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# Biocontrol potential of aqueous and methanolic extracts of Russian knapweed, *Acroptilon repens*, L. (Asteraceae) against *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)

Seyedeh Sahar Sarboland[1], Fariba Mehrkhou[1]✉, Mohammad Fattahi[2] & Maryam Forouzan[3]

**Plant based natural products could be used as new alternative of chemical insecticides due to their co-friendly, safety to non-target organisms, and low-level resistance properties. In this study, the contact toxicity and sublethal effects of *Acroptilon repens* L., Russian knap weed, extracts (aqueous and methanolic) were evaluated on the biological, population traits and population projection of greenhouse whitefly, *Trialeurodes vaporariorum*. The essential oil was extracted from the plant by steam distillation using a Clevenger apparatus and the chemical compounds were identified by gas chromatography-mass spectrometry. Twenty-three chemical components of *A. repens* were characterized, in which caryophyllene oxide (30.67%) and α-copaene (15.05%), were identified as the predominant compounds of Russian knapweed. Moreover, the results illustrated that some secondary metabolites (e.g. flavonoids, alkaloids and polyphenols) were rich in methanolic extract compared to the control. The leaf dipping method was used for the bioassay tests against whiteflies. According our findings, the aqueous extract ($LC_{50}$: 1802.59 ppm) was more toxic than the methnolic treatment ($LC_{50}$: 3849.15 ppm) on the *T. vaporariorum* adults. The age-stage, two-sex life table theory was used to analyze the life table data. The sublethal concentration of either aqueous or mehanolic extracts of *A. repens* significantly affected the biological and population growth parameters of *T. vaporariorum* compared to the control by prolongation the developmental period, adult longevity, reducing the survival rate, fecundity and decreasing the net reproductive rate ($R_0$), intrinsic rate of increase ($r$), and finite rate of increase (λ) of adults, as well. The overall results demonstrated that both of the Russian knapweed extracts could be considered in management programs of greenhouse whitefly. However, the cost-effective property of aqueous extract should not be neglected.**

**Keywords** Russian knapweed, Secondary metabolites, Greenhouse whitefly, Two-sex life table

Whiteflies are the most common and problematic insect pests on vegetable and ornamental crops[1]. Among them, the greenhouse whitefly, *Trialeurodes vaporariorum,* Westwood (Hemiptera: Aleyrodidae), is a common pest and well established in the greenhouse ecosystem, and it is one of the most important economic pests of various greenhouse vegetables, particularly in tomatoes, cherry tomatoes, and cucumber crops[2–4]. Either adults or nymphs cause substantial damages to plants directly, through ingestion of phloem sap, and indirectly, by secretion large amounts of honeydew which supports the growth of sooty mold[2]. Furthermore, it is known as a carrier of some plant viruses' pathogens including, Bean Golden Mosaic Virus (BGMV), Tomato Yellow Leaf Virus (TYLCV), and Beat Pseudo Yellow Virus (BPYV), which resulted in losses to tomato and cucurbit crops[3,5].

[1]Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran. [2]Department of Horticulture, Faculty of Agriculture, Urmia University, Urmia, Iran. [3]Plant Protection Research Department, West Azarbaijan Agricultural and Natural Resources Research Center, AREEO, Urmia, Iran. ✉email: f.mehrkhou@urmia.ac.ir

Control of whiteflies is primarily based on chemical insecticides in Iran[3–6]. The overuse of insecticides developed the resistance of whiteflies population to numerous conventional insecticides especially pyrethroids[7], neonicotinoids[8] and ketoenols[9]. Additionally, the extensive usage of chemical insecticides led to the reduction of non-target insects' population (e.g., natural enemies, pollinators), environmental pollution and increasing the toxicological risks for farmers and consumers[6,10]. That is why, the worker safety and attempt to produce organic and healthy products has gained importance in enclosed system such as greenhouse than outdoor or field spaces, therefore, it is essential to develop alternative strategies for pest management in greenhouse system[11].

Given the resistance potential of the greenhouse whitefly to conventional insecticides, much effort has been focused on the use of biopesticides with plant-based origin phytochemicals as potential sources of commercial insect control agents in integrated pest management (IPM) program[2,3]. Over the past two decades, sources for remedies and control of insect pests were shifted to the natural resources and numerous extracts or essential oils from indigenous plants and have evaluated against pests. The use of plant compounds, such as plant extracts, is an increasing interest due to their fewer side effects on non-target organisms and low-risk to environment compared to conventional insecticides and are easily decomposed in soil and are not stored in plants or animals[12,13].

*Acroptilon repens*, Russian knapweed, (Asteraceae) belongs to the Asteraceae family. *A. repens* not only known as a persistent weed but also it could be used as an herbal insecticide. It has been widely grown and reported from northwestern of Iran such as West Azarbayjan, Zanjan, and Tehran[14,15]. The aerial tissues of *A. repens* possess aromatic amines, and sterols[16], as well as sesquiterpene lactones[17,18]. The potential of *A. repens* refers to the existence of polyphenolic and triterpene constituents, which in turn enhance its biological features, including repellency, contact, fumigant toxicity, anti-oviposition, and antifeedant activity against insect pests[19–22], by altering the insect behavior and promote physiological changes, in addition to causing mortality in insect pest populations.

All of the previous reports considered the insecticidal activity of medicine plants or edible plants. There are a few studies on the insecticidal activity of *A. repens* extracts, as a weed, against agricultural and medical pests. For instance, the insecticidal properties of *A. repens* extract were investigated in China against some species of Lepidopteran larvae[20]. In the other study, Toolabi et al.[21] surveyed on the larvicidal activity of methanolic extract of *A. repens* against third instar larvae of some medical pests (e.g., *Anopheles stephensi* Liston, *Culex pipiens* Linnaeus and *C. quinquefaciatus* Say), in which *An. stephensi*, was more susceptible than other mosquitoes.

