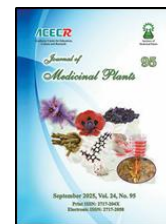




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### Research Article

## Efficacy of *Salvia hydrangea* DC. ex Benth essential oil and extract against *Phthorimaea operculella* (Lepidoptera: Gelechiidae) across multiple life stages

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### ABSTRACT

**Background:** *Phthorimaea operculella* (potato tuber moth) is among the most destructive pests of Solanaceous crops. Excessive use of chemical pesticides poses environmental and health concerns. Plant-derived materials offer environmentally friendly alternatives with lower ecological risk. **Objective:** In this research, the insecticidal toxicity of *Salvia hydrangea* DC. ex Benth essential oil and extract was evaluated on multiple life stages of *P. operculella*. Additionally, larval penetration and adults' oviposition preferences were assessed. **Methods:** Bioassays were conducted at  $25 \pm 2$  °C,  $65 \pm 5$  % relative humidity (RH), and a photoperiod of 8:16 (L:D) in a completely randomized design with five replications per treatment. Probit analysis was used to estimate lethal concentrations (LC<sub>50</sub>). **Results:** Adults were the most sensitive to *S. hydrangea* essential oil (LC<sub>50</sub> = 0.33 µl/L air). The plant extract was more toxic to adults (LC<sub>50</sub> = 219.72 mg/L) than to eggs (LC<sub>50</sub> = 490.24 mg/L). Both the essential oil and the extract reduced larval penetration rates and significantly affected oviposition preferences of adult *P. operculella*. In addition, among homeopathic preparations tested, Silicea 30 C showed the greatest effect on potato growth parameters. **Conclusion:** *Salvia hydrangea* essential oil and extract exhibit substantial activity against *P. operculella* and can contribute to protecting stored potatoes from infestation. When used in combination with Silicea 30 C, these plant-derived products may be integrated into broader pest management programs. Further studies should evaluate field efficacy, non-target effects, and compatibility with compatible storage practices.

### 1. Introduction

Potato, *Solanum tuberosum* L., is ranked as the fourth most significant food crop after rice, wheat, and maize worldwide [1]. Asia and Europe are the largest potato-producing regions

globally, accounting for 80 % of world production [2, 3].

*Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) is a worldwide major pest of potato damage in fields and during potato storage [4]. Larvae of *P. operculella*

**Abbreviations:** L, light; D, darkness; mg/L, milligram per liter; N and E, north and east; µl/L, microliter per liter; RH, relative humidity; h, hours; L, liter; GC-MS, Gas chromatography–mass spectrometry; eV, electron volts; ANOVA, Analysis of variance; g, gram; nm, nanometer; mm, millimeter; µm, micrometer

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damage potatoes by mining into the tubers and discharging dark brown feces outside the borehole. This results in severe qualitative and quantitative losses, potentially reaching as high as 80 % [5-7].

This pest is considered very difficult to control. Over the years, the primary approach to managing *P. operculella* has been the application of broad-spectrum chemical insecticides [8, 9]. Chemical insecticides not only kill insect pests but also contribute to environmental, agricultural, and aquatic pollution, as well as contaminate food [10-12]. Additionally, the development of insecticide resistance by pest insects is another disadvantage of insecticide usage [12]. So, seeking and using other alternative safe methods, such as medicinal plants, for pest control is crucial.

The genus *Salvia* (sage), which includes approximately 900 species worldwide, is one of the most economically valuable genera of the Lamiaceae family. In Iran, about 60 species of this genus are described [13]. *Salvia hydrangea* DC. ex Benth, known by its common Persian name "Gol-e Arooneh," is a most popular medicinal plant that grows extensively throughout Iran [14]. The plant extract and essential oil of this species demonstrated potent antimicrobial, insecticidal, antifungal, and anti-inflammatory properties [15]. Plant essential oils and extracts can prevent the insect's growth via biochemical mechanism, and they can cause lethal effects on insects, as well as deter feeding and oviposition. Some studies described reductions and disruptions in the levels of biochemical compositions in treated larvae with these compounds [16].

Agrohomeopathy is a novel method in the agricultural field that attempts to change the physiological activities of plants, including

increasing their levels of protein, sugar, chlorophyll, and enzyme activity [17, 18].

This study aimed to evaluate, for the first time, the insecticidal activity of *S. hydrangea* essential oil and extract against *P. operculella*. We also examined the efficacy of these compounds on oviposition-preference activity and larval penetration of *P. operculella*. Additionally, we assessed several homeopathic remedies concerning potato plant growth characteristics. The results obtained can open new horizons for researchers to investigate innovative approaches for developing integrated *P. operculella* management.

