#### **ORIGINAL ARTICLE**





# Effects of plant extracts and essential oils on the behavior of *Acrobasis advenella* (Zinck.) caterpillars and females

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#### Abstract

At present, *Acrobasis advenella* (Zinck.) (Lepidoptera, Pyralidae) is the most dangerous pest of black chokeberry (*Aronia melanocarpa* [Michx.] Elliot). Damaged flower buds may decrease yield, while pest feeding inside fruits causes deterioration in their quality. The aim of the study was to determine and compare the effect of water and acetone plants extracts and essential oils using free choice tests of feeding site of larvae and oviposition of females. The following species were examined: *Achillea millefolium*, *Cymbopogon citratus*, *Nepeta cataria*, *Origanum vulgare*, *Satureja hortensis*, *Tagetes patula nana*, *Tanacetum vulgare* and *Thymus vulgaris*. The results showed that plant species had a significant influence on the choice of oviposition and feeding site of *A. advenella*. Moreover, the type of formulation affected the number of laid eggs. Females did not lay eggs on infructescences treated with acetone extracts. Among the test preparations, the highest percentage larvae and adults of *A. advenella* was recorded for plants soaked in *C. citratus*, and therefore it can be a potential attractant for this pest species. The lowest number of eggs and larvae was observed for *T. vulgare*.

**Keywords** Botanical insecticides · Repellents · Attractants · Choice test · Cymbopogon citratus · Acrobasis advenella

#### Introduction

The introduction of chemical plant protection products into the natural environment results in the disruption of biological balance, and poses a great threat to human health, animals and the environment. They cause major damage to ecosystems and groundwater. In addition, chemical methods contribute to the destruction of useful fauna as well as contamination of agricultural products (Nicolopoulou-Stamati et al. 2016; Carvalho 2017). Due to the unfavorable effect of pesticides, non-chemical methods play an increasingly important role in pest control. The use of natural compounds in place of synthetic insecticides can reduce environmental pollution, and they are safe for human and animals health (Liao et al. 2017; Kunbhar et al. 2018). Plant extracts and

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essential oils have emerged as an excellent alternative to synthetic insecticides for the management of insect pests. Botanical insecticides are naturally occurring insecticides that are derived from plants and contain a range of bioactive chemicals (Isman 2000; Govindarajan et al. 2016; Sammour et al. 2018). Non-chemical insecticides can affect various insects in different ways, depending on physiological characteristics of insect species as well as the type of plant. Plant extracts and essential oils (EOs) show a wide range of action against insects: they can act as repellents, attractants or antifeedants; they also may inhibit respiration, hamper the identification of host plants, inhibit oviposition and decrease adult emergence by ovicidal and larvicidal effects (Sarwar and Salman 2015; El-Sheikh et al. 2016; Ali et al. 2017). Repellents are substances that deter insects through their sense of taste, smell or touch. They act locally or at a distance, deterring an arthropod from biting, flying to or landing on the plant. Generally, repellents form a barrier preventing insects from coming into contact with plant areas (Choochote et al. 2007; Nerio et al. 2010). Volatile plant secondary metabolites are detected by olfactory system employed by insects to locate suitable plants as hosts and to avoid unsuitable hosts, so they in combination with plant extracts can also act as attractants. It is a method of

