REPELLENT AND INSECTICIDAL EFFECTS OF ESSENTIAL OILS ON COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* (SAY, 1824))

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Abstract. The Colorado potato beetle (*Leptinotarsa decemlineata* Say, 1824) is one of the most important pests of potatoes worldwide. Larvae and adults feed on the leaf mass, causing damage. Controlling the pest is much more difficult because there are no effective chemical protection agents and there is also the problem of resistance development. The aim of the work is to determine the reaction of adult individuals of the Colorado potato beetle to different types of essential oils and the effectiveness of essential oils on adult mortality. In 2022, adult forms were collected at two locations in untreated fields. Attractiveness/repellence was determined using an olfactometer and the efficacy of the essential oils on the adults using IRAC test method 007. The results showed that star anise essential oil has the highest efficacy while basil essential oil has the highest repellence, but there are no significant differences between the essential oils in determining attractiveness or repellence. Considering the problem of Colorado potato beetle resistance to synthetic chemical insecticides, essential oils have good properties and could be an efficient substitute for chemical insecticides.
Keywords: *resistance, biological control, star anise, basil, potato*

Introduction

The Colorado potato beetle (*Leptinotarsa decemlineata* Say, 1824) poses a significant threat to potato crops. The females lay eggs on the underside of the leaves. The rate of development of the eggs is highly dependent on temperature, with optimum growth occurring at around 25°C. The larvae pass through four stages and feed voraciously on the host plants. The defoliation caused by larvae and beetles can lead to varying degrees of yield loss. Studies show that some level of defoliation (up to 40%) can occur, resulting in lower yields and possibly crop destruction if not managed effectively (Coombs et al., 2003; Alyokhin and Atlihan, 2005; Huseth et al., 2014). After the larval stage, the beetles pupate in the soil. The adult beetles hatch and can go into diapause, a dormant state that improves survival in severe winters (Piiroinen et al., 2011). The Colorado potato beetle can be controlled with various protective measures. The most utilized control method is the application of chemical agents, i.e. insecticides. Throughout history, insecticides have been used extensively in all developed countries. Due to the frequent and inappropriate use of insecticides in pest control, problems have arisen due to the development of resistance. There are more than 300 proven cases of resistance to 56 active ingredients worldwide from the groups of organophosphates, pyrethroids, neonicotinoids, spinosyns insecticides against Colorado potato beetle (Bažok et al., 2017). As a result, farmers are worried because they cannot find high-quality products and the resistance problem has significantly affected their potato yields and quality. The constant use of pesticides around the world also leads to environmental pollution and therefore damage to human health. In the face of these control methods,

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farmers are concerned about the control of the Colorado potato beetle and, in turn, consumers are concerned about human health. As a result of these problems, farmers are forced to use ecological and efficient methods. Effective protection has become a major challenge in recent years, mainly because harmful species have already become resistant to certain preparations. New control methods and technologies are needed and have been discussed (Laznik et al., 2010; Göldel et al., 2020; Kadoić Balaško et al., 2020). Biological agents are a suitable substitute for synthetic chemical pesticides, mainly because of their lower negative impact on human health and environmental pollution (Mohan et al., 2011; Saroukolai et al., 2014). One of the most promising control methods are the naturally occurring substances that have been used throughout history in human medicine and to repel harmful organisms (Isman, 2006). More acceptable methods of potato cultivation and the Colorado potato beetle are emphasizing the use of biopesticides. These agents include essential oils, natural complex secondary metabolites with low efficacy on beneficial insects and with repellent and insecticidal properties, for which they are considered safe biopesticides.

In the last 25 years, hundreds of isolated metabolites showing repellent and insecticidal effects on insects have been described in the scientific literature; however, natural insecticides based on essential oils are only the subject of few researches (Isman, 2006; Mahdi et al., 2011; Rafiee-Dastjerdi et al., 2013; Sablon et al., 2013; Ropek and Kołodziejczyk, 2019). Essential oils can act as repellents, fumigants and insecticides (Shaaya et al., 1997). According to their chemical composition, essential oils are classified into monoterpenes (hydrocarbons and oxygenated derivatives) and sesquiterpenes (hydrocarbons and oxygenated derivatives), while aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) are responsible for characteristic odors (Isman et al., 2001; Kim and Ahn, 2001; Kordali et al., 2007). The chemical properties depend mainly on the host plant, i.e. the leaves, the color of the flower and the origin of the plant itself. Plant-based preparations do not have a long-term effect like chemical preparations, which is why the plants should be treated more frequently (Sajjadi, 2006).

