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Toxicity of essential oils on cabbage seedpod weevil (*Ceutorhynchus obstrictus*) and a model parasitoid (*Nasonia vitripennis*)

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Plant essential oils are being increasingly studied as a potential environmentally friendly alternative to synthetic insecticides. The insecticidal efficacy of essential oils on the cabbage seedpod weevil (*Ceutorhynchus obstrictus*), an important oilseed rape pest, has not been previously tested. We examined the impact of six essential oils on *C. obstrictus* via contact with dry residues on leaf and flower surfaces. We also examined the effect of these essential oils on a model non-target parasitoid wasp, *Nasonia vitripennis*. Exposure to dry residues of cumin (*Cuminum cyminum*) and cinnamon (*Cinnamomum verum*) essential oils (applied to oilseed rape leaves) resulted in significant loss of mortality and immobility in *C. obstrictus* adults. Treatment with *C. cyminum* essential oil at 1.5% resulted in 50.71% mortality and 87.3% combined mortality and immobility in *C. obstrictus*. *Cinnamomum verum* oil, at 1.5% concentration, resulted in 88.8% mortality and immobility among *C. obstrictus* 24 h post-treatment. All treatments studied with essential oil dry residues at 0.3% concentration caused high mortality and immobility in *N. vitripennis*. The greatest mortality and immobility were observed at 0.3% concentration in *F. vulgare* and *C. verum* treatments (54 and 53% loss respectively). At 0.1% concentration, *F. vulgare* and *T. vulgaris* significantly reduced parasitoids mobility and at 1.5% concentration all essential oils resulted in 100% mortality of *N. vitripennis* after 3 h. Our study revealed that *C. cyminum* and *C. verum* essential oils may have potential in the management of *C. obstrictus*. However, their impact on non-target organisms, including parasitoids, needs to be studied more thoroughly to determine the potential of essential oil main compounds in integrated pest management.

KEYWORDS

Ceutorhynchus obstrictus, Hymenoptera, biopesticide, biosafety, endoparasitoid, *Brassica napus*, IPM

1 Introduction

The cabbage seedpod weevil (*Ceutorhynchus obstrictus* Marsham) is widely distributed and one of the most important oilseed rape pests in Europe (Williams, 2010) and North America (Buntin, 1998; Dossdall et al., 2006; Dossdall and Mason, 2010). Adult *C. obstrictus* feeds on flower buds and young pods of oilseed rape plants, but the main economic loss is caused by larvae feeding within the seedpods (Bonnemaison, 1957; Williams and Free, 1978). Management of *C. obstrictus* is mostly based on synthetic insecticides that have harmful effects on biodiversity, pollute the environment (Geiger et al., 2010) and may leave pesticide residues on products (Yigit and Velioglu, 2020). Broad-spectrum insecticides have fatal effects on naturally occurring predatory arthropods such as lacewings, spiders, ladybirds, carabid beetles, rove beetles and parasitoids, which otherwise can effectively control the abundance of agricultural pests and reduce the need to apply insecticides (Tschumi et al., 2016; Begg et al., 2017; Albrecht et al., 2020). For example, key parasitoids of *C. obstrictus* can substantially contribute to biocontrol services, as the parasitism rate of *C. obstrictus* can reach up to 90% (Veromann et al., 2011; Kovács et al., 2019). In the light of the European Union's Farm to Fork Strategy which aims to diminish the negative impacts of agriculture on the environment, there is a great need to find environmentally sustainable pest control measures (European Commission, 2020).