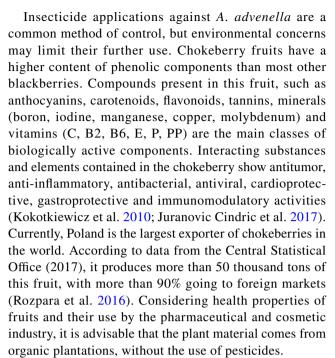


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luring and eventual chemical control in a specific area. The attractiveness of plant volatile compounds depends on insect species. The same substance can act as an attractant to one species and as a repellent in relation to another (Boczek et al. 2013). Therefore, their impact should be studied separately for each species of insect. Antifeedants are substances that partly or completely inhibit feeding or disrupt insect feeding, making the treated plants unattractive or unpalatable (Dancewicz et al. 2006). Kesdek et al. (2014) reported that acetone extracts of Achillea wilhelmsii C. Koch, Nepeta meyeri Benth., Satureja hortensis L., Origanum onites L., Origanum rotundifolium Boiss. and Tanacetum argyrophyllum (C. Koch) have a larvicidal effect on the second, third and fourth instar larvae of pine processionary moth, Thaumetopoea pityocampa (Den. and Schiff.). Wawrzyniak (2009) found that the water extracts from Geranium pratense L. and Geranium sanguineum L. protected cabbage plants against egg lying and showed negative influence on Pieris brassicae L. development causing high mortality of laid eggs. One of the most widely known plant species is Azadirachta indica A. Juss, which is an excellent source of potent plant insecticide used in pest control. According to many authors, this plant species had adverse effects on survival, fecundity, oviposition, feeding, larval, pupae and adults development, and also they greatly increased mortality of different species of pest insects (Charleston et al. 2006; Seljåsen and Meadow 2006; Schneider et al. 2017). Biopesticides possess multiple pest control properties; however, their mode of action has not been completely elucidated. For this reason, it is very important to use the appropriate dose, concentration and frequency of application of natural pesticides. In particular, there are fears of potential mutagenic or genotoxic adverse effects and negative effects on the human endocrine system. However, the most EOs and their main compounds have been reported to be not mutagenic or genotoxic (Bakkali et al. 2008).

Acrobasis advenella has been the biggest threat on black chokeberry plantations in recent years. It is a moth of the family Pyralidae. Larvae feed on plants of the family Rosacea, mainly the genera Crataegus L. and Sorbus L. (Slamka 1997). A. advenella is highly widespread in Europe, reaching as far as Russia eastward. The moth is spread throughout Poland. A. advenella has been described for the first time in 2004 as a pest of black chokeberry plantations in southeastern Poland (Górska-Drabik 2009). Since then, it has become a moth of the highest economic significance in all black chokeberry plantations. In the spring, larvae damage flower buds, negatively affecting yield quantity and quality. Moths appear at the end of June and remain until the first days of August. Females of A. advenella lay their eggs in the remnants of the calyx on immature fruits. When the larvae hatch, they bore into the fruits, forming shallow corridors (Górska-Drabik 2009, 2013).



In the available literature, there is no information about the natural preparations used to limit *A. advenella* population. The aim of this study was to determine the effect of water and acetone plant extracts and essential oils on the choice of feeding site of larvae and oviposition of *Acrobasis advenella*.

# **Materials and methods**

#### Insects

Larvae of *Acrobasis advenella* feeding in inflorescences were collected on a black chokeberry (Aronia melanocarpa [Michx.] Elliot) plantation in Samoklęski near Lublin (Poland) (51.4500°N–22.4333°E). The larvae were collected in the first half of May. They were then further reared in laboratory conditions in the Department of Plant Protection at the University of Life Sciences in Lublin (Poland). They were kept separately in Petri dishes (9 cm in diameter) and fed on chokeberry inflorescences. Feces were collected daily, and fresh inflorescences were also provided daily. After a few days, soil was added to ensure the best conditions for pupation. The adults were sexed and fed with a 20% honey solution. Part of the larvae was used for antifeedant experiments, and the rest were further reared for oviposition experiments. The experiment was conducted in the laboratory at  $22 \pm 1$  °C with  $70 \pm 5\%$  relative humidity and 16:8 h (L:D) photoperiod. The experiments were conducted from May to August 2016–2017.