The aim of this research is to determine the reaction of the adult forms of the Colorado potato beetle to different types of essential oils and their effectiveness on the mortality of the adult forms.

Material and methods

Adult forms of the Colorado potato beetle were used for the study, which were collected at two locations. The beetles were collected by hand every day in the morning and evening on the untreated potato plants.

Six types of essential oils were used for the study: Star anise (*Illicium verum* L.), basil (*Ocimum basilicum* L.), eucalyptus (*Eucalyptus globulus* L.), lavender (*Lavandula angustifolia* L.), lemon (*Citrus limonum* L.) and peppermint (*Mentha spicata* L.).

The attraction of the essential oils to the adult forms of the Colorado potato beetle was determined in three replicates using a six-channel olfactometer (Andrieu, 2013). Before and after each replicate, the arena of the olfactometer was cleaned with alcohol to avoid the influence of previously tested individuals, and new adult beetles were used for each replicate. For each replicate, 10 adult forms of Colorado potato beetles were used and released into the arena for 60 s at an air flow rate of 0.9 l/min. The essential oils were prepared by dropping five drops from the original packaging onto a filter

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paper, which was then placed in a container and sealed with the lid. An empty container with no odor source served as a control. The results were recorded visually after a period of 60 s. The number of adult Colorado potato beetles was recorded after each repetition for each variant at the odor sources of the olfactometer. The number of adult forms of Colorado potato beetles was recorded after each replicate for each variant at the odor sources of the olfactometer device. It was then determined which oil had a greater attraction. The greater attractiveness was determined with a larger number of individuals at a particular odor source.

The study of the effectiveness of essential oils on the adult forms of the Colorado potato beetle was carried out according to the modified method IRAC No. 007 and Inđić et al. (2013) by dipping potato leaves for insect feeding in solutions of essential oils. The experiment was conducted in three replicates, using ten adult forms of Colorado potato beetle in each replicate. The experiment was carried out with six previously mentioned types of essential oils at three concentrations of 2%, 2.5% and 3% mixed with acetone. Whole undamaged and untreated potato leaves were used for the experiment. Each leaf was immersed in a solution of essential oil and acetone and left for a few seconds to soak up the same solution, then placed on a grid to dry. The dried leaf was placed on a filter paper at the bottom of the Petri dish in the center, and ten beetles were placed on it, after which the Petri dish was sealed. Each Petri dish was labeled with a waterproof marker on the lid with the type of essential oil, concentration and number of replicates. The control was performed by dipping the leaf in pure acetone, drying it and placing it in a Petri dish on filter paper at the bottom. The recording of the experiment was carried out on three consecutive days, i.e. 24, 48 and 72 h after the start of the experiment. For each day after treatment, all adult forms of Colorado potato beetle in each Petri dish were examined. Dead and live adult beetles were recorded for each experimental variant. A live individual is one that responds to a specific movement stimulus with a coordinated step, while a dead individual is one that shows no response to a specific touch stimulus (IRAC, 2009).

Based on the number of dead and live adult forms of Colorado potato beetles when the petri dishes were examined, the efficiency was calculated for each variant and for each replicate. The mortality of the control was calculated according to the Schneider-Orelli formula (1947):

$$\text{corrected \%} = \left(\frac{\text{mortality \% in treated plot - mortality \% in control plot}}{\text{100 - mortality \% in control plot}}\right) \times 100$$

The data on the efficacy of the essential oils used on the adult forms of Colorado potato beetle were statistically analyzed by one-way analysis of variance, and the mean values were compared with the Tukey rank test using ARM software (GDM Solutions, 2024).

Results and discussion

Research on the attraction/repellency of essential oils

The proportion of adult forms of the Colorado potato beetle depending on the attractiveness of the essential oils used is shown in *Figure 1*.

The results show very small differences in the attractiveness of the essential oils used for the adult forms of the Colorado potato beetle. The highest attractiveness was found

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for lemon essential oil (12.20%), followed by lavender (10.50%), star anise (10.23%), eucalyptus (10.23%) and peppermint (10.20%) essential oils, while basil essential oil attracted the lowest number of adult forms of Colorado potato beetle (9.33%).