## 2. Materials and methods

### 2.1. Insect

The initial population of *Phthorimaea operculella* was collected from an infested potato field located in Ardabil Province, Ardabil, Iran (38.1242° N, 48.3954° E). The stock culture was reared in plastic containers (10 × 20 × 30 cm) containing tubers of *Agria* potato cultivar, maintained at 25 ± 2 °C, 65 ± 5 % RH, and an 8:16 h (Light: Darkness) photoperiod. A thin layer of clean sand was added to the bottoms of the containers to serve as a pupation substrate. Females laid their eggs on filter papers that were set on top of the cylindrical containers [9].

### 2.2. Essential oil and its chemical characterization

The dried and powdered aerial parts (100 g) of *S. hydrangea* with marketing code of 36 (IMPHM) and deionized water (1 L) were subjected to essential oil extraction through hydrodistillation using a modified Clevenger-type apparatus. The extracted essential oil was kept at 4 °C [3, 12].

Gas chromatography (GC Agilent, 7890 USA) with a mass selective detector (Agilent 5975 A, USA) and a capillary column (BP-5 MS, 30 m length, inner diameter of 0.25 mm, thin-film of 0.25  $\mu$ m) were used to perform the GC-MS analysis of the essential oil. The GC temperature program began at 80 °C (held for 3 min), then raised up to 180 °C at the rate of 8 °C/min. The flow rate of carrier gas (helium) was 1 ml/min. All mass spectra were recorded in the electron ionization mode (70 eV) and scanned the 40-500 m/z range. The components of *S. hyrangeta* essential oil were determined by comparing their mass spectra with reference data in the instrument libraries [12].

### 2.3. Methanolic extract and its total phenolic content

A Soxhlet extractor was used to isolate the components of *S. hydrangea* aerial parts. About 20 g of plant powder was put into the Soxhlet apparatus. About 300 ml of methanol was used to charge the device [19]. Bioassays were done with 5% methanolic extract, and concentrations of 1 %, 3 %, and 5 % were used for evaluating the larval penetration and oviposition preference assays. For bioassays, a 5 % methanolic extract solution was prepared, with additional concentrations of 1 %, 3 %, and 5 % used for larval penetration and oviposition preference assays.

For measuring the total phenolic content of methanolic extract, 1 mL of diluted extract was combined with 5 mL of distilled water. Then, Folin-Ciocalteu's reagent (0.5 mL) was added to the mixture as well. After 3 min, 1 mL of 20 % (w/v) anhydrous sodium carbonate solution was added and vortexed vigorously. After 30 min of dark incubation, the absorbance at 725 nm was measured. The regression equation of standard

gallic acid was used to determine the content of phenol compounds [20].

### 2.4. Bioassays with essential oil

#### 2.4.1. Adults

This bioassay was followed by Rafiee et al. [21] protocol. A 250 mL glass vial with a sealing lid was used as the fumigation chamber. Filter papers (Whatman No. 1, 2 cm diameter) were attached to the undersurface of the lids and loaded with concentrations of essential oil (0.10, 0.18, 0.33, 0.60 and 1.10  $\mu$ L/L air). The lids were tightly sealed with parafilm on the vials containing 20 *P. operculella* adults (< one-day-old). Five replicates were set up for each treatment and control (distilled water). Mortality was assessed 24 hours after initial exposure.

#### 2.4.2. Eggs

Twenty newly laid eggs were placed in 250 mL glass vials. The filter papers (Whatman No. 1, 2 cm diameter) were infused with appropriate concentration (12.50, 17.00, 22.40, 29.50 and 38.60  $\mu$ L/L air) of *S. hydrangea* essential oil, then affixed to the vial caps' inside surface. Parafilm was used to seal the vial caps. Eggs in the group of control, only received distilled water. Following an 8-day incubation period, the number of hatching eggs was noted. Each treatment was replicated five times [3, 22].

#### 2.4.3. Larval penetration

Each potato tuber (mean weight =  $31.18 \pm 0.10$  g) was individually dipped in 0.10 %, 0.25 %, and 0.50 % concentrations of essential oil. For example, to prepare a 0.25 % concentration of essential oil, 5  $\mu$ L of essential oil was dissolved in 2 mL acetone. After drying, the treated potato tubers were placed in plastic containers with ventilated lids and maintained under growth chamber conditions. A soft-haired brush was then

used to transfer 20 neonate larvae (< 5h old) on to each tuber. Each treatment was replicated five times. The same procedure was done for the control (distilled water). Larval penetration was measured by counting adult emergences in the experiments [9].