# **Plant material**

The plants used in this study were purchased from DARY NATURY company (Grodzisk, Poland). The study used dry comminuted aerial parts of *Satureja hortensis* L. (summer savory), *Nepeta cataria* L. (catnip), *Achillea millefolium* L. (yarrow), *Origanum vulgare* L. (oregano), *Cymbopogon citratus* L. (lemon grass), *Thymus vulgaris* L. (thyme) and *Tanacetum vulgare* L. (tansy) and ornamental parts of *Tagetes patula nana* L. (marigold). Water and acetone extracts and essential oils were prepared from these plant species and called "type of diluent." Ornamental parts from *T. patula nana* were collected from cultivation locations from Kielce (south-central Poland) between June and August in 2015. Plants from *T. patula nana* were dried in the shade and then pulverized by manual grinding.

#### **Essential oil extraction**

Essential oils (EOs) were extracted from the aerial parts of *S. hortensis*, *N. cataria*, *A. millefolium*, *O. vulgare*, *C. citratus*, *Th. vulgaris* and *T. vulgare* by hydrodistillation using a Deryng apparatus for 4 h. The obtained oils were stored in amber bottles. For bioassays, essential oils (0.5 g with each EO) were prepared from stock solutions and distilled water with Tween 80 as an emulsifier (0.005%) and 2% (v/v) ethanol. The concentration of each essential oil applied to the insects during the assays was 0.1% (w/v). A mixture with the same composition, but without the essential oil was used as a control solution

# **Extracts preparation**

The experiments included water and acetone plants extracts obtained from the following plants: *S. hortensis*, *N. cataria*, *A. millefolium*, *O. vulgare*, *C. citratus*, *Th. vulgaris*, *T. vulgare* and *T. patula nana*. Ten grams of each type of dried plant material was soaked in 100 ml of distilled water. The mixtures were stored at room temperature in the dark for 24 h. After this period, they were filtered through (cotton fabric) a filter paper, thereby obtaining 10% aqueous extracts and immediately used to perform the experiments.

Acetone extracts were prepared by placing 10 g of each type of dried plant material in 100 ml of acetone for 24 h. Subsequently, the extract was filtered through a filter paper. Next, the mixtures were evaporated at a temperature not exceeding 40 °C to obtain 1/3 of the initial volume. Mixtures without plant materials served as controls.

#### **Oviposition assay**

For the choice test, nine or eight fresh black chokeberry infructescences were soaked for 30 s in an emulsion or

a control and dried at room temperature for 30 min. One infructescence consisted of ten individual immature fruits of black chokeberry. After drying, nine infructescences treated with water and acetone extracts and eight plants treated with essential oils were placed in insect rearing cages (31 cm $\times$ 21 cm $\times$ 21 cm) at the edges of the cage, which were covered with a mesh screen. The insect rearing cages were purchased from TRIXIE company (Kobylarnia, Poland). The infructescences were inserted into the floral foam to keep them fresh for as long as possible. One pair of newly emerged A. advenella adults (male and female) were released into each cage. The moths were fed with a 20% honey solution. Immature fruits were checked every day, and the number of eggs was recorded on each plant. Ten replicates (cages) were set up for each treatment. The test was carried out for 30 pairs (10 for each plant extracts and essential oil). The experiment was conducted starting late June and continuing until the first days of August, during the moths' flight season.

### Larval free choice test

Fresh and developing black chokeberry inflorescences were immersed for 30 s in plant extracts (water and acetone, respectively) and essential oils. The inflorescences were subsequently left to dry at room temperature for 30 min. The plants were then put in plastic, round insect boxes with a diameter of 15 cm. Filter paper was placed on the bottom to avoid drying. One larva was transferred into each box and allowed to feed on treated and control inflorescences. Each *A. advenella* larva was placed at the bottom center of each box. After 2, 5, 12 and 24 h, the inflorescences were inspected and the number of larvae on each inflorescence was recorded. The experiments were conducted on 30 larvae for four instars larvae ( $L_1$ ,  $L_2$ ,  $L_3$  and  $L_4$ ) and inflorescences soaked with water and acetone extracts, essential oils and controls (N=360).