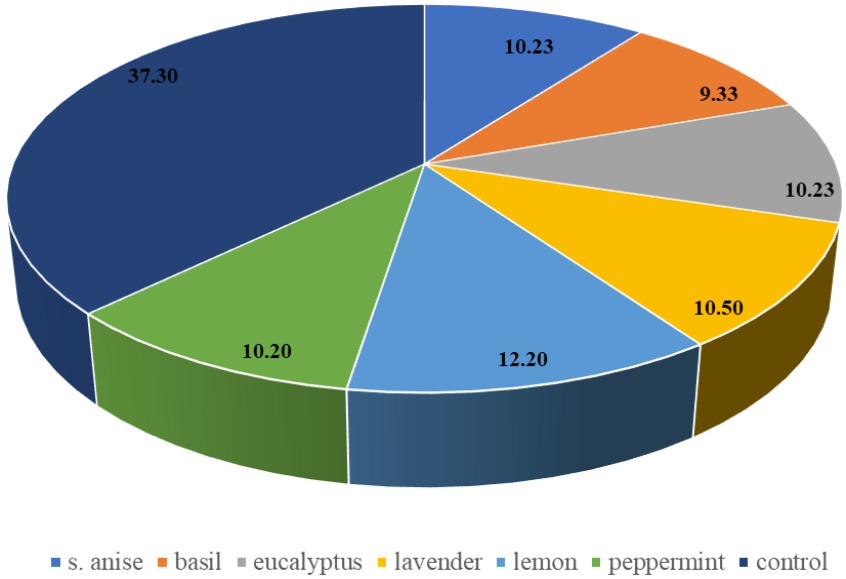

Figure 1. *Attractiveness of essential oils for adult forms of the Colorado potato beetle (percentage of adult forms of the Colorado potato beetle toward each essential oil odor, determined by olfactometer)*

Research on the efficacy of essential oils

The results of the analysis of variance performed to test the differences between treatments in the efficacy of a 2% concentration of essential oils on the adult forms of Colorado potato beetle 24 h after treatment are shown in *Figure 2*, 48 h after treatment are shown in *Figure 3* and 72 h after treatment are shown in *Figure 4*.

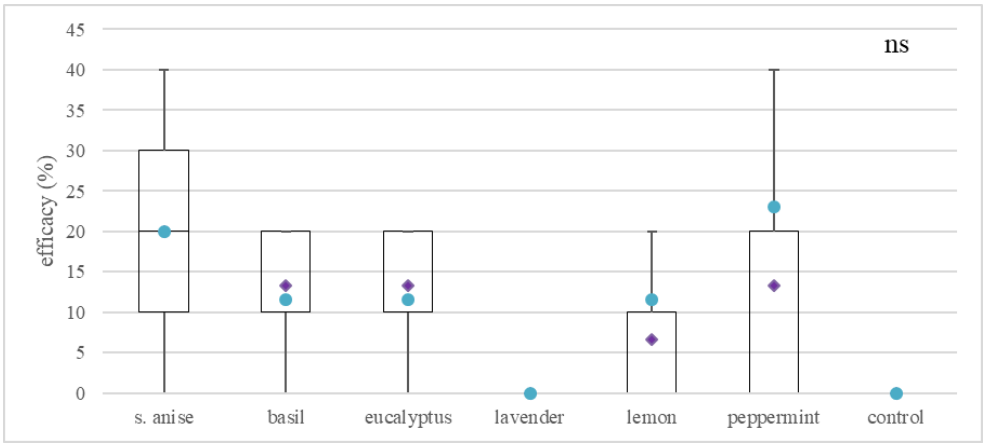

Figure 2. *Results of the analysis of variance for the efficacy (%) of a 2% concentration of essential oils 24 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are given; the center line indicates the median, the diamond indicates the mean and the circle the standard deviation; ns – no statistically significant differences between treatments)*