#### 2.4.4. Oviposition preference activity

Three potato tubers of uniform weight and shape were selected to investigate the effect of *S. hydrangea* essential oil on the oviposition preference of *P. operculella* adults. Each tuber was treated with 0.10 %, 0.25 %, and 0.50 % concentrations of essential oil. Three replicates were conducted for each concentration. After the solvent evaporated, each tuber was placed into a 500 mL container, and 10 pairs of adults were introduced. The number of oviposited eggs on the potato tubers was calculated after 24, 48, and 72 hours. For the control, only the solvent was used [3].

### 2.5. Bioassays with plant extract

#### 2.5.1. Adults

Potato leaflets (6-8 cm diameter) were dipped for 10 s in either distilled water (control group) and serial concentrations of *S. hydrangea* extract (120.50, 158.49, 213.80, 288.40 and 386.80 mg/L), then air-dried at room temperature. Each treated leaflet was placed in a plastic petri dish containing a moistened filter paper. 20 adults of *P. operculella* (<one-day-old) were chosen at random and put into each petri dish. Petri dishes were maintained under growth chamber conditions for 24 h for dose-response data collection [21].

#### 2.5.2. Eggs

For each treatment and distilled water (control group), filter papers containing 20 one-day-old eggs individually dipped in appropriate

concentration of *S. hydrangea* extract (348.79, 426.58, 512.86, 616.59 and 750.68 mg/L). Dried filter papers were placed within plastic containers (8 cm diameter × 4 cm height) with ventilated lids and containing potato tubers. Egg hatching rates were investigated after 8-days. Replication for each experiment was done five times [3, 8].

#### 2.5.3. Larval penetration

Each potato tuber (mean weight =  $31.18 \pm 0.10$  g) was individually dipped in 1 mL of 1 %, 3%, and 5% *S. hydrangea* extract. After drying, the tubers were transferred into plastic containers (8 cm in diameter × 4 cm in height). 20 neonate larvae (< 5 h old) were placed on each tuber. The percentage of larval penetration was measured by the number of adult emergences in the experiments. This assay was replicated five times [3].

#### 2.5.4. Oviposition preference activity

This assay was done with 1 %, 3 %, and 5 % methanolic extracts of *S. hydrangea*. Three tubers were individually immersed in 2 ml of the aforementioned extracts, while a fourth tuber (control group) was immersed in 2 ml of solvent. After the evaporation of methanol, these tubers were placed on an exposure cage's four corners (35 × 35 × 35 cm). Ten pairs of one-day-old adults were placed into the cage, and their oviposition rates were noted at 24, 48, and 72 h. This experiment consisted of three replicates [6, 21].

### 2.6. Homeopathic remedies

Potato tubers (cv. Agria) were grown in pots under greenhouse conditions ( $24 \pm 2$  °C,  $65 \pm 5$  % relative humidity, and 16: 8h (light: dark) photoperiod). Homeopathic remedies (Ferrum Met. 30 C, Coccinella 30 C, Pyrethrum Parth. 30

C and Silicea 30 C) were applied 2 weeks after planting. Distilled water was used as a control group. After 90 days of treatment, the effect of different homeopathic remedies on the potato plant growth parameters (stem length, stem diameter, and number of leaves and stems) was measured. From 30 plants per treatment were grown, ten uniformly growing potato plants were randomly selected for evaluation [23, 24].

2.7. Data analysis

The experiments carried out following a completely randomized design. In order to determine lethal concentrations (LC<sub>50</sub> and LC<sub>95</sub>), the data were analyzed using the probit analysis in SPSS software (version 17.0). The percentage transformation of data was done by arcsin√x followed by statistical analysis. Analysis of

variance (ANOVA) and correlation analysis were used to evaluate differences between the data. Means separations were performed using Tukey's HSD test at a significance level of α = 0.05.

3. Results

3.1. Toxicity of essential oil and extract of *Salvia hydrangea* on eggs and adults of *Phthorimaea operculella*

The results demonstrated that *S. hydrangea* essential oil was more toxic by fumigation against *P. operculella* (Table 1). Adults (LC<sub>50</sub> = 0.33 µl/L air) were more sensitive to the fumigant effect than eggs (LC<sub>50</sub> = 21.75 µl/L air). In similarly, the methanolic extract of *S. hydrangea* was more toxic to *P. operculella* adults (LC<sub>50</sub> = 219.72 mg/L) than to eggs (LC<sub>50</sub> = 490.24 mg/L).