Plant based insecticides cause toxic effects at both lethal and sublethal levels which refer to the existence of secondary plant metabolites. They are involved in the cellular and physiological processes by disruption in redox balance, hormonal regulation, neuronal signalization or reproduction in exposed individuals of insect pest population, which in turn led to prolonged lifespan, reduced the fecundity, survival rate and deformities in parental and offspring generations[23]. Demographic parameters estimation through life table analysis is an essential approach for ecological predictions of population growth, which are valuable for pest management[24–26]. However, there were previous reports about the contact activity of essential oils, such as *Thymus vulgaris*, *Mentha piperita*[1,2], *Trachyspermum ammi*, *Withania coagulans* and *Murraya koenigii*[27], *Piper marginatum* Jacq. and *Manosa alliaceae* Miers[10] against the different whitefly species. Insecticidal activity of 53 essential oils was assessed against on developmental stage of *T. vaporariorum* (eggs, nymphs, and adults) by Choi et al.[28]. They found that the oil type, usage dose, and developmental stage of the insect pests are involved in their susceptibility to essential oils. Mahmoodi et al.[29] stated that *Petroselinum crispum* L. (Apiaceae) essential oil has fumigant toxicity on *T. vaporariorum* adults under greenhouse conditions. A survey on the anti-oviposition and repellency activities of essential oils and aqueous extracts from five aromatic plants against greenhouse whitefly[30] illustrated that the most significant repellency effect occurred 3 and 6 days after infestation with aqueous extracts of *Cuminum cyminum* L. and *Tymus vulgaris*.

Due to the specific biological properties of greenhouse whitefly (high dispersion potential, competitive ability, and resistance to conventional insecticides), it is necessary to study alternative tactics to control this pest[7–9]. Our literature review show that the effects of *A. repens* has already been studied in terms of lethal toxicity for *T. vaporariorum* but not for sublethal effects. In light of this, this study aimed to assess the biological features of Russian knapweed for on the biological and population growth parameters of greenhouse whitefly.

## Results

### Chemical constituents of *A. repens*

The chemical constituents, Kovats index, retention time, and percentage of *A. repens* are shown in Table 1. The results indicated about 23 compounds in which, caryophyllene oxide (30.67%) and α-copaene (15.05%) were two predominant abundant constituents of *A. repens* (Table 1).

### The flavonoid, phenol, and alkaloid contents of the aerial parts of aqueous and methanolic extracts of *A. repens*

The total flavonoids, phenol, and alkaloid contents in the aerial parts of the methanolic extract of *A. repens* are shown in Table 2. The results showed that methanolic extract of Russian knapweed affected the amount of total flavonoids, phenol, and alkaloid. Based on the results of the t-test (α = 5%), these differences were significant (Table 2). The average flavonoid concentration in methanolic extract of *A. repens* (11.16 ± 0.13 mg/g DW), was significantly more than control treatment (6.21 ± 0.01 mg/g DW). Similarly, Comparison of showed a significant difference based on the t-test (α = 5%) between the control and methanolic extract of *A. repens*. The lowest amount of the phenol and alkaloid contents were obtained in the control group.

| Components | Kovats Index | Retention time (min) | Percentage (%) |
|---|---|---|---|
| α-Cubebene | 1350 | 25.70 | 4.71 |
| α-Copaene | 1372 | 27.03 | 15.05 |
| β-Cubebene | 1385 | 27.44 | 2.61 |
| *E*-caryophyllene | 1415 | 28.83 | 2.57 |
| E-β ionone | 1490 | 30.94 | 1.15 |
| γ-Muurolene | 1482 | 31.46 | 0.84 |
| A-*Muurolene* | 1502 | 31.87 | 2.20 |
| γ-Cadinene | 1520 | 32.70 | 4.17 |
| δ-Cadinbene | 1525 | 32.89 | 1.53 |
| Dimethyl ionone | 1565 | 34.08 | 1.14 |
| Spathulenol | 1580 | 34.61 | 1.83 |
| Caryophyllene oxide | 1585 | 35.47 | 30.67 |
| β-Copaene-4-α-ol | 1591 | 35.58 | 4.25 |
| Salvia-4 (14)-en-l-one | 1597 | 35.73 | 0.93 |
| Humulene epoxide II | 1613 | 36.08 | 2.03 |
| Cisisolongifolanone | 1618 | 36.31 | 4.28 |
| l-epi-cubenol | 1625 | 36.40 | 2.14 |
| Muurola-4,10-dien-l-ß-ol | 1635 | 36.97 | 1.60 |
| Selina-3,11-dien-6α-ol | 1650 | 38.03 | 1.95 |
| 14-Hydroxy-Z-caryophyllene | 1675 | 38.62 | 5.42 |
| Mustakone | 1680 | 38.74 | 1.61 |
| Heptadecene | 1700 | 39.02 | 2.28 |
| Hexahydrofarnesyl acetone | 1860 | 44.17 | 1.56 |

**Table 1**. Chemical constituents of the essential oil isolated from aerial parts of *Acroptilon repens* L.

| Parameters | Treatments (Mean ± SE) | | No | F | Sig | t | df | Sig |
|---|---|---|---|---|---|---|---|---|
| | Control | Methanolic extract | | | | | | |
| Flavonoid (mg/g DW) | 6.21 ± 0.01 | 11.16 ± 0.13 | 3 | 14.50 | 0.019 | − 12.35 | 4 | 0.006 |
| Phenol (mg/g DW) | 14.87 ± 0.70 | 69.80 ± 1.43 | 3 | 13.30 | 0.022 | − 40.62 | 4 | 0.001 |
| Alkaloid (mg/g DW) | 194.696 ± 3.56 | 340.454 ± 1.892 | 3 | 0.943 | 0.386 | − 36.13 | 4 | 0.000 |

**Table2**. Mean (± SE) flavonoids, polyphenol and alkaloid contents of methanolic extract of aerial parts of *Acroptilon repens*. *DW* dry weight of aerial parts of *Acroptilon repens*.

| Treatments | $LC_{50}$ (ppm)[1] | $LC_{25}$ (ppm)[1] | Slope ± SE | Intercept (a) + 5 | $\chi^2$ (df) | No |
|---|---|---|---|---|---|---|
| Aqueous extract | 1802.593 (1352.06–2611.52) | 904.360 (511.284–1220.863) | 2.252 ± 0.52 | − 7.331 + 5 | 4.660 (3) | 300 |
| Methanolic extract | 3849.15 (2002.39–19,822.39) | 1221.788 (308.71–2374.04) | 1.353 ± 0.187 | − 4.852 + 5 | 6.625 (3) | 300 |

**Table 3**. Susceptibility of *Trialeurodes vaporariorum* adults to aqueous and methanolic extracts of *Acroptilone repens* 24 h after treatment. *L. C.* lethal concentration, *CL* confidence limits, $X^2$ Chi-square value, *df* degrees of freedom.

## Insecticidal activity of the aqueous and methanolic extracts of *A. repens* against greenhouse whitefly

The median lethal ($LC_{50}$) and sublethal ($LC_{25}$) concentrations of the aqueous and methanolic extracts of *A. repens* against *T. vaporariorum* adults are shown in Table 3. Probit analysis revealed that the median lethal ($LC_{50}$) and sublethal ($LC_{25}$) concentrations of the aqueous and methanolic extracts of *A. repens* against *T. vaporariorum* adults were obtained (1802.60 and 904.360 ppm) and (3849.15 and 1221.78 ppm), respectively. The aqueous extract was more toxic against greenhouse whitefly than the methanolic extract (Table 3).