# Statistical analysis

Statistical analyses were based on two-factor and three-factor analysis of variance models and Tukey's multiple T tests, at the assumed significance level of  $\alpha\!=\!0.05$ . ANOVA with Repeated Measures Analysis of Variance was used for data obtained from the measurements in subsequent time points (from the same objects) (data are correlated). Analysis of variance models with the main effects of the studied factors and their interactions with the final indication of the calculated probabilities related to the applied F test functions (F-Snedecor or Fisher-Snedecor) were applied. Tukey's multiple comparison HSD tests enabled detailed comparative analyses of means by separating statistically homogeneous medium groups (homogeneous groups). The



detailed data of the statistical analysis are presented in Table (Supplementary data).

#### Results

# Oviposition preference of *Acrobasis advenella* females

In total, during the experiment, *A. advenella* females laid 2751 eggs (average 137.55 eggs/female). No eggs were found on fruits treated with acetone extracts. Statistical analysis of the results did not confirm a significant difference in the number of laid eggs in relation to the diluent (aqueous extracts and essential oils) (Table: Supplementary data). There was also no interaction between the applied diluent and the plant species used for spraying the inflorescences. However, plant species had a significant impact

on the choice of females ( $F_{8.160} = 20.4910$ ;  $P = 1 \times 10^{-7}$ ). The highest number of eggs was laid by females on fruits treated with *C. citratus* (32.36%) (Fig. 1). A large number of them were also recorded on fruits sprayed with *O. vulgare* (19.68%), but this difference was not statistically significant compared to control (17.15%). Significantly fewer eggs were observed on fruits treated with other plant species. The lowest number of eggs was found on fruits treated with *T. vulgare* (2.43%) and *S. hortensis* (3.89%).

# Effects of essential oils and extracts on free choice test of *Acrobasis advenella* larvae

The results showed that plant species had a significant influence on the selection of feeding site by larvae  $(F_{7.192}=45.255; P=1\times10^{-7})$  (Fig. 2). Most larvae selected control inflorescences (27.35%), while from the remaining ones they chose *C. citratus* (25.68%) and *O. vulgare* 

Fig. 1 Mean percentage  $(\pm SE)$  of eggs laid by females of *Acrobasis advenella* on infructescences of *Aronia melanocarpa* treated with different species of plant extract (N=20). Bars marked by different letters are significantly different (Tukey's HSD test  $P \le 0.05$ )

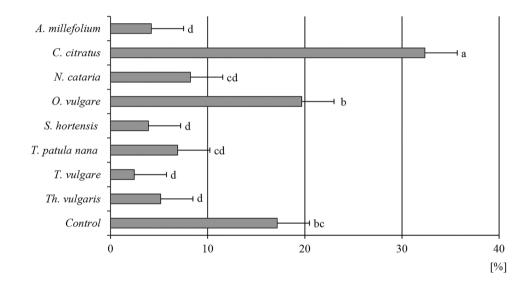
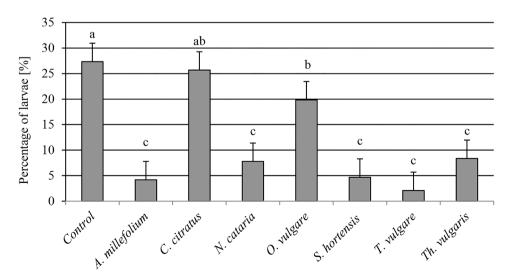


Fig. 2 Mean percentage ( $\pm$  SE) of *Acrobasis advenella* larvae on inflorescences of *Aronia melanocarpa* treated with different species of plant extract (N= 360). Bars marked by different letters are significantly different (Tukey's HSD test  $P \le 0.05$ )





(19.84%) most willingly. The lowest number of larvae was observed on inflorescences treated with T. vulgare (2.09%), A. millefolium (4.19%) and S. hortensis (4.69%). Statistical analyses did not show statistically significant differences in the selection of feeding site by particular larval stages ( $L_1$ – $L_4$ ) (Table: Supplementary data).