**Table 1.** Results of bioassay of *S. hydrangea* essential oil and extract on eggs and adults of *Phthorimaea operculella* in laboratory conditions

| Compound      | growth stage | days after treatment | n   | χ <sup>2</sup> (df) | Slope ± SE  | LC <sub>50</sub> *<br>(Lower-Upper 95% CI) | LC <sub>95</sub> *<br>(Lower-Upper 95% CI) | R <sup>2</sup> |
|---------------|--------------|----------------------|-----|---------------------|-------------|--------------------------------------------|--------------------------------------------|----------------|
| Essential oil | egg          | 8                    | 500 | 0.82 (3)            | 3.11 ± 0.36 | 21.75<br>(19.90 - 23.73)                   | 73.58<br>(58.27 - 105.56)                  | 0.99           |
|               | adults       | 1                    | 500 | 2.73 (3)            | 1.39 ± 0.16 | 0.33<br>(0.27 - 0.40)                      | 5.01<br>(2.92 - 11.71)                     | 0.97           |
| Extract       | egg          | 8                    | 500 | 0.34 (3)            | 4.43 ± 0.52 | 490.24<br>(459.73 - 520.63)                | 1151.71<br>(978.54 - 1486.96)              | 0.99           |
|               | adults       | 1                    | 500 | 0.07 (3)            | 2.66 ± 0.33 | 219.72<br>(198.64 - 243.71)                | 911.06<br>(676.06 - 1478.13)               | 0.99           |

\*LC<sub>50</sub> and LC<sub>95</sub> for essential oil: (µl/L air) and for extract: (mg/L), LC: lethal concentration, CL: confidence limits, χ<sup>2</sup>: Chi-square value, df: degree of freedom

Significant difference was observed among different concentrations and the effectiveness of *S. hydrangea* essential oil (adults: F = 147.57, df = 4, P < 0.05; eggs: F = 141.88, df = 4, P < 0.05) and methanolic extract (adults: F = 174.43, df = 4,

P < 0.05; eggs: F = 178.33, df = 4, P < 0.05) in their effects on mortality of *P. operculella*. Their toxicity was increased by increasing the concentration (Table 2 and Table 3).

**Table 2.** ANOVA table for the effect of *S. hyrango* extract on eggs and adults of *Phthorimaea operculella* in laboratory conditions

| Source          | df | SS        | MS        | F         | P-value |
|-----------------|----|-----------|-----------|-----------|---------|
| <b>Egg</b>      |    |           |           |           |         |
| Corrected Model | 4  | 3437.791  | 859.448   | 178.334   | < 0.001 |
| Intercept       | 1  | 54897.586 | 54897.586 | 11391.179 | < 0.001 |
| Concentration   | 4  | 3437.791  | 859.448   | 178.334   | < 0.001 |
| Error           | 20 | 96.386    | 4.819     |           |         |
| Total           | 25 | 58431.763 |           |           |         |
| Corrected total | 24 | 3534.177  |           |           |         |
| <b>Adult</b>    |    |           |           |           |         |
| Corrected Model | 4  | 2885.784  | 721.446   | 174.426   | < 0.001 |
| Intercept       | 1  | 49308.858 | 49308.858 | 11921.573 | < 0.001 |
| Concentration   | 4  | 2885.784  | 721.446   | 174.426   | < 0.001 |
| Error           | 20 | 82.722    | 4.136     |           |         |
| Total           | 25 | 52277.364 |           |           |         |
| Corrected total | 24 | 2968.506  |           |           |         |

df: degree of freedom, SS: Sum of squares, MS: Mean square

**Table 3.** ANOVA table for the effect of *S. hyrango* essential oil on eggs and adults of *Phthorimaea operculella* in laboratory conditions

| Source          | df | SS        | MS        | F         | P-value |
|-----------------|----|-----------|-----------|-----------|---------|
| <b>Egg</b>      |    |           |           |           |         |
| Corrected Model | 4  | 3650.501  | 912.625   | 141.881   | < 0.001 |
| Intercept       | 1  | 52487.288 | 52487.288 | 8159.927  | < 0.001 |
| Concentration   | 4  | 3650.501  | 912.625   | 141.881   | < 0.001 |
| Error           | 20 | 128.646   | 6.432     |           |         |
| Total           | 25 | 56266.436 |           |           |         |
| Corrected total | 24 | 3779.147  |           |           |         |
| <b>Adult</b>    |    |           |           |           |         |
| Corrected Model | 4  | 3104.131  | 776.033   | 147.573   | <0.001  |
| Intercept       | 1  | 49654.846 | 49654.846 | 11170.153 | <0.001  |
| Concentration   | 4  | 3104.131  | 776.033   | 147.573   | <0.001  |
| Error           | 20 | 88.906    | 4.445     |           |         |
| Total           | 25 | 52847.884 |           |           |         |
| Corrected total | 24 | 3193.038  |           |           |         |

df: degree of freedom, SS: Sum of squares, MS: Mean square

### 3.2. Chemical characterization of *S. hydrangea* essential oil

GC-MS analysis (Table 4) revealed that the constituents with the highest concentrations in *S. hydrangea* aerial parts essential oil (Fig. 1) were included: caryophyllene oxide (23.62 %), eucalyptol (14.82 %),  $\alpha$ -pinene (9.97 %),  $\beta$ -pinene (7.45 %), and (*E*)-caryophyllene (6.89 %).