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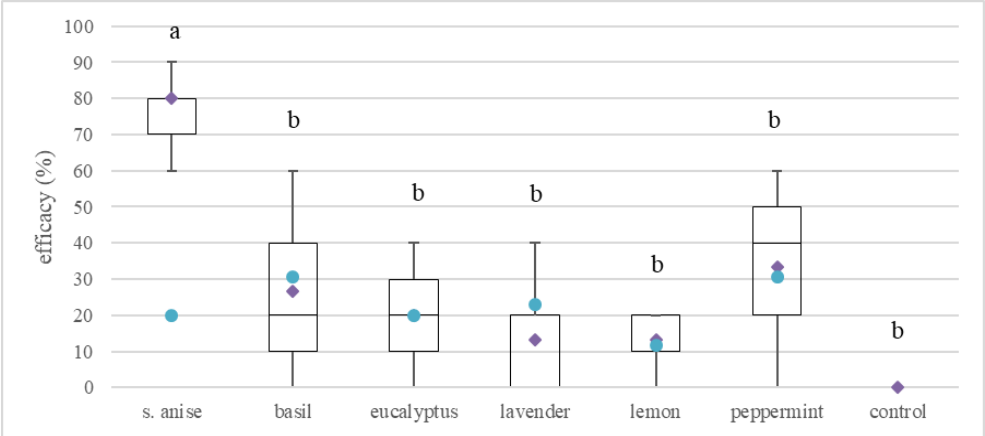

Figure 3. *Results of the analysis of variance for the efficacy (%) of a 2% concentration of essential oils 48 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are indicated; the center line indicates the median, the diamond indicates the mean and the circle indicates the standard deviation; ranks not connected by the same letter are significantly different)*

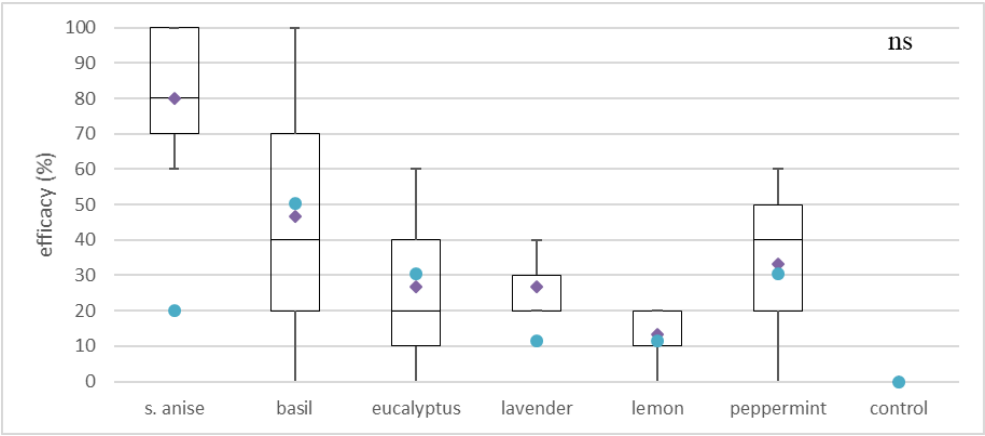

Figure 4. *Results of the analysis of variance for the efficacy (%) of a 2% concentration of essential oils 72 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are given; the center line indicates the median, the diamond indicates the mean and the circle the standard deviation; ns – no statistically significant differences between treatments)*

Statistical analysis of the data to test the differences between treatments in the efficacy of a 2% concentration of essential oils on the adult forms of Colorado potato beetle 24 and 72 h after treatment revealed no significant differences. Statistical analysis of the data to test for differences between treatments in the efficacy of a 2% concentration of essential oils on the adult forms of Colorado potato beetle 48 h after treatment revealed a significant difference between treatments ($F = 4.038$; $P < 0.0001$).

The results of the analysis of variance performed to test the differences between treatments in the efficacy of a 2.5% concentration of essential oils on the adult forms of Colorado potato beetle 24 h after treatment are shown in *Figure 5*, 48 h after treatment are shown in *Figure 6* and 72 h after treatment are shown in *Figure 7*.

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Statistical analysis of the data to test the differences between treatments in the efficacy of a 2.5% concentration of essential oils on the adult forms of Colorado potato beetle 24 h after treatment revealed no significant differences. Statistical analysis of the data to test for differences between treatments in the efficacy of a 2.5% concentration of essential oils on the adult forms of Colorado potato beetle 48 h after treatment revealed a significant difference between treatments ($F = 4.629$; $P < 0.0001$). Statistical analysis of the data to test for differences between treatments in the efficacy of a 2.5% concentration of essential oils on the adult forms of Colorado potato beetle 48 h after treatment revealed a significant difference between treatments ($F = 8.286$; $P < 0.0001$).