Essential oils are of interest in pest science, as a possible alternative to synthetic plant protection products (Menossi et al., 2021; Devrnja et al., 2022). Several studies have highlighted their insecticidal effects on important agricultural pests (Das et al., 2021; Devrnja et al., 2022). An added complexity of essential oil studies is due to the variable chemical composition, even within the same plant species, as a result of different cultivars, growing conditions, production methods, plant parts used and harvesting times (Figueiredo et al., 2008; Baser and Buchbauer, 2009; Turek and Stintzing, 2013). Previously, several studies have examined the potential of essential oils to control another oilseed rape pest – pollen beetle (*Brassicogethes aeneus* Fabricius) (Mauchline et al., 2005; Pavela, 2011; Mauchline et al., 2013; Dorn et al., 2014; Willow et al., 2020). Pavela (2011) found that *Carum carvi* L., *Thymus vulgare* L. and *Foeniculum vulgare* Miller had an insecticidal effect against the pollen beetle, while Willow et al. (2020) showed only a slight insecticidal effect of residual exposure using very high dosage of *Cinnamomum verum* J. Presl oil; Mauchline et al. (2005); Mauchline et al. (2013) and Dorn et al. (2014) found repellent and lethal efficacy with *Lavandula angustifolia* Miller while Cook et al. (2007a) indicated no behavioural response from the main parasitoids *Phradis interstitialis* Thomson and *P. morionellus* Holmgren. However, it is unknown whether these essential oils are effective against another oilseed rape pest, *C. obstrictus*, as well which could potentially aid in the control of these two major pests simultaneously. While botanical insecticides are considered to be less toxic to humans and the environment compared to synthetic pesticides, their impact on the natural enemies of pests should be assessed, as they can interfere with their behavior or biology (Rampelotti-Ferreira et al., 2017; Parreira et al., 2018; Lima et al., 2020; Stenger et al., 2021).

Biosafety of plant protection products is a major concern in agriculture. Ideally, plant protection product applications should not be at the expense of non-target organism populations, especially those

contributing to biological control of the target pest. Some essential oils have been evidenced as safe for such beneficial insects. For instance, essential oil residues of *Origanum vulgare* L. and *T. vulgare* showed no sublethal or lethal effects in the parasitoid *Trissolcus basalis* Wollaston (Platygastridae) in direct contact and fumigation bioassays (Werding González et al., 2013). Essential oil from *Piper aduncum* L. showed promising results to control the stink bug *Euschistus heros* Fabricius, while parasitism and emergence of the egg parasitoids *Telenomus podisi* Ashmead (Platygastridae) and *Trissolcus urichi* Crawford (Scelionidae) were unaffected (Turchen et al., 2016). However, decreases in parasitism rate have been observed in other species (Boeke et al., 2003; Stenger et al., 2021), leaving the biosafety profile of essential oils questionable with regard to parasitoids.

In this study, we aimed to investigate whether the dry residues of six plant essential oils affect the mortality and immobility of *C. obstrictus*, as well as mortality and immobility, and next generation development of the model parasitoid *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae). We show that *Cuminum cyminum* L. and *C. verum* essential oils have potential for use in *C. obstrictus* management, but also that their impact on parasitoids needs further study to determine the actual potential of these essential oils within integrated oilseed rape protection.

2 Materials and methods

2.1 Insects

We collected *C. obstrictus* adults from an untreated oilseed rape field (58.36377°N, 26.66145°E, Tartu County, Estonia) using a plant tapping method and collecting insects into a ventilated plastic bottle. In the laboratory we identified *C. obstrictus* via Morris (2008) and allowed weevils to feed *ad libitum* on oilseed rape leaves and flowers also collected from the same field. As the key parasitoids of *C. obstrictus* are of the family Pteromalidae, we used *N. vitripennis* as a model non-target biocontrol species in this study. *Nasonia vitripennis* were reared in a climate chamber (Sanyo MLR-351H, Japan) at 20 ± 2°C, 60% RH and 16:8 h light:dark cycle, using blow fly (*Calliphora* sp.) pupae as the host. Blow fly larvae were bought from a commercial fishing store and were allowed to pupate in the laboratory. To produce new *N. vitripennis* adults, we placed approximately 20 blow fly pupae into transparent, polystyrene, ventilated insect breeding dishes (diameter 10 cm x height 4 cm; SPL Life Sciences, Gyeonggi-do, South Korea; hereafter referred to as cages), and introduced approximately 20–50 fast moving (a proxy for insect health) *N. vitripennis* adults into the cages. Prior to introducing the parasitoids to the cages, parasitoids were fed 50% sugar water to optimize their reproductive potential.