### 3.3. Total phenol content of *S. hydrangea* extract

According to the results, the amount of total phenolic content in methanolic extract of *S. hydrangea* aerial parts was determined to be  $6.83 \pm 0.23$  mg gallic acid per ml of extract.

### 3.4. Larval penetration

Results related to the effect of different concentrations of *S. hydrangea* essential oil and extract on *P. operculella* larval penetration are shown in Fig. 2.

**Table 4.** Chemical composition of *S. hydrangea* essential oil

| No.   | Compound                         | RT    | Composition (%) | KI   | Type |
|-------|----------------------------------|-------|-----------------|------|------|
| 1     | $\alpha$ -Thujene                | 10.87 | 1.73            | 930  | MH   |
| 2     | $\alpha$ -Pinene                 | 11.25 | 9.97            | 939  | MH   |
| 3     | Camphene                         | 12.13 | 2.28            | 954  | MH   |
| 4     | Sabinene                         | 13.31 | 0.77            | 975  | MH   |
| 5     | $\beta$ -Pinene                  | 13.58 | 7.45            | 979  | MH   |
| 6     | $\beta$ -Myrcene                 | 14.15 | 0.26            | 991  | MH   |
| 7     | $\alpha$ -Terpinene              | 15.62 | 0.14            | 1017 | MO   |
| 8     | Cymene                           | 16.13 | 5.62            | 1029 | MH   |
| 9     | Limonene                         | 16.28 | 1.45            | 1029 | MH   |
| 10    | Eucalyptol                       | 16.47 | 14.82           | 1031 | MO   |
| 11    | $\gamma$ -Terpinene              | 17.82 | 0.27            | 1060 | MH   |
| 12    | Terpinolene                      | 20.03 | 0.89            | 1089 | MH   |
| 13    | Camphor                          | 22.65 | 2.38            | 1146 | MO   |
| 14    | Borneol                          | 23.86 | 1.60            | 1169 | MO   |
| 15    | Terpinen-4-ol                    | 24.20 | 0.45            | 1177 | MO   |
| 16    | $\alpha$ -Terpineol              | 24.99 | 0.83            | 1188 | MO   |
| 17    | Bornyl acetate                   | 29.00 | 3.48            | 1285 | MO   |
| 18    | Carvacrol                        | 30.00 | 0.30            | 1299 | MO   |
| 19    | $\beta$ -Bourbonene              | 33.33 | 0.29            | 1388 | SH   |
| 20    | ( <i>E</i> )-Caryophyllene       | 34.93 | 6.89            | 1419 | SH   |
| 21    | ( <i>Z</i> )- $\beta$ -Farnesene | 36.17 | 0.80            | 1442 | SH   |
| 22    | $\alpha$ -Humulene               | 36.49 | 0.33            | 1454 | SH   |
| 23    | $\beta$ -Bisabolene              | 38.55 | 0.15            | 1505 | SH   |
| 24    | Spathulenol                      | 41.61 | 3.32            | 1578 | SO   |
| 25    | Caryophyllene oxide              | 41.80 | 23.62           | 1583 | SO   |
| 26    | Ar-Turmerone                     | 44.92 | 4.26            | 1669 | SO   |
| 27    | Valeranone                       | 45.35 | 0.96            | 1674 | SO   |
| Total | -                                | -     | 95.00           | -    | -    |

RT: Retention Time (min), KI: Kovats Index, MH: Monoterpene Hydrocarbons, SH: Sesquiterpene Hydrocarbons, MO: Oxygenated Monoterpenes, SO: Oxygenated Sesquiterpenes