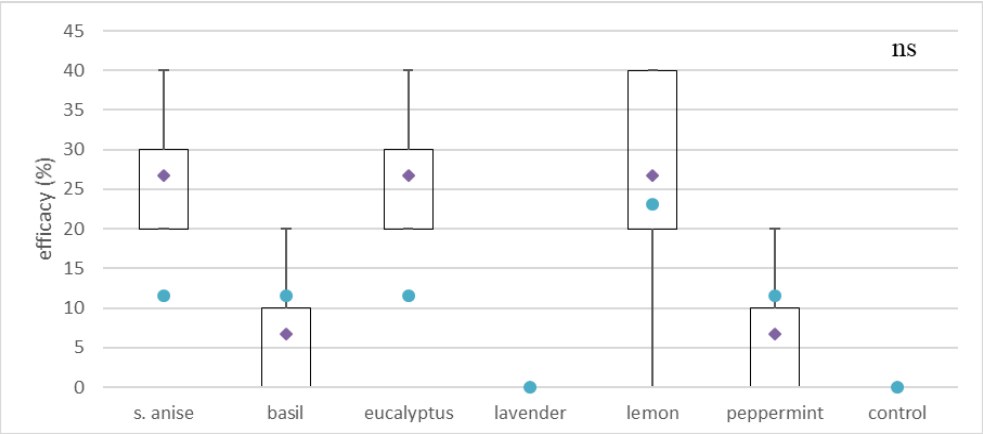

Figure 5. *Results of the analysis of variance for the efficacy (%) of a 2.5% concentration of essential oils 24 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are given; the center line indicates the median, the diamond indicates the mean and the circle the standard deviation; ns – no statistically significant differences between treatments)*

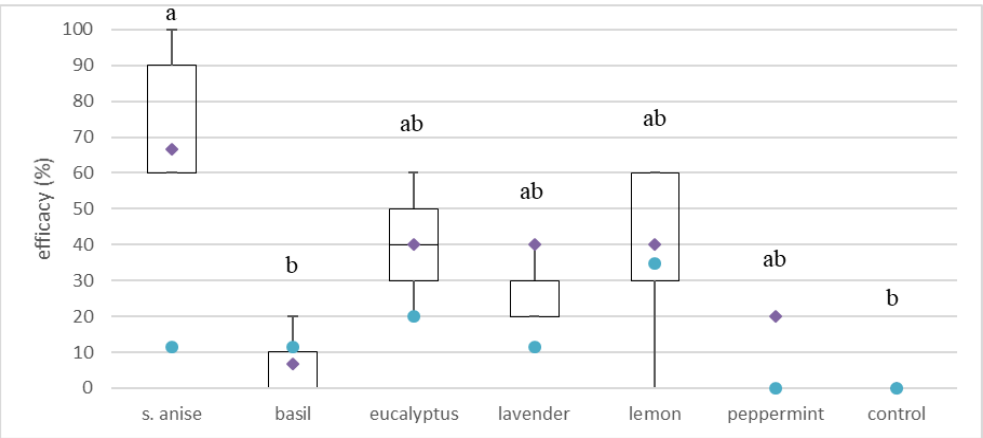

Figure 6. *Results of the analysis of variance for the efficacy (%) of a 2.5% concentration of essential oils 48 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are indicated; the center line indicates the median, the diamond indicates the mean and the circle indicates the standard deviation; ranks not connected by the same letter are significantly different)*

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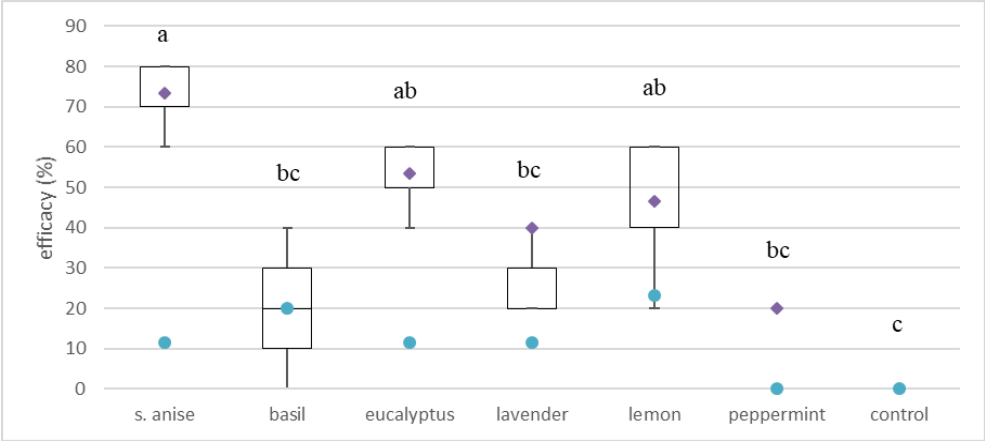

