LC <sub>50</sub> | LC <sub>90</sub> | Slope± S.E. | Toxicity index LC <sub>50</sub> | LC <sub>90</sub> /LC <sub>50</sub> | R     | P     |
|-------------|-------------|----------------------|------------------|------------------|-------------|---------------------------------|------------------------------------|-------|-------|
| Menthol     | 500         | 50                   | 455.6            | 2542.19          | 1.7± 0.22   | 17.97                           | 2.47                               | 0.988 | 0.382 |
|             | 1000        | 76.67                |                  |                  |             |                                 |                                    |       |       |
|             | 3000        | 90                   |                  |                  |             |                                 |                                    |       |       |
|             | 5000        | 96.66                |                  |                  |             |                                 |                                    |       |       |
| Costunolide | 100         | 56.67                | 81.86            | 727.7            | 1.4± 0.2    | 100                             | 8.89                               | 0.942 | 0.087 |
|             | 500         | 80                   |                  |                  |             |                                 |                                    |       |       |
|             | 750         | 90                   |                  |                  |             |                                 |                                    |       |       |
|             | 1000        | 96.67                |                  |                  |             |                                 |                                    |       |       |
| Ottelione   | 500         | 20                   | 1974.04          | 20372.38         | 1.26± 0.17  | 4.15                            | 10.32                              | 0.987 | 0.465 |
|             | 1000        | 40                   |                  |                  |             |                                 |                                    |       |       |
|             | 3000        | 56.67                |                  |                  |             |                                 |                                    |       |       |
|             | 5000        | 70                   |                  |                  |             |                                 |                                    |       |       |

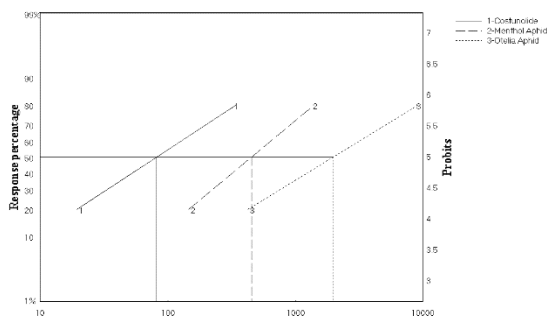


Figure 4: Probit regression lines for menthol, costunolide and ottelione against the adult stages of cotton aphid (*A. gossypii*)

## Dissection

Chemical pesticides are widely employed to control a variety of bioaggressors, including bacteria, fungus, viruses, and insects. But, their toxicological side effects and the pests' emergence of resistance have compelled a constant quest for better alternatives (Mansour *et al.*, 2012). Today, plant extracts are thought to be a source of bioactive chemicals that may be effective against a variety of insect pests (Pavela, 2014).

In recent years, there has been a lot of research on the utilization of natural resources from plant species to manage plant viral infections and associated insect vectors. Several plant extracts in particular have drawn attention because of the crucial role they play in protecting plants in nature as antibacterials, antivirals, antifungals, insecticides, and insect repellents. For current agricultural systems around the world, it is imperative that a remedy based on a natural substance has properties that are both safe for human health and the environment, effective in controlling plant viruses, and repels their insect vectors (Taglienti *et al.*, 2023). Numerous plant extracts or products have been identified as effective against insect pests, exhibiting properties such as insecticidal, repellent, antifeedant, attractant, ovicidal, oviposition deterrent, growth inhibition, and reproductive inhibition (Ulrichs *et al.*, 2008; Dubey *et al.*, 2010; Rai and Carpinella, 2006; Ben Hamouda *et al.*, 2015a,b; Ekesi, 2000). To prevent environmental contamination, insect resistance and resurgence, as well as harmful impacts on non-target organisms, plant extracts and natural remedies have garnered a lot of attention in pest management programs. A

recent focus has been on discovering new antifeedants sourced from plant extracts as a feasible approach for creating "eco-friendly insecticides" (Wheeler *et al.*, 2001). In summary, assessing the antifeedant properties of botanicals is essential for effective insect pest management (Pavunraj *et al.*, 2012).