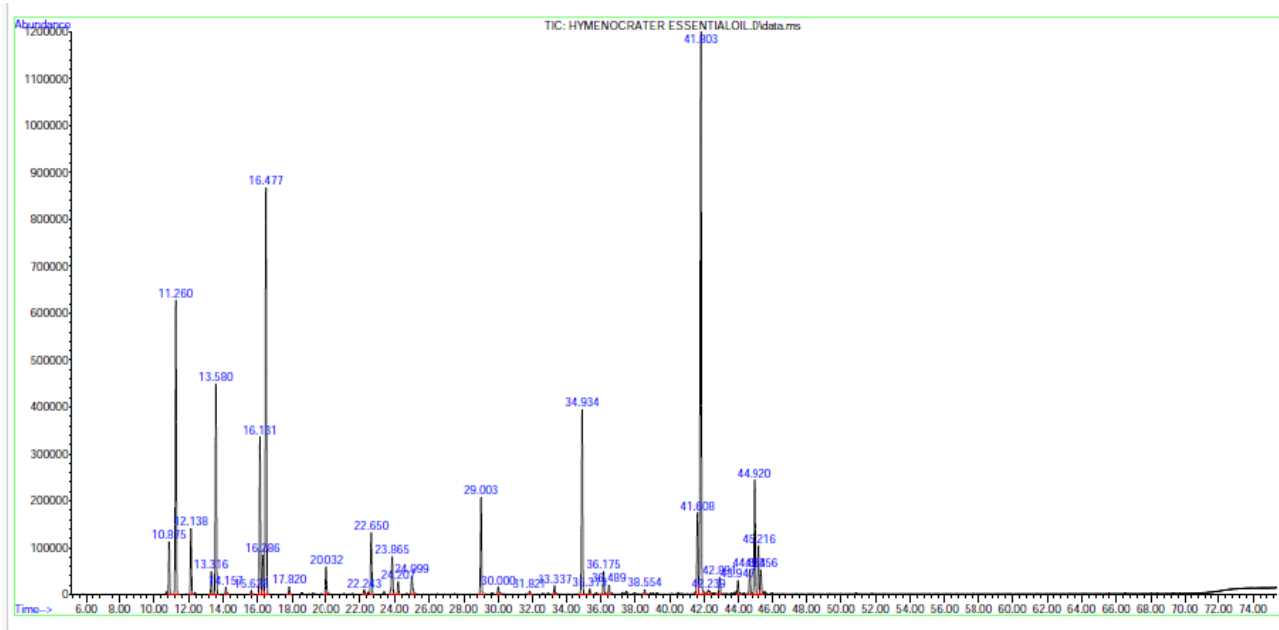


Fig. 1. GC–MS chromatogram of *S. hydrangea* essential oil with recognized peaks indexed in Table 2.

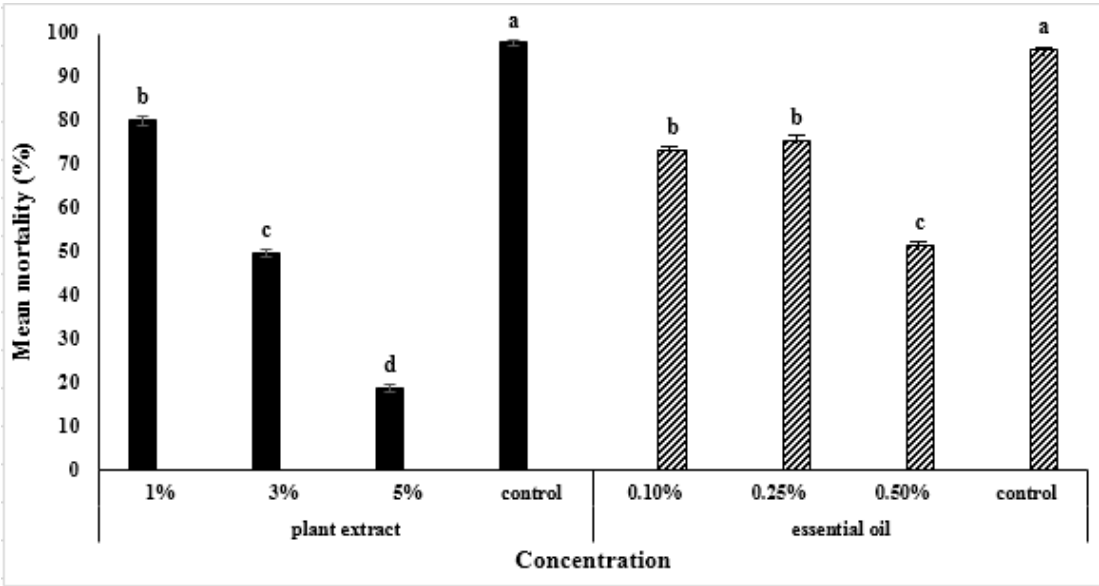


Fig. 2. Effect of different concentrations of *S. hydrangea* essential oil and extract on *P. operculella* larval penetration rate

Comparing the effects of different concentrations of *S. hydrangea* extract on the first-instar larval penetration of *P. operculella* revealed a significant difference among the various concentrations of the extract and the control group, with the 5 % extract showing the

highest negative effect on the percentage of larval penetration ( $F = 282.58$ ,  $df = 3$ ,  $P < 0.05$ ). Also, based on the results (Fig. 2), a concentration of 0.50 % essential oil exhibited the highest preventive effect on larval penetration. The concentrations of 0.25 % and



0.10 % did not differ significantly from each other, and their effects were similar ( $F = 45.47$ ,  $df = 3$ ,  $P < 0.05$ ).