## Sublethal effects of the aqueous and methanolic extracts of *A. repens* on the biological traits of F1 generation of the greenhouse whitefly

The sublethal effects of *A. repens* on the biological traits of greenhouse whitefly exposed to the aqueous and methanolic extracts of *A. repens* are illustrated in Table 4. The shortest egg incubation period in the greenhouse whitefly's F1 generation observed in control group. However, there were no significant differences between

| Biological parameters | | Treatments | |
|---|---|---|---|
| Traits | Control | Aqueous extract | Methanolic extract |
| Egg (days) | $5.04 \pm 0.08^{b}$ | $6.64 \pm 0.18^{a}$ | $6.41 \pm 0.13^{a}$ |
| N1 (days) | $3.63 \pm 0.07^{b}$ | $4.96 \pm 0.13^{a}$ | $5.18 \pm 0.13^{a}$ |
| N2 (days) | $3.74 \pm 0.09^{c}$ | $4.19 \pm 0.12^{b}$ | $4.55 \pm 0.14^{a}$ |
| N3 (days) | $4.54 \pm 0.08^{a}$ | $4.72 \pm 0.12^{a}$ | $4.48 \pm 0.13^{a}$ |
| Pupa (days) | $5 \pm 0.09^{a}$ | $4.45 \pm 0.15^{b}$ | $4.00 \pm 0.16^{c}$ |
| Pre-adult (days) | $21.74 \pm 021^{b}$ | $24.66 \pm 0.34^{a}$ | $24.78 \pm 0.55^{a}$ |
| Preadult survival rate (%) | $0.92 \pm 0.23^{a}$ | $0.61 \pm 0.05^{c}$ | $0.66 \pm 0.05^{b}$ |
| Male longevity (days) | $4.41 \pm 0.39^{a}$ | $2.84 \pm 0.24^{b}$ | $2.5 \pm 0.25^{b}$ |
| Total male longevity (days) | $26.23 \pm 0.44^{b}$ | $27.69 \pm 0.45^{b}$ | $26.25 \pm 0.66$ |
| Female longevity (days) | $7.36 \pm 0.32^{a}$ | $4.12 \pm 0.31^{b}$ | $4.18 \pm 0.29^{b}$ |
| Total female longevity (days) | $29.04 \pm 0.39^{a}$ | $28.54 \pm 0.74^{b}$ | $30.65 \pm 0.94^{a}$ |
| APRP (days) | $1.00 \pm 0.10^{a}$ | $2.80 \pm 0.53^{a}$ | $0.71 \pm 0.19^{a}$ |
| TPRP (days) | $22.68 \pm 0.29^{c}$ | $25.73 \pm 0.74^{b}$ | $27.18 \pm 0.76^{a}$ |
| Reproductive days (days) | $6.28 \pm 0.32^{a}$ | $2.41 \pm 0.26^{b}$ | $2.53 \pm 0.24^{b}$ |
| Reproduction (eggs/female) | $35.52 \pm 2.47^{a}$ | $7.23 \pm 1.04^{b}$ | $6.35 \pm 1.41^{b}$ |

**Table 4**. Sublethal effects ($LC_{25}$) of aqueous and methanolic extracts of *Acroptilone repense* on developmental time adult longevity and fecundity of *Trialeurodes vaporariorum*. The means followed by different letters in each row are significantly different (paired-bootstrap at 5% significance level). *N* Nymphal stage, *APRP* adult pre-reproductive period, *TPRP* total pre-reproductive period.

| Population parameters | Control | Aqueous extract | Methanolic extract |
|---|---|---|---|
| $R_0$ (off spring/individual) | $11.10 \pm 1.98^{a}$ | $1.89 \pm 0.43^{b}$ | $1.47 \pm 0.44^{c}$ |
| r ($day^{-1}$) | $0.0920 \pm 0.0070^{a}$ | $0.0230 \pm 0.0080^{b}$ | $0.0120 \pm 0.0010^{b}$ |
| λ ($day^{-1}$) | $1.090 \pm 0.007^{a}$ | $1.020 \pm 0.008^{b}$ | $1.010 \pm 0.010^{b}$ |
| T (day) | $26.09 \pm 0.28^{c}$ | $27.63 \pm 0.63^{b}$ | $30.55 \pm 0.92^{a}$ |
| GRR (off spring/individual) | $41.49 \pm 6.09^{a}$ | $11.70 \pm 2.33^{b}$ | $11.67 \pm 3.09^{b}$ |

**Table 5**. The sublethal ($LC_{25}$) effects of aqueous and methanolic extracts of *Acroptilon repens* on population growth parameters of *Trialeurodes vaporariorum*. The means followed by different letters in each column are significantly different (paired-bootstrap at 5% significance level). $R_0$ net reproductive rate, *r* intrinsic rate of increase, λ finite rate of increase, *T* mean generation time, *GRR* Gross reproductive rate.

extracts of *A. repens* in terms of egg incubation period. Exposure to sublethal concentration of *A. repens* extracts resulted in prolonged developmental time of the greenhouse whitefly and it was shorter in the control group than those exposed to the both extracts of *A. repens*. The longest female ($7.36 \pm 0.32$) and male ($4.41 \pm 0.39$ days) longevity were recorded in the control group, while the sublethal concentrations of either aqueous or methanolic extracts led to shortened adult longevity of greenhouse white fly. The shortest and longest TPRP were obtained by control ($22.68 \pm 0.29$ days) and methanolic extract of *A. repens* ($27.18 \pm 0.76$ days) populations, respectively (Table 4). Additionally, the highest reproduction rate ($35.52 \pm 2.47$ eggs/female) was obtained in control groups compared to both treatments.

## Sublethal effects of *A. repens* extracts on the population growth parameters of *T. vaporariorum*

Our results indicated that exposure to sublethal concentration of aqueous and methanolic extracts of *A. repens* decreased the population growth parameters including the net reproductive rate, the intrinsic rate of increase, and the finite rate of increase of the greenhouse whitefly. However, the treatment with methanolic extract led to prolonged mean generation time of greenhouse whiteflies (Table 5).