A.advenella larvae had the possibility to change the feeding site. At all time points, the number of larvae in nontreated inflorescences (control) and soaked in solutions of C. citratus and O. vulgare increased, reaching the highest value on C. citratus and control after 24 h (Fig. 3). The highest changes (increase or decrease) were most noticeable after 12 h from the beginning of the experiment (Fig. 3). After 2 and 5 h, the lowest number of larvae inhabited inflorescences soaked with T. vulgare and A. millefolium. After 12 h, all larvae left the inflorescences treated with T. vulgare, and several still inhabited the inflorescences soaked with S. hortensis and A. millefolium. Although part of the caterpillars changed their feeding sites during the experiment, statistical analysis did not confirm that time significantly influenced the choice of location by larvae, but multivariate statistical analysis showed that there are interactions between the species of plants used in the experiment and time  $(F_{21.576} = 33.528; P = 1 \times 10^{-7})$ . The experiments also showed that the type of diluent used (essential oil, water and acetone extract) had no effect on the choice of larvae (Table: Supplementary data).

#### Discussion

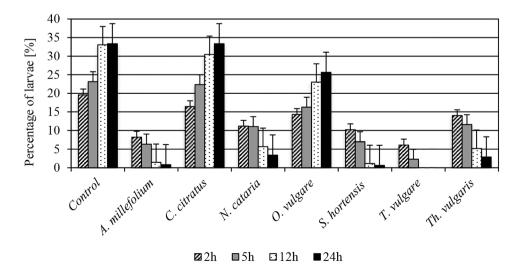
Essential oils (EOs) contain organic compounds that are plant secondary metabolites. Chemically, these substances are mixtures of compounds such as monoterpenes, sesquiterpenes, phenols, aldehydes, alcohols or other compounds (Olayemi 2017). Plant volatiles can contain many

components. Generally, oil is determined by one, two or three of its main active compounds, but it also happens that the residual oil components are important (Mossa 2016; Moghaddam and Mehdizadeh 2017). Chemical variations in EO composition are rather common even within the same species. Mainly, it depends on the type of genotype, plant organ, harvest, geographical region, season, plant nutritional status and climatic conditions—temperature, humidity and light intensity (Webster et al. 2010; Dhifi et al. 2016).

Volatiles emanating from plants play different ecological

and behavioral roles in the life of insects. They can contribute to indicate food, decide about mating sites and control reproduction processes; furthermore, they are involved in food selection and acceptance of individual plants or only their parts, but also plants that are inappropriate for adult or larval food. Female insects also use plant volatiles to select oviposition sites (Nansen and Phillips 2003; Borrero-Echeverry et al. 2018). Oviposition by females is particularly important for the survival of their progenies, mainly because the quality of food and its availability to larvae are determined by oviposition of the female on a suitable host plant (Honda 1995). Antifeedants inhibit insect feeding, through sensory perception, but they do not directly kill insects (Li et al. 2005). Female moths select plants for oviposition based on visual, olfactory and gustatory information (Fei et al. 2017). These cues that induce or inhibit oviposition play an important role in the survival of herbivorous insects, since hatching larvae have often restricted activity. Moreover, they play a role in host selection, because choosing oviposition site depends on the presence or absence of stimulants or deterrents (Renwick and Radke 1988; Renwick 1994). Essential oils of the family Lamiaceae, including, Satureja, Thymus and Origanum contain mainly aromatic monoterpenes, carvacrol, thymol and p-cymene (Tabari et al. 2015). Insecticidal and acaricidal activities are often attributed to these compounds (Bakkali et al. 2008; Senthil-Nathan

Fig. 3 Mean percentage (±SE) of larvae of *Acrobasis advenella* on inflorescences of *Aronia melanocarpa* treated with different species of plant extract after 2, 5, 12 and 24 h (N=360)