### 3.5. Oviposition preference activity

As shown in Table 5, applying 5 % extract of *S. hydrangea* on potato tubers resulted in a

significantly higher reduction in egg laying of the *P. operculella* ( $F = 215.17$ ;  $df = 3$ ,  $P < 0.05$ ). Additionally, the number of eggs laid by pest on tubers treated with concentration of 0.50 % essential oil of *S. hydrangea* was the lowest compared to other treatments and the control group ( $F = 61.05$ ;  $df = 3$ ,  $P < 0.05$ ).

**Table 5.** The mean number of laid eggs of *P. operculella* on treated and non-treated tubers with extract and essential oil of *S. hydrangea* after 24, 48 and 72 hours in comparison with control

| The mean number of eggs $\pm$ SE |               |                  |                  |                  |                    |
|----------------------------------|---------------|------------------|------------------|------------------|--------------------|
|                                  | Concentration | 24 h             | 48 h             | 72 h             | Total              |
| Extract                          | 1%            | $4.12 \pm 0.99$  | $8.44 \pm 0.15$  | $15.47 \pm 0.11$ | $28.03^b \pm 0.16$ |
|                                  | 3%            | 0                | $4.33 \pm 0.87$  | $14.77 \pm 0.56$ | $19.10^c \pm 0.32$ |
|                                  | 5%            | 0                | 0                | $10.46 \pm 0.01$ | $10.46^d \pm 0.14$ |
| Control                          | 0             | $18.33 \pm 0.46$ | $22.56 \pm 0.99$ | $28.44 \pm 0.50$ | $69.33^a \pm 0.12$ |
| Essential oil                    | 0.5%          | $10.66 \pm 0.11$ | $12.33 \pm 0.10$ | $17.57 \pm 0.65$ | $40.56^b \pm 0.25$ |
|                                  | 0.25%         | 0                | $10.00 \pm 0.16$ | $18.58 \pm 0.78$ | $28.58^c \pm 0.16$ |
|                                  | 0.50%         | 0                | $7.50 \pm 0.12$  | $11.10 \pm 0.56$ | $18.60^d \pm 0.45$ |
| Control                          | 0             | $16.67 \pm 0.11$ | $20.12 \pm 0.16$ | $26.66 \pm 0.18$ | $63.45^a \pm 0.08$ |

### 3.6. Homeopathic remedies effects

Regarding the plant growth parameters, a significant impact of the homeopathic remedies was observed (Table 6). In all evaluated growth

parameters, Silicea exhibited the highest positive effect compared to the other remedy treatments and the control group.

**Table 6.** Effects of homeopathy remedies on plant growth parameters of potato in comparison with control

| Homeopathy remedies   | Potato growth parameters               |                                         |                                        |                                       |
|-----------------------|----------------------------------------|-----------------------------------------|----------------------------------------|---------------------------------------|
|                       | Plant length (cm)                      | Branch No per plant                     | Leaf No per plant                      | Stem diameter (mm)                    |
| Ferrum Met. 30 C      | $57.20^b \pm 0.56$                     | $4.53^b \pm 0.18$                       | $47.00^b \pm 0.89$                     | $13.51^b \pm 0.18$                    |
| Coccinella 30 C       | $52.90^{bc} \pm 0.19$                  | $4.01^c \pm 0.57$                       | $44.33^c \pm 0.16$                     | $12.84^c \pm 0.11$                    |
| Pyrethrum Parth. 30 C | $51.48^d \pm 0.36$                     | $3.65^d \pm 0.11$                       | $45.60^{bc} \pm 0.56$                  | $12.60^c \pm 0.99$                    |
| Silicea 30C           | $63.16^a \pm 0.36$                     | $5.65^a \pm 0.56$                       | $48.50^a \pm 0.23$                     | $14.85^a \pm 0.35$                    |
| Control               | $48.41^d \pm 0.18$                     | $3.11^e \pm 0.62$                       | $43.20^c \pm 0.46$                     | $12.94^c \pm 0.42$                    |
| One-Way ANOVA         | $F = 24.93$ , $df = 4$ ,<br>$P < 0.05$ | $F = 169.70$ , $df = 4$ ,<br>$P < 0.05$ | $F = 11.92$ , $df = 4$ ,<br>$P < 0.05$ | $F = 125.01$ $df = 4$ ,<br>$P < 0.05$ |

cm: centimeter, mm: millimeter

## 4. Discussion

The chemical profile of *S. hydrangea* ascertained that the main components were caryophyllene oxide (23.62 %), eucalyptol (14.82 %),  $\alpha$ -pinene (9.97 %),  $\beta$ -pinene (7.45 %), and (E)-caryophyllene (6.89 %), which accounted for 95 % of the identified compounds.