## Sublethal ($LC_{25}$) effects of *A. repens* extracts on the age-stage specific survival rate ($s_{xj}$), fecundity ($m_x$), and maternity ($l_x m_x$), life expectancy ($e_{xj}$) and reproductive value ($v_{xj}$) of F1 generation of the greenhouse whitefly

The sublethal effects of *A. repens* aqueous and methanolic extracts on the age-stage specific survival rate ($s_{xj}$) of greenhouse whiteflies' F1 generation are presented in Fig. 1. The survival probability of preadult greenhouse whiteflies was considerably lower in the aqueous extract of Russian knapweed and the adult females emerged with delay in both treatments compared to control group. The age-specific survival rate ($lx$), fecundity ($m_x$) and the maternity ($l_x m_x$) of the greenhouse whiteflies are demonstrated in Fig. 2. The appearance of the peaks of the age-specific fecundity ($m_x$) and age-specific maternity ($l_x m_x$) occurred with delay in the methanolic group.

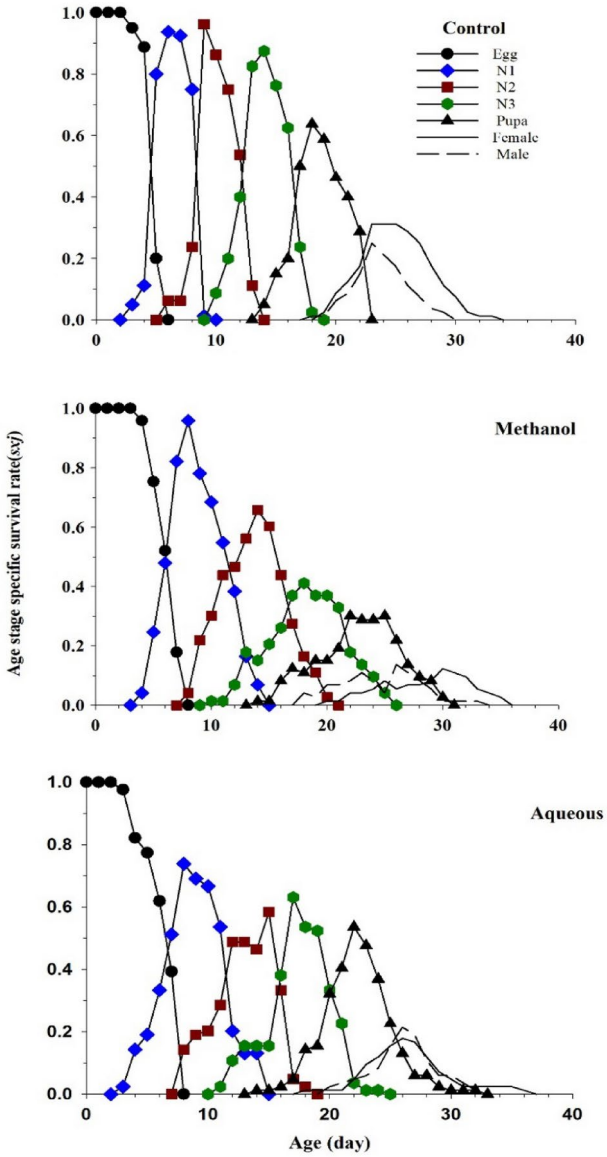


**Fig. 1**. Sublethal effects of methanolic and aqueous extracts of *Acroptilon repens* on age-stage specific survival rate ($s_{xj}$) of *Trialeurodes vaporariorum*.

The age-stage-specific life expectancy ($e_{xj}$) of *T. vaporariorum* which treated with sublethal concentration of *A. repens* extracts, was decreased compared to control group (Fig. 3). The life expectancy in the first day of the adult emergence were obtained 10.04, 6.51 and 3 days in the control, methanolic and aqueous populations, respectively. Exposure to the sublethal effects of *A. repens* aqueous and methanolic extracts affected the age-stage specific reproductive value ($v_{xj}$) of greenhouse whiteflies' F1 generation (Fig. 4). The earliest and highest reproductive value peaks were observed in the control group ($v_{21} = 27.81$). The peak values obtained for the greenhouse whiteflies populations exposed to extracts of *A. repens* were close to each other, although occurred on different days ($v_{27} = 7.58$, $v_{22} = 7.5$ for methanolic and aqueous extracts, respectively).

## Population projection

Population projection results illustrated that methanolic and aqueous extracts of *A. repens* have a notable effect on the predicted population growth of the greenhouse whitefly. Exposing *T. vaporariorum* to methanolic extract of Russian knapweed resulted in decreasing the population size in the greenhouse whitefly. However, the population size of the greenhouse whitefly was increased in the control group after 60 days (Figs. 5 and 6).

## Discussion

According to GC–MS analysis, 23 components were identified in *A. repens*, in which caryophyllene oxide (30.67%) and α-copaene (15.05%), were characterized as the predominant compounds of Russian knapweed. Previously published studies also identified some of the same main compounds, too (e.g., caryophyllene oxide,

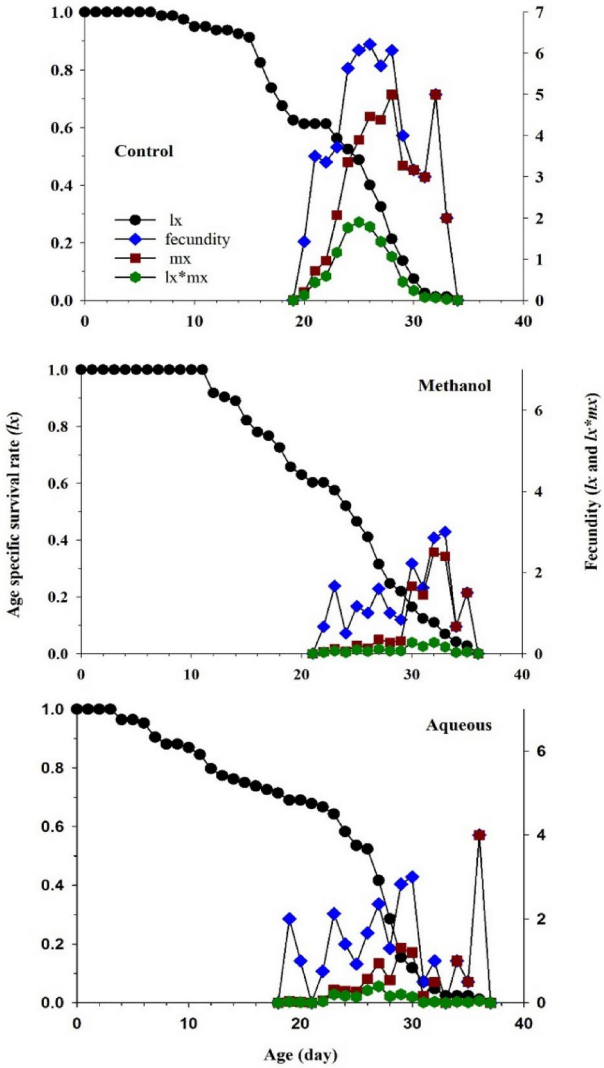


**Fig. 2**. Sublethal effects of methanolic and aqueous extracts of *Acroptilon repens* on age-specific survival ($l_x$), age-stage specific fecundity ($m_x$) and net maternity ($l_x m_x$) of *Trialeurodes vaporariorum*.