Figure 7. *Results of the analysis of variance for the efficacy (%) of a 2.5% concentration of essential oils 72 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are indicated; the center line indicates the median, the diamond indicates the mean and the circle indicates the standard deviation; ranks not connected by the same letter are significantly different)*

The results of the analysis of variance performed to test the differences between treatments in the efficacy of a 3% concentration of essential oils on the adult forms of Colorado potato beetle 24 h after treatment are shown in *Figure 8*, 48 h after treatment are shown in *Figure 9* and 72 h after treatment are shown in *Figure 10*.

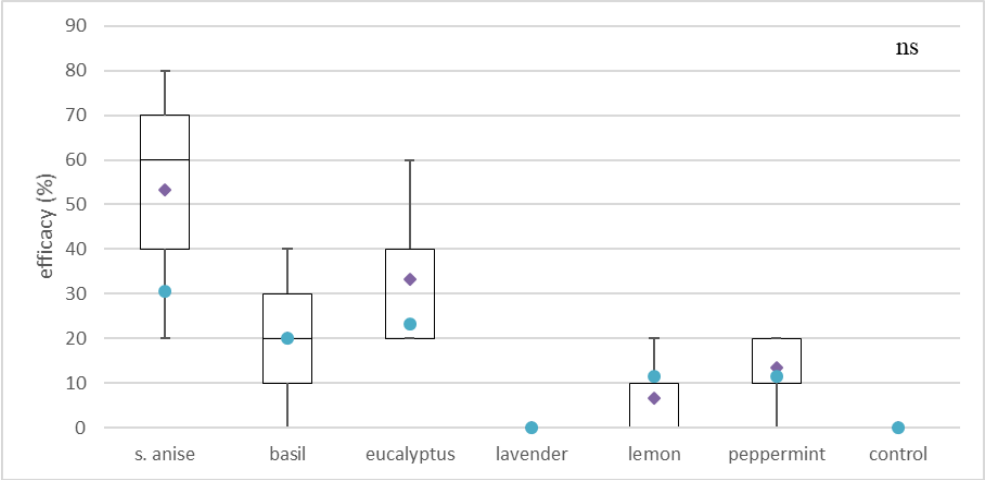

Figure 8. *Results of the analysis of variance for the efficacy (%) of a 3% concentration of essential oils 24 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are given; the center line indicates the median, the diamond indicates the mean and the circle the standard deviation; ns – no statistically significant differences between treatments)*

Statistical analysis of the data to test the differences between treatments in the efficacy of a 3% concentration of essential oils on the adult forms of Colorado potato beetle 24 h after treatment revealed no significant differences. Statistical analysis of the data to test for differences between treatments in the efficacy of a 3% concentration of

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essential oils on the adult forms of Colorado potato beetle 48 h after treatment revealed a significant difference between treatments ($F = 11.247$; $P < 0.0001$). Statistical analysis of the data to test for differences between treatments in the efficacy of a 3% concentration of essential oils on the adult forms of Colorado potato beetle 48 h after treatment revealed a significant difference between treatments ($F = 7.947$; $P < 0.0001$).