2015). According to Honda (1995), secondary compound from plants also stimulates or inhibits oviposition of females. The reduction in fecundity may be due to the direct effect of essential oils, disruption of reproductive behavior by compounds in EOs or a combination of these two processes on adults. Essential oils often have stronger insecticidal activity than any of their individual constituents (Tak et al. 2016). Kim et al. (2010) demonstrated that the essential oil from O. vulgare was more effective against Tribolium castaneum (Herbst) than carvacrol, i.e., its pure secondary metabolite. On the other hand, it has been observed that complex essential oil compounds are more effective than pure EOs (Bakkali et al. 2008). Our results showed that O. vulgare extracts and EOs were rather attractant both for the females and larvae of A. advenella. Thymol may contain up to 80% of the main compounds of thyme essential oils (Archana et al. 2011). For example, Tabari et al. (2015) reported that carvacrol and thymol showed strong acaricidal activity against Dermanyssus gallinae (De Geer). Park et al. (2017) demonstrated that thymol exhibited insecticidal activity against Pochazia shantungensis Chou & Lu adults and nymphs. In our experiment, by far the strongest repellent activity was shown by the tansy, but a low percentage of larvae were found on plants treated with extracts and oils from S. hortensis. Magierowicz et al. (2019) proved that carvacrol was the dominant substance in S. hortensis essential oil (73.24%). We have noted that they are characterized by insecticidal activity against A. advenella. S. hortensis essential oil reduced the occurrence of moths' longevity and increased mortality of larvae. Shahab-Ghayoor and Saeidi (2015) reported significant inhibition of Plodia interpunctella (Hübner) feeding even at low concentrations of the summer savory EO.

Essential oils and extracts belonging to plants of the families Poaceae and Lamiaceae are commonly used as insect repellents. Cymbopogon citratus is one of the most widely used natural repellents against various insect pests, but mainly it has been used to control mosquitoes and houseflies (Aidaross et al. 2005; Pushpanathan et al. 2006; Maia and Moore 2011). Lemon grass exerts both repellant and toxic effects against insects. Geranial (a-citral) and neral (b-citral) are the two main active components of lemon grass oil, but other compounds, which are often present in small amounts, such as geraniol and citronellol, are also known repellents (Baldacchino et al. 2013). Extracts from C. citratus exhibited high toxicity against Anopheles arabiensis Patton (Karunamoorthi and Ilango 2010). The repellent effect of C. citratus was also noted against Coleoptera insects, such as Sitophilus oryzae L. (Saljoqi et al. 2006) and Sitophilus zeamais Motschulsky (Parugrug and Roxas 2008). Rafeeg et al. (2016) also reported the repellent effect of C. citratus against Luprops tristis F., and the rate of this effect was higher with increasing oil dose. Hussein et al. (2015) observed that lemon grass extract and essential aromatic oil reduced the population of *Tuta absoluta* Povolny on tomato plants. In the available literature, we have not found information about attractiveness of C. citratus for insects. Our results showed that both adults and larvae of A. advenella definitely and preferably chose plants treated with lemon grass. Females laid the highest number of eggs on these plants, and they had the highest percentage of all A. advenella larval stages. The oldest larvae selected significantly more frequently inflorescences treated with C. citratus than controls, and therefore it could be a potential attractant for A. advenella. However, further studies are necessary to confirm the attractiveness of C. citratus EO in higher concentrations. Our results also showed that extracts and essential oils of O. vulgare were frequently chosen by A. advenella. However, this EO is also an effective repellent against pests (Yazdani et al. 2014; Nasr et al. 2017).