α-copaene)[17,18]. The results reported by Norouzi-Arasi et al.[17] were approximately in the same range (36.6%; 15.6%) for caryophyllene oxide and α-copaene, respectively. However, the amount of caryophyllene oxide and α-copaene (6.4%; 22.8%) reported by Tunalier et al.[18], were different than those of our findings. The geographical position, may be one of the reasons for these discrepancies[31]. Plants origin, harvesting time, the procedure of essential oils extraction and geographical position are involved in quantitative differences of *A. repens* chemicals[2,31].

Due to the existence of complex chemical composition in plant-based insecticides, they have multiple modes of action and broad physiological activity, which in turn reduce the probability of developing resistance of polyphagous pests such as greenhouse whitefly to their host plants[2,15,32].

Russian knapweed has a various biological features including repellency, contact, fumigant toxicity, anti-oviposition, and antifeedant activity against insect pests[19–22] which related to the abundance of plant secondary metabolites (e.g., alkaloids, glycoalkaloids, terpenoids, organic acids, phenolic compounds, terpenes, essential oils, saponins, flavonoids, and alcohols) in *A. repens*[19–21].

There are some documents in terms of insecticidal properties of *A. repens* against medical pests[20,33] and some limited agricultural pests[20,22]. In the current study, the total content of some secondary metabolites of Russian knapweed (polyphenolics, alkaloids, flavonoids, and terpenes) were the highest in methanolic extract than aqueous extract, but according to obtained results by GC–MS analysis, the sesquiterpenes, class of terpenoids, were more effective in the lethal activity of *A. repens*, which is consistent with Tunalier et al.[18] findings. They stated that the sesquiterpenes, were the most amount (56%) among identified compounds of *A. repens*. In the current study, the contact toxicity of Russian knapweed may be due to the major amount of caryophyllene oxide as a sesquiterpene. There are previous reports about contact activity of caryophyllene oxides[34–37] against agricultural insects, and the results some of them are consistent with our findings. However, for the contact

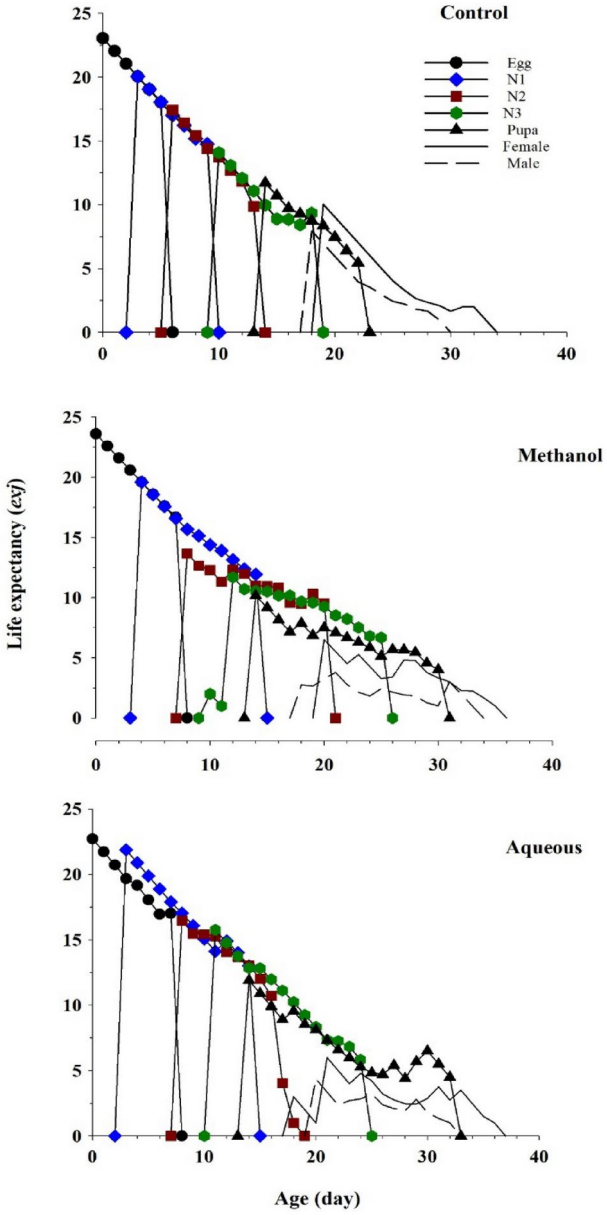


**Fig. 3**. Sublethal effects of methanolic and aqueous extracts of *Acroptilon repens* on age-stage life expectancy ($e_{xj}$) of *Trialeurodes vaporariorum*.

toxicity of *T. vaporariorum*, it is not found in previous studies. Ma et al.[38] isolated and identified five insecticidal compounds from *Cephalotaxus sinensis* essential oil, in which caryophyllene oxide was one of the main active constituents exhibited potent contact and fumigant activity against broad spectrum agricultural pests (e.g., *Megoura japonica* Okamoto & Takahashi, *Sitophilus zeamais* Motschulsky and *Plutella xylostella* (Linnaeus). According to our obtained results, it is surprising that the aqueous extract of *A. repens* was more active and toxic than the methanolic extract of Russian knapweed. It may be due to the chemical structure of sesquiterpenes. Wang et al.[20] using immersion method, demonstrated that the existence of ethyl acetate and petroleum ether fraction from the extract of *A. repens* in florescence stage of whole plant had very strong contact toxicity against fifth instar larva of *M. separate*.