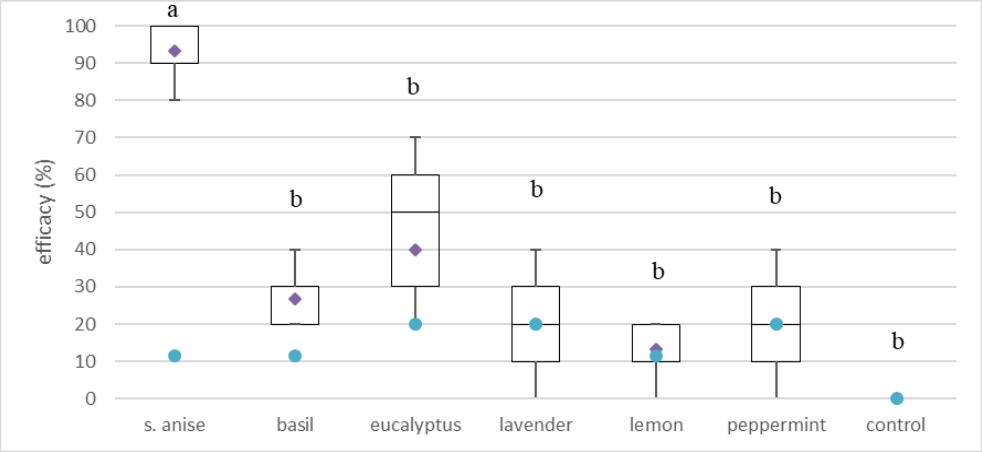

Figure 9. *Results of the analysis of variance for the efficacy (%) of a 3% concentration of essential oils 48 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are indicated; the center line indicates the median, the diamond indicates the mean and the circle indicates the standard deviation; ranks not connected by the same letter are significantly different)*

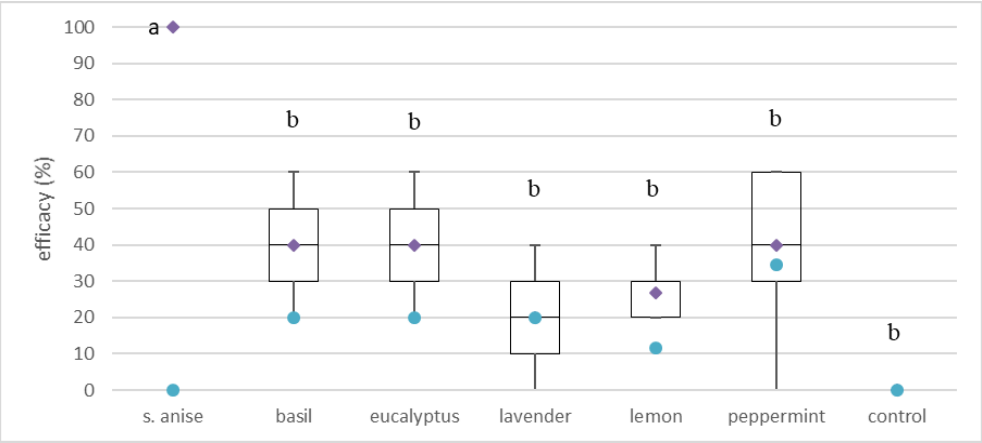

Figure 10. *Results of the analysis of variance for the efficacy (%) of a 3% concentration of essential oils 72 h after treatment on the adult forms (n = 210) of Colorado potato beetle (for each treatment, the upper and lower quartiles and the minimum and maximum values are indicated; the center line indicates the median, the diamond indicates the mean and the circle indicates the standard deviation; ranks not connected by the same letter are significantly different)*

According to the results obtained after 24 h at a concentration of 2% (*Fig. 2*), no significant differences were found between the efficacy of the essential oils. After 48 (*Fig. 3*) and 72 h (*Fig. 4*) at the same concentration, star anise essential oil showed the highest efficacy of 80%, followed by basil essential oil, which showed an efficacy

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of 26.67% after 48 h and 46.67% after 72 h, and peppermint essential oil, which showed the same efficacy of 33.33% after 48 and 72 h. Lavender essential oil showed no efficacy at all three concentrations (2, 2.5 and 3%), i.e. the efficacy was 0%. At a concentration of 2.5%, no significant efficacy of the essential oils was observed after 24 h (*Fig. 5*). After 48 and 72 h (*Figs. 6* and 7), star anise essential oil showed the highest efficacy compared to the other oils at 66.67. At a concentration of 3%, there was no significant difference between the effectiveness of the essential oils after 24 h (*Fig. 8*). After 48 h, star anise essential oil showed the highest efficacy of 93.33% (*Fig. 9*). After 72 h, star anise essential oil showed an efficacy of 100% (*Fig. 10*). The essential oils of basil, eucalyptus and peppermint also showed a significant efficacy of 40% after 72 h (*Fig. 10*), but star anise essential oil showed a significantly higher efficacy (from 66.67% to 100%) on adult forms of Colorado potato beetle.