In our study, we aimed to develop a sustainable management strategy for controlling *A. gossypii* larvae using three plant extracts (menthol, costunolide, and ottelione) at varying concentrations. Mortality percentages of the tested extracts were recorded after 1, 3, 5, and 7 days of treatment, given that the mortality potency of plant extracts is different from conventional pesticides, and no initial change was observed. Our findings showed that costunolide and menthol were the most effective extracts against *A. gossypii* mortality. *A. gossypii*, commonly known as the cotton aphid, is a major pest of cotton crops and can cause significant damage.

Menthol is a natural organic compound that is found in several plants, including peppermint and spearmint. Menthol has been studied for its effects on *A. gossypii*, and it has been found to have a number of important effects on this pest. One of the most important effects of menthol on *A. gossypii* is its insecticidal activity. Menthol has been shown to be toxic to *A. gossypii* at certain concentrations, causing mortality and reducing the fecundity of the surviving aphids. This insecticidal activity is thought to be due to the disruption of the aphid's nervous system, which can lead to paralysis and death. The mechanism of action of menthol against the mites was thought to be due to its ability to disrupt the mite's cell membranes and interfere with its metabolic processes (Souza *et al.*, 2014). In addition to its insecticidal activity, menthol has also been found to have repellent effects on *A. gossypii* (Srivastava *et al.*, 2002). When exposed to menthol, aphids have been observed to avoid areas treated with the compound and to move away from the source of the menthol. This effect can be useful in controlling the spread of aphids in agricultural settings. Overall, the effects of menthol on *A. gossypii* can be important in controlling this pest in agricultural settings. However, further research is needed to determine the most effective concentrations and application methods of menthol for controlling *A. gossypii* infestation. Zamani Verdi *et al.*, (2018) indicated that essential oil of *M.*

*longifolia* is highly toxic to the melon aphid.

Costunolide is a natural compound found in Crep ginger, a plant commonly used in traditional medicine (Kishore *et al.*, 2014). Some studies have investigated the potential use of costunolide as an insecticide against agricultural pests, including *A. gossypii*. The mechanism of action of costunolide against *A. gossypii* is not fully understood, but some studies suggest that it works by disrupting the aphid's nervous system and reducing its ability to feed and reproduce. Costunolide has also been shown to have repellent effects on *A. gossypii*, causing the aphids to avoid areas treated with the compound. Costunolide may have strong insecticidal activity against *A. gossypii*, causing mortality and reducing the fecundity of the surviving aphids. The costunolide may have a long residual effect, remaining effective for up to 14 days after application. Costunolide had a synergistic effect when used in combination with other natural compounds, such as eucalyptol and limonene. The combination of these compounds was found to have even stronger insecticidal activity against *A. gossypii* than costunolide alone. Overall, the mechanism of action of costunolide against *A. gossypii* is thought to involve disruption of the aphid's nervous system and repellent effects, leading to reduced feeding and reproduction. Costunolide has been found to have strong insecticidal activity against *A. gossypii* and may be a promising natural insecticide for use in agriculture.

*Ottelia alismoides* is a species of aquatic plant that belongs to the family Hydrocharitaceae. It is commonly known as duck lettuce, water lettuce or water cabbage (Jiang and Kadono, 2001 and Wang *et al.*, 2022). *Ottelia alismoides* potential as a natural pesticide is still under study. Ottelione is not typically used as a pesticide for crops, though some studies have suggested that it may have antimicrobial and antioxidant properties. Little data on the internet has shown that *Ottelia alismoides* have insecticidal and acaricidal properties, as the ethanol extract of *Ottelia alismoides* was found to be effective against the cotton bollworm, a common pest that attacks crops such as cotton, corn, and soybean (Tan *et al.*, 2001). The study found that the extract had a mortality rate of 96.3% on the cotton bollworm larvae after 48 hours of exposure. Another study by (Zhang *et al.*, 2019) evaluated the insecticidal activity of *Ottelia*