Secondary plant metabolites cause toxic effects that can be observed at either lethal or sublethal levels, which can ultimately lead to the death of insect pests[32]. The results of previous investigations evaluated the short-term toxicity of *A. repens* and there is no documents regarding the sublethal effects of Russian knapweed against any insect pests in long term. Hence, it is essential to obtain a comprehensive perception, regards the ecotoxicological effects of Russian knapweed in the management of insect pests' population[3,25]. Our research showed that, both sublethal concentration of aqueous and methanolic extracts of Russian knapweed led to delay in incubation period of eggs, extending the developmental time, prolonged the preadult oviposition period, reduction of survival rate and fecundity compared to control group. It may be due to the antifeedant

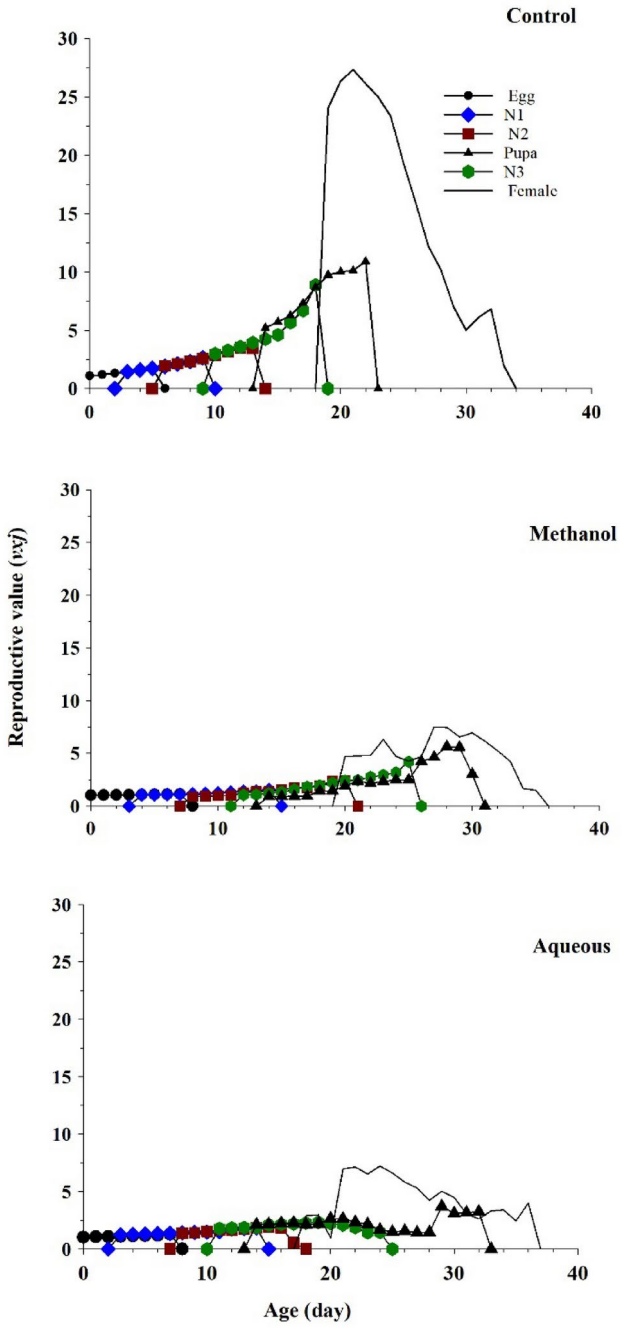


**Fig. 4**. Sublethal effects of methanolic and aqueous extracts of Acroptilon repens on age-stage-specific reproductive value (vxj) of Trialeurodes vaporariorum.

and repellency effects of phytochemical compounds of *A. repens*, which in turn cause disruption in cellular and physiological processes in exposed individuals of insect pest by altering the hormonal regulation, neuronal signalization or reproduction[3,22,25,32]. Similarly, Sharifiyan et al.[2] reported the same sublethal biological effects of *M. pulegium* essential oil and its nanoformulation against greenhouse whitefly.

Our results demonstrated that exposure of the adults to sublethal concentrations of both used extracts had noticeable effects on the population growth parameters by reducing the net reproductive rates ($R_0$), the intrinsic rates of increase ($r$), the finite rates of increase ($\lambda$). However the methanolic extract of *A. repens* prolonged the mean generation time of the greenhouse whitefly progeny more than the aqueous extract, then followed by the control group.The sublethal effects of *A. repens* on the aforementioned population growth parameters in this research was more effective than *M. pulegium* and its nanofrmulation on the adults of *T. vaporariorum*[2]. This variation could be due to differences in the bioassay method, the plant species and the chemical structure of the used essential oil, the exposure time of insects, as well[2,28]. Surprisingly, in the current study, both studied extracts of Russian knapweed were more effective than even those of flonicamide as a chemical insecticide and

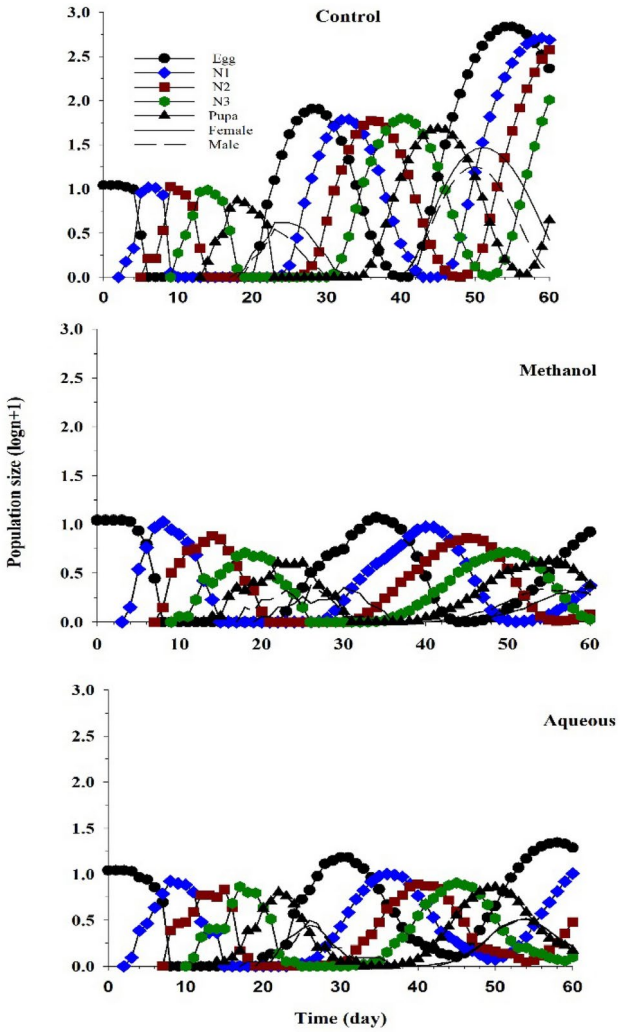


**Fig. 5**. Projection of population growth potential and stage structure of *Trialeurodes vaporariorum* treated with $LC_{25}$ of methanolic and aqueous extracts of *Acroptilon repens* in comparison with control during 60 days.