The previous data on the chemical scents that attract adult forms of the Colorado potato beetle are based exclusively on substances secreted by the host plant. It is known that host plant volatiles attract the Colorado potato beetle and allow it to forage, causing considerable economic damage (Visser et al., 1979). There is very little data on the effect of essential oils on the attraction/repulsion of the Colorado potato beetle, which has great potential as one of the preventive measures in the integrated control of this pest. The attractiveness/repellency potential of essential oils can also be used in various forms of odor baits to prevent the transfer of pests to the crop. Although a very small difference in the attractiveness of essential oils to the adult forms of Colorado potato beetle was observed (*Fig. 1*), basil essential oil showed the greatest repellent potential, while lemon essential oil attracted the most adult forms. Lazarević et al. (2021) performed similar odor tests with the essential oils of two plant species from the Asteraceae family (*Tanacetum parthenium* L. and *Tanacetum vulgare* L.), but the tests were performed on larvae and there is a possibility that they react differently to the same volatiles than the adult forms. However, the results showed that the essential oil of *T. parthenium* at higher concentrations caused greater repellency than that of *T. vulgare*. The results suggest that essential oils have the potential to act as repellents or attractants for their odorants, which needs to be further explored.

The high efficiency of essential oils in insect pests control is demonstrated by their dual action as repellents and insecticides. Given their very favorable ecotoxicological properties and their wide range of applications, they are the subject of numerous studies (Farahani and Bandani, 2023; Sulg et al., 2023; Awad et al., 2024; Shan et al., 2024) as an alternative to synthetic chemical insecticides. The use of essential oils for Colorado potato beetle control is increasingly being investigated and is of interest to many researchers. The most effective are the essential oils of *Camphorosma monspeliaca* L. (Çakmak, 2023), *Camphora officinarum* Ness, *Tanacetum parthenium* (L.) Sch.Bip, *Tanacetum vulgare* L. (Lazarević et al., 2021), *Cymbopogon citratus* (DC.) Stapf (Ebadollahi et al., 2017) and *Pimpinella anisum* L. (Skuhrovec et al., 2017, 2019). From the results of this study, it is evident that the efficacy of the essential oils used depends on the concentration, which is also supported by the study by Lazarević et al. (2021). Star anise essential oil proved to be the most effective, showing 55% efficacy as early as 24 h after application, while at the end of the experiment (72 h after application) the mortality was total (100%). Similar results were obtained by Skuhrovec et al. (2017), although in their study they used the essential oil of anise (*Pimpinella anisum* L.); however, both species (anise and star anise) contain anethole, which could also have an insecticidal effect in addition to antimicrobial, antioxidant, hypoglycemic,

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hypolipidemic and estrogenic properties (Vecchio et al., 2016). After 24 h of treatment, all essential oils did not achieve a satisfactory insecticidal activity and no statistically significant differences were found between treatments, which was expected as essential oils have a slow and cumulative effect (Skuhravec et al., 2017). A very potential insecticidal effect could be provided by the essential oil of *C. citratus*, i.e. lemongrass, obtained by extraction from the leaves, which showed a remarkable efficacy of 95% on adult Colorado potato beetles 24 and 48 h after treatment (Sajjadi, 2006). The efficacy of essential oils depends not only on the concentration but also on the developmental stage of the pest; however, in this study, the efficacy of essential oils was tested only on adult forms. Considering that Colorado potato beetle larvae cause more damage, research should be extended to the larval and egg stage, which is confirmed by the results of Saroukolai et al. (2014), Skuhrovec et al. (2019), Lazarević et al. (2021) and Çakmak (2023).

Essential oils are an alternative to synthetic chemical insecticides, as certain essential oils are highly effective and have certain favorable ectotoxicological properties. However, essential oils also have a negative side, namely the effects on non-target organisms (natural enemies – predators and parasitoids), which raises the question of their safety for biodiversity (Danna et al., 2023; Sulg et al., 2023). In addition, questions remain about the use of essential oils under field conditions, considering the very rapid loss of stability (Maurya et al., 2021), the effects on host plants and the application problems (Singh et al., 2021) in agricultural production. Therefore, further research is needed to ensure the use of essential oils in practice.

Conclusion

In this study, the essential oils showed considerable efficacy despite extremely low concentrations of the preparations. Essential oils, in combination with other available control measures, could be an effective solution for controlling resistant populations of Colorado potato beetle, but further laboratory tests and studies on their efficacy in field trials are needed.

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