Studies by many authors have proved that plants of the Asteraceae family are a potential source of biopesticides with insecticidal properties (Umpiérrez et al. 2012; Czerniewicz et al. 2018). Extracts and essential oil from T. vulgare exhibit strong insecticidal and antifeedant effects (Panasiuk 1984; Schearer 1984; Hough-Goldstein and Hahn 1992). Mainly, these activities depend on their chemical composition. There are about 30 chemotypes of tansy in the world, but β-thujone is the main component of its essential oil (Kleine and Müller 2011). Szołyga et al. (2014) showed that T. vulgare EO and its main components,  $\alpha$ - and  $\beta$ -thujone inhibited growth, slowed development, and increased mortality of young larvae (10-day-old) of Alphitobius diaperinus Panzer. On the other hand, they also acted as strong attractants, but the younger larvae were more susceptible to all tests than the older ones. Many authors emphasize that larvae become less susceptible to essential oils and their active ingredients with age (Mondal and Khalequzzaman 2010; Szczepanik et al. 2012), but our results did not show statistically significant differences in the selection of feeding site by particular larval stages (L<sub>1</sub>-L<sub>4</sub>). In addition, during all time points of the experiment, we showed that the lowest number of A. advenella larvae was obtained for inflorescences treated with T. vulgare plant extracts. T. vulgare definitely had the strongest repellent effect for larvae and females, although the statistical analysis did not confirm this. Gabel and Thiery (1994) reported a similar result that T. vulgare EO had a deterrent effect on oviposition and it inhibited the mating behavior in the moth Lobesia botrana (Den. and Schiff.). They showed that egg laying was reduced by 30-80% and the longevity of adults was decreased. In addition, tansy essential oil had an oviposition deterrent effect on Choristoneura rosaceana (Harris) females (Larocque et al. 1999). According to Szołyga et al. (2014), stronger action of EOs may be the result of the dose applied. Hough-Goldstein (1990) tested an aqueous extract of T. vulgare against Colorado potato beetles in laboratory choice tests. Tansy showed



the most potent antifeedant effect compared to other test plants. The authors proved that the extracts deterred feeding by both larval and adult Colorado potato beetles (*Leptinotarsa decemlineata* Say). Moreover, Hough-Goldstein and Hahn (1992) reported that aqueous extract of tansy exerted antifeedant effects on *Plutella xylostella* L. larvae. Additionally, cabbage leaves treated with tansy extract caused longer growth of *P. xylostella* larvae. According to Ertürk et al. (2004), the alcohol extract of *T.vulgare* showed the strong deterrent effect against the larvae of *Yponomeuta malinellus* Zell., but it did not show toxicity effect against the pupae and larvae of *Y. malinellus*.

Previous studies have reported that the use of Asteraceae plants with repellent properties could be a useful method of reducing aphid populations. Halbert et al. (2009) found high repellency of *A. millefolium* essential oil against corn leaf aphids (*Rhopalosiphum maidis* Fitch). Czerniewicz et al. (2018) also demonstrated that repellent properties were enhanced with increasing EO concentrations. These authors showed that, among the used oils, *A. millefolium* oil had the highest settling inhibitory activity against *M. persicae*. Ebadollahi and Ashouri (2011) reported the essential oil from *A. millefolium* was highly effective against adults of *Plodia interpunctella* (Hübner), and the mortality values reached 100% after 48 h. Our results also showed that yarrow extracts had rather repellent properties and it was the second most rarely chosen plant species.

Research shows that the effect of compounds contained in essential oils is very diverse. Some substances can simultaneously attract and deter various insect species. Substances that occur in large quantities do not necessarily determine the acceptance of the insect. Biopesticides are a promising source of pest control compounds. They have aroused great interest in recent years as potential sources of natural insect control agents mainly due to the insects repelling properties. A thorough investigation of attractive components in plant volatiles would allow the development of effective plant protection products. Attractants would help in catching male and female moths, monitoring the population and eventually reducing the number of pests. The use of plant extract or EO as biopesticides can reduce damage to chokeberry by lowering the number of eggs laid and reducing A. advenella population. The application of natural pesticides can be combined with different biological methods in an integrated management strategy against A. advenella. This approach could also reduce the use of synthetic insecticides. However, further research is necessary to investigate the impact of extracts and essential oils of plant species that have the strongest effect on A. advenella in field conditions.

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# **Compliance with ethical standards**

Conflict of interest All authors declare that they have no conflict of interest.

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