*alismoides* against the red flour beetle, a pest that infests stored grains. The study found that the ethanol extract of *Ottelia alismoides* had significant mortality rates on the red flour beetle, with a mortality rate of 100% observed after 48 hours of exposure. Overall, the findings suggest that *Ottelia alismoides* extract may have potential as a natural pesticide for controlling pests in crops. However, further research is needed to determine the optimal formulation and application methods to ensure its effectiveness in real-world agricultural settings. Generally, it can be said some recommendations based on these authors as follows; Instead of using chemical pesticides to manage insect pests, consider using natural plant extracts that have been shown to have various properties such as insecticidal, repellent, antifeedant, attractant, ovicidal, oviposition deterrent, growth inhibition, and reproductive inhibition. These extracts can effectively control insect pests without harming the environment or non-target organisms. Menthol and costunolide have been found to be effective against *A. gossypii* mortality. To control the cotton aphid in agricultural settings, consider using these natural plant extracts. Further research is needed to determine the most effective concentrations and application methods of menthol and costunolide for controlling *A. gossypii* infestation. To enhance the insecticidal activity of costunolide against *A. gossypii*, consider using a combination of natural compounds such as eucalyptol and limonene. To effectively manage insect pests while reducing the use of chemical pesticides, it is important to assess the antifeedant properties of botanicals. This can help prevent environmental contamination, insect resistance and resurgence, as well as harmful impacts on non-target organisms. Explore other natural plant extracts and compounds that have potential insecticidal or repellent properties against other insect pests in agricultural settings. Consider implementing an integrated pest management (IPM) program that includes the use of natural plant extracts, cultural practices, and biological control. This can help effectively manage insect pests while reducing the use of chemical pesticides. Our results are in harmony with those of Abdel-Hamid *et al.*, (2016); Wahba *et al.*, (2017); El-Genaidy and Dar (2019); Shehawy *et al.*, (2019).

## Conclusion

The results of the study suggest that the tested plant extracts have the potential as an alternative to synthetic insecticides for sustainable pest control in the long term. However, further investigation is required to determine their efficacy on other insect species. The study found that costunolide and menthol had the highest mortality rates among the extracts tested. The results imply that the studied plant extracts are less harmful to the environment and natural enemies compared to synthetic pesticides and may have a significant role in managing pests and reducing the need for highly toxic pesticides in the future.

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## الملخص العربي

### عنوان البحث: تقييم التأثيرات السامة لبعض المستخلصات النباتية الجديدة على حشرة من القطن

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في التقنيات البيئية المعاصرة، المبيدات النباتية هي بديل للمبيدات الكيميائية الاصطناعية في مكافحة الآفات. لا تشكل هذه المنتجات خطر على البيئة أو صحة الناس. تُفضل مستخلصات النبات في الزراعة العضوية المعاصرة بسبب عدد من الفوائد. تزداد تلك المجموعة من المنتجات باستمرار، لذلك من المهم فهم كيفية عملها. تم إجراء هذا البحث لتحديد التأثير التسممي لـ *Costunolide* و *Ottelione* و *Menthol* كمستخلصات نباتية ضد المن *Aphis gossypii*. وأظهرت النتائج أن الموت الإجمالي كان ٩٦,٦٦٪ و ٧٠٪ عند تركيز ٥٠٠٠ و ١٠٠٠ و ٥٠٠٠ من *Menthol* و *Costunolide* و *Ottelione* على التوالي. وعمومًا، فإن زيادة تركيز المستخلصات النباتية التي تم دراستها يؤدي إلى مزيد من الوفيات. وكان *Costunolide* المركب الأكثر فعالية الذي لديه تركيز قاتل جزئي (LC50) 81.9ppm، تليه *Menthol* LC50 455.6ppm ثم *Ottelione* LC50 1615.8ppm. كان أكثر مستخلص نباتي فعالية هو *Costunolide* (Ti = 100٪)، في حين بلغ مؤشر السمية ١٧,٩٧ و ٤,١٥٪ على التوالي لـ *Menthol* و *Ottelione*. الآن لدينا خيار آخر للقضاء على هذه الآفة بفعالية قوية مثبتة من خلال استخدام المستخلصات النباتية.