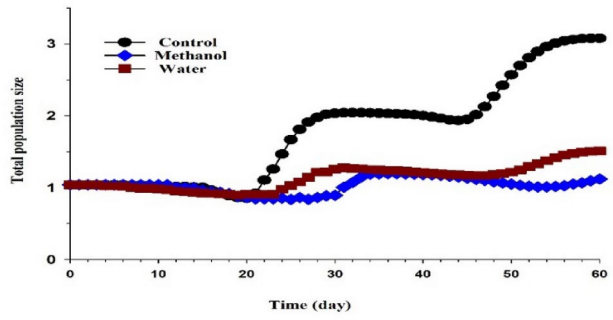


**Fig. 6**. Population projection of *Trialeurodes vaporariorum* (total stage) treated with $LC_{25}$ of methanolic and aqueous extracts of *Acroptilon repens* in comparison with control treatment during 60 days.

*Sophora flavescens* on population dynamics of greenhouse whitefly[3], which shows that it could be consider in management of *T. vaporariorum*.

We also used the obtained life table results to illustrate the dynamics of stage structure and variability of population growth whitefly based on a computer projection program[39]. The results showed that population growth of the greenhouse whitefly in different treatments was exactly in consistent with the estimated population growth parameters. Therefore, the population projection results would be a good evidence for the population

growth rate of the progeny whose parents were exposed to the sublethal concentration of *A. repens* extracts. For example, insects treated with methanolic extract of Russian knapweed, had slower growth rate and, thereby prolonged mean generation time (*T*).

In conclusion, the sublethal effects of methanolic and aqueous extracts of Russian knapweed against *T. vaporariorum* were reported in the current study for the first time. Based on the obtained $LC_{25}$ and $LC_{50}$ values, either aqueous or methanolic extracts of *A. repens* have the potential to be developed as an active plant natural insecticide for the control of greenhouse whitefly. However, due to the cost-effective being of aqueous extract it could be more considered in pest management programs. Further study should be conducted to determine the side effects of *A. repens* on the greenhouse whiteflies' enemies and its nanoformulated efficacy against *T. vaporariorum* in greenhouse and open field, as well.

## Material and methods

### Sample collection

Aerial parts of *A. repens* was collected from Vaqasluy-e Sofla (45° 1′ E, 37° 39′ N), West Azerbaijan, Iran during the summer season 2021. The identification of *A. repens* was occurred by Dr. Heydari, and deposited at the herbarium at Agricultural Research, Education and Extension Organization (AREEO), West Azerbaijan, Urmia, Iran with voucher specimen 106606.

### Sample preparation for GC–MS analysis and identification of chemical constituents of *A. repens*

The plants were dried at room temperature until they were crispy. Then, they were powdered entirely by an electric mill and stored in a dark glass container in a refrigerator. Then, they were subjected to hydrodistillation. The eextraction was done using a modified Clevenger-type apparatus. Five hundred fifty grams of the powdered plant was added in 500 ml of distilled water and the extraction processes was continued for 4 h. After extraction, anhydrous sodium sulfate was used to dehydrate the EO. The isolated oils were poured in darkness vials and stored at 4 °C in a refrigerator. Analysis of chemical constituents of *A. repens* were performed phytochemistry laboratory in Research Institute of Forests and Rangelands, Tehran, Iran. The identification of *A. repens* was performed on an Agilent 7890A/5975C GC–MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was programmed as follows: The initial temperature of 60 °C was increased immediately to 220 °C at a rate of 3 °C/min, subsequently the temperature was increased to 260 °C at 20 °C/min and held in this temperature for 5 min. The injector and transfer line temperatures were 260 °C and 280 °C, respectively. Helium gas was selected as a carrier gas with linear velocity of 30.6 cm/s; split ratio 1:100; ionization energy 70 eV, scan times 1s; and mass range 40–300 a.m.u.

### Measurement of some secondary metabolites

To sample preparation of flavonoids and phenols, we used the method of Golizadeh et al.[40] with a slight modification. Briefly, 0.5 g of milled sample were dissolved in 5 ml of 80% methanol, then extracted using an ultrasonic device at a temperature of 30 °C for 30 min. After that, they were centrifuged at 10,000 × g for 15 min and 4 °C. The supernatant was used for the measurement of flavonoid and polyphenol content.

The flavonoid content of *A. repens* extracts was determined according to the procedure of Chang et al.[41] with some modifications using Quercetin as a standard (0.627–10 mg $ml^{-1}$). Briefly, 15 µl of extracted sample of *A. repens*, 500 µl of 10% Aluminium chloride ($AlCl_3$), and 500 µl of Potassium acetate 1M were dissolved in 1.4 ml distilled aqueous and incubated in darkness at room temperature for 30 min, then centrifuged at 10,000 × g for 10 min. The absorbance was read at 415 nm using a spectrophotometer (UV-2100).

Folin-Ciocalteu and Gallic acid (0.627–10 mg $ml^{-1}$) were used as reagent and standard, respectively to measure polyphenol content of *A. repens*, as described by Slinkard and Singleton[42]. Briefly, 5 µl of extracted sample of *A. repens*, 1200 µl of 10% Folin-Ciocalteu reagent, 960 µl 7% Sodium carbonate ($Na_2CO_3$), were dissolved in 180 µl distilled aqueous and incubated in darkness at room temperature for 30 min, then centrifuged at 10,000 × g for 10 min. The absorbance was measured at 760 nm for polyphenol using a spectrophotometer. Three technical replications were carried out for each treatment.

To measure alkaloid content of *A. repens*, Boromocresole green and Hyoscyamine (0.4–1.2 mg $ml^{-1}$) were used as reagent and standard, respectively[43]. Briefly, 1 g of milled sample was dissolved in 20 ml of 80% methanol, then shake on shaker for 24 h. After that, it was filtered with filter paper. The evaporation of methanol was conducted using a rotary at 45 °C in a dark condition. Next, the concentrated sample was dissolved in 1ml Hydrogen chloride (HCl 2N) and filtered. After that, 1 ml of the obtained sample was washed in 10 ml Chloroform (pH = 7, NaOH 0.1N). Finally, 5 ml Boromocresole green reagent, 5ml Phosphate buffer (pH = 4.7) and 4ml Chloroform were added to the aforementioned solution and shake well. The absorbance was measured at 470 nm for alkaloids using a spectrophotometer (Halo-DB-20 UV visible). Three technical replications were carried out for each treatment.