These results align with several studies on *S. hydrangea* essential oil conducted by other Iranian researchers, who reported that the essential oil of *S. hydrangea* contained the same compounds, albeit in different concentrations [25, 26]. However, variations in the components and their relative concentrations in the essential oil of *S. hydrangea* have also been documented.

For instance, camphor (46.0 %) was identified as the primary constituent of the essential oil of *S. hydrangea* gathered from Turkey [27], with 1,8-cineole (7.5 %), camphene (6.8 %), limonene (6.5 %),  $\alpha$ -pinene (5.6 %), and  $\beta$ -pinene (6.1 %) following. In another study, Kotan *et al.* assessed the composition of the essential oil of *S. hydrangea* that was gathered from Iğdır [13] and discovered that the predominant ingredients were  $\alpha$ -humulene (4.0 %) and camphor (54.2 %). Numerous earlier investigations have indicated that the chemical profile of *Salvia* species varies depending on factors such as genotype, harvesting period, phenological stage, method of essential oil extraction, collection location, and climate conditions [27, 28].

In this study, for the first time, the insecticidal activity of *S. hydrangea* essential oil and extract was evaluated against *P. operculella*. Contact and fumigant toxicity experiments indicated that the insecticidal activities of these compounds against *P. operculella* were dose-dependent. The insecticidal activity of *S. hydrangea* essential oil and extract against other insect pests such as *Tribolium confusum* Jacquelin du Val [13], *Spodoptera frugiperda* J.E. Smith [29] and *Sitophilus granarius* L. [30] has already been verified.

According to our results, the number of *P. operculella* eggs laid on tubers treated with essential oil and extract of *S. hydrangea* was significantly lower than that of the control. These results are consistent with Soltanpour *et al.* [3], who investigated the effects of essential oil and plant extract of *Syzygium aromaticum* L. on the oviposition preference of adult *P. operculella*. Additionally, another study evaluated how the oviposition rate of *P. operculella* was affected by leaf powder and a 5% extract of *Sambucus ebulus* (L.), *Artemisia annua* (L.), and *Pterocarya*

*fraxinifolia* (Lam.). Their results showed that the aforementioned plants' leaf powder or extracts have deterrent properties [21]. In future research, the efficacy of the plant powder of *S. hydrangea* on the oviposition preference of *P. operculella* could be examined.

In the current study, the tubers treated with *S. hydrangea* essential oil and extract had the lowest percentage of *P. operculella* larval penetration as compared to the control group, suggesting that the under-evaluation compounds had detrimental effects on the feeding and mobility of larvae. These findings corroborate those of Naghizadeh *et al.* [31] who found that potato tubers treated with essential oils of *Artemisia absinthium* L., *Achillea millefolium* L., and *Artemisia dracunculus* L. had a lower larval penetration rate by *P. operculella*. Another study also found that powder, essential oil and extract of *S. aromaticum* were effective in reducing the larval penetration rate of *P. operculella* [3].

The results regarding the effects of homeopathy remedies on certain potato growth parameters demonstrated that these compounds had a significant positive impact compared to the control group. Silicea exhibited the greatest effect. The potato plant length, number of leaves per plant, number of branches per plant, and stem diameter increased following treatment with this remedy. Future research should carefully evaluate these results under semi-field and field conditions, and all information regarding the effects of Silicea and other homeopathy remedies on the physiological parameters of potatoes should be disclosed.

In comparison to the control treatment, the coumarin content rose when some homeopathic remedies such as Humic Acid 200CH, Sulphur, *Ageratum conyzoides* L. 2CH, 6CH, and 30CH were used during the growth of *Justicia pectoralis* Jacq. and *Ageratum conyzoides* L. [32]. For *Talinum triangulare* (Jacq.) Willd., similar

outcomes were noted; the application of phosphorus (3CH, 12CH, and 30CH) raised the amounts of flavonoids that give antioxidant properties to the plant [33]. Additionally, Capra et al. [34] found increases of 30 % and 47 % in the quercetin content of *Baccharis trimera* after treatment with Silicea terra (6 cH, 7 dH) and Equisetum (7 dH).

## 5. Conclusion

Given the current study's findings, considering the destructive environmental effects of chemical insecticides and the low risk of plant-derived compounds for human and environmental health, it seems that conducting further studies (biochemical and field assays) on *S. hydrangea* and Silicea, as the most effective homeopathy remedy, could enable these compounds to be used in an integrated control of the potato tuber moth.

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## Author contributions

Research conception and design: NA, OV and AS. Data collection: AS and NA. Review and editing: OV and AS. The submitted version of the article was approved by all authors who contributed to it.

## Conflicts of interest

Each author affirms that there were no financial or commercial relationships that might be interpreted as a conflict of interest during the conduct of this study.

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