### Aqueous and methanolic extraction to study lethal and sublethal

According to Samareh-Fekri[22], the immersion method was used to obtain the methanolic and aqueous extracts of *A. repens*. In this method, 50 g of the powdered plant was soaked in 300 ml of solvents (aqueous and 80% methanol) and placed on a shaker at room temperature for 48 h, then the obtained extracts were passed through filter paper twice and concentrated by a rotary vacuum distillation machine at 40 °C with speed of 100 rpm. The resulting concentrated liquid was spread on a watch glass and placed in a dark place until the solvent was removed entirely, and the extract was obtained as a powder. Concentrated extracts were stored in dark-colored lidded jars inside the refrigerator at 4 °C.

## Host plant breeding and insect rearing

The host plant used in this study, was tomato (variety Sun-seed 6189) which obtained from the Plant and Seed Modification Research Institute (Urmia, Iran). Tomato seeds were grown in plastic seedling trays (29 mm in height and 42 mm in diameter) containing a mixture of manure, peat moss, and vermiculite (in a ratio of 1:1:1) in a greenhouse. Finally, the seedlings (6–8 leaflets) were transferred into plastic pots (15 × 20 cm).

The rearing colony of *T. vaporariorum* was performed by gathering the adults of greenhouse whitefly using aspirator on tomatoes from the greenhouse of Department of Plant Protection, Urmia University, Urmia, Iran. The adults were released into cages (80 × 80 × 100 cm) on tomato pots. To obtain the same-aged greenhouse whitefly, the released adults were removed after 24 h. The colony was monitored until they reached the desired stage for carrying out the bioassays. The insect rearing was done for three generations prior to bioassay tests and demographic studies. The colony was kept in a growth chamber at 25 ± 2 °C and 60 ± 10% RH at a photoperiod of 16: 8 h (L:D).

## Dose-mortality response bioassay to obtain the $LC_{50}$ and $LC_{25}$ of both aqueous and methanolic extracts of Russian knapweed

To obtain dose-mortality response for adult stage of greenhouse whitefly, we followed the bioassay methods described by Reshadat-Salvanagh et al.[3] with some modifications. Five final concentrations in the ranges of (39.06–2500 and 312–7000 ppm) were used for aqueous and methanolic extracts, respectively, which were obtained based on preliminary tests causing the mortality range between 10 and 90%. The leaf dipping method was used in bioassay tests. For this purpose, the tomato leaves were dipped in 500 ml of each aforementioned concentration of aqueous and methanolic extracts of *A. repens* for 10 s and the treated leaves were air-dried for 10 min at room temperature, then put into the ventilated plastic Petri dishes (8 cm in diameter). Next, 15 adults of greenhouse whiteflies released onto the treated leaves. Distilled aqueous was used as a control. Four replicates were set up for each treatment and control. The number of dead adults were counted 24 h after treatment. The basal ends of the tomato leaflets were wrapped in moistened cotton wicks, to avoid the desiccation of the leaves. The lethal ($LC_{50}$) and sublethal ($LC_{25}$) concentrations, and their 95% confidence limits in each treatment were obtained using the Probit analysis[44] which conducted by SPSS software (Ver. 20, 2011). The bioassay test was conducted in a growth chamber at 25 ± 2 °C and 60 ± 10% RH at a photoperiod of 16: 8 h (L:D).

## Sublethal effects ($LC_{25}$) of aqueous and methanolic extracts of *A. repens* on demographic parameters of *T. vaporariorum*

The sublethal effects of methanolic (1221.788 ppm) and aqueous extraction (904.36 ppm) were studied on the life history and population growth rate of *T. vaporariorum*, which was carried out according to Reshadat-Salvanagh et al.[3] method with some modifications. Briefly, tomato leaflets were dipped in aforementioned sublethal concentrations of treatments for 10 s and transferred into Petri dishes (8 cm diameter), then 150 numbers of the same-aged adults of *T. vaporariorum* were released on treated leaves for each treatment (50 numbers of adult with three replicates). After 24 h, all alive adults were separated with aspirator and released on untreated tomato-pots and allowed them to laid their eggs for 24 h, then the adults were removed. To demographic and life table studies, 75, 84, and 73 the same-aged of their eggs were used as a cohort for control, aqueous and methanolic extract of *A. repens*, respectively. Due to the some biological properties of greenhouse whitefly (eggs attachment on the surface of the leaves and slow movement behavior of pre-imaginal stages), and to avoid any hurt to eggs during transferring, the leaves containing one egg were preserved on tomato pots. Then, they were bounded by the clip cages and, the incubation period, developmental period and survival of preadult stages were recorded for each individual daily. Without any manipulating of the old leaf which containing the preimaginal stages of greenhouse whitefly, the new and fresh leaf was placed on the underside of the old leaf so that the insect would move to the lower leaf automatically. After adult emergence, they were differentiated sexually by Gerling and Sinai[45] method. Each pair was transferred in a transparent plastic container (5 cm in diameter × 7 cm in height) containing tomato leave. The total pre-reproductive period (TPRP), adult pre-reproductive period (APRP), the number of eggs produced, number of reproductive days, adult longevity (in days), and total lifespan of the progeny of the treated parents were recorded daily. The experimental conditions were used as the same conditions which mentioned earlier.

## Data analysis

The comparison between methanolic extract of *A. repens* and control in terms of flavonoid, phenols, and alkaloid contents was performed by T-test at the 95% probability level using (SPSS, Ver. 20, 2011). The life history data of the greenhouse whitefly were analyzed according to the age-stage, two-sex life table theory[46,47] using TWOSEX–MSChart computer program[48]. According to Efron and Tibshirani[49] method, the variance and standard errors of the development time, reproduction time, fecundity, and population parameters were analyzed via a the bootstrap approach with 100,000 replicates. To assign the differences among treatments, we used the paired bootstrap test at a 5% significance level based on the confidence interval of differences. Bootstrap method and paired bootstrap test are included in the TWOSEX–MSChart of the computer program. Also, the life table data was used to project the population growth of *T. vaporariorum* using the computer program TIMING-MSChart[50] based on Chi[40] theory. Additionally, the life table's data based on the 0.025th and 0.975th bootstrap results of the net reproductive rate were used to obtain the uncertainty of population growth of *T. vaporariorum*[51].

## Data availability

All data generated or analyzed during this study are included in this published article.

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## Author contributions
S.S.S. performed the experiments, F.M. designed the experiments and wrote the manuscript, F.M. and M.F. analyzed the bioassay and life table data, and M.F. designed the secondary metabolite measurements.

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## Declarations

## Competing interests
The authors declare no competing interests.

## Additional information
**Correspondence** and requests for materials should be addressed to F.M.

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