2.2 Examining the effect of six essential oils on *C. obstrictus* via contact with dry treatment residues on leaf and flower surfaces

To examine the effect of essential oil applications on *C. obstrictus*' mortality and immobility, we treated oilseed rape leaf and flower

surfaces with *T. vulgaris*, *F. vulgare*, *C. cyminum*, *C. verum*, *C. carvi* and *Cannabis sativa* L. essential oils. Pure essential oils were ordered from Talia (Rome, Italy; www.taliaessenze.com) in 2019, and once received stored in a refrigerator at +4°C, in small separate boxes in darkness. Details regarding the origin of plants, plant parts used, extraction method, and the major relevant compounds in each essential oil used, are described in detail in [Willow et al. \(2020\)](#). The gas chromatography–mass spectrometry (GC-MS) used for analyzing the essential oils, is described in detail in [Kännaste et al. \(2014\)](#). In the present study, essential oils were used at a concentration of 1.5%, with acetone as the solvent and polysorbate Tween80 (0.05%) as a wetting agent. The negative control treatment contained only acetone and Tween80; the positive control treatment was analytical grade lambda-cyhalothrin applied at the recommended field concentration (7.5 g active compound/ha) in acetone and Tween80. For each treatment, oilseed rape leaves were individually placed on a petri dish and using a pipette, at 1000 µl of treatment solution were applied onto each oilseed rape leaf (~ 12 cm x 9 cm). In each petri dish we included four oilseed rape flowers dipped in the respective [acetone + Tween80 + essential oil] solution. After that, treated leaves and flowers were allowed to air dry for 1 h, and then one leaf and four flowers were placed into each cage, followed by the introduction of eight *C. obstrictus* adults into each cage. Five cages per each treatment were prepared. Cages were kept in a ventilated room with an ambient air temperature of 22 ± 2°C, away from direct sunlight. Survival and mobility of weevils were assessed after 3 h and 24 h of exposure to dry residues of each treatment. In each sample, the number of weevils displaying immobility effects, including erratic movements or loss of mobility, was recorded, and all dead weevils were counted. This experiment was repeated twice, in total ten cages per treatment (N=10), total of 80 weevils per treatment.

The two most effective essential oils from the abovementioned tests were then evaluated for *C. obstrictus* control efficacy at four different concentrations. Here, we examined the dry residues of *C. verum* and *C. cyminum* essential oils applied at 0.5%, 1%, 1.5% and 2% concentrations. The experimental setup was the same as previously described, but the experiment was not repeated (N=5), total of 40 weevils per treatment.

2.3 Examining non-target effects of six essential oils on *N. vitripennis*

To examine the effects of essential oils on *N. vitripennis*, we treated filter paper, *via* pipette, with the same six plant essential oils as were tested on *C. obstrictus*, using acetone as the solvent, as well as Tween80 (0.05%). The positive controls were the same as used in the *C. obstrictus* assays. Here, all six essential oils were applied at four concentrations: 0.1%, 0.3%, 0.5% and 1.5%. For each treatment, we pipetted 1000 µL of treatment solution onto five pieces of filter paper (~ 7 cm x 3 cm) individually on a petri dish. After treatment, the filter papers were allowed to air dry for 1 h, and subsequently placed into cages, one piece of filter paper per cage. Eight *N. vitripennis* adults were introduced to each cage, and the cages were kept in a ventilated room with an ambient temperature of 22 ± 2°C. At 3 h and 24 h post-exposure to treatment residues, *N. vitripennis* mortality and immobility were monitored. This experiment was repeated twice, in

total ten cages per treatment (N=10), total of 80 parasitoids per treatment.

To assess the potential impact of essential oils on the developmental success of *N. vitripennis*, we used already-parasitized blow fly pupae. For that, the parasitoids we allowed freely to lay eggs into the blow fly pupae for 7 days. After 7 days, adult parasitoids were removed, and blow fly pupae were dipped into the treatment solutions for 2 seconds, allowed to air dry for 1 h, and then placed into cages, 10 pupae per cage. Cages were kept in a ventilated room with an ambient temperature of 22 ± 2°C, away from direct sunlight, for three weeks. After that, all emerged parasitoids were counted, and all pupae were dissected to indicate the presence of unemerged parasitoids. The experiment was repeated twice and in total ten cages per treatment (N=10), total of 80 pupae per treatment.

2.4 Statistical analysis

Statistical analyses were performed in R v3.6.1 ([R Core Team, 2018](#)), using the R packages “car”, “emmeans”, “MASS”, “DHARMA” and “dunn.test” ([Venables et al., 2002](#); [Dinno and Dinno, 2017](#); [Hartig and Hartig, 2017](#); [Fox and Weisberg, 2019](#); [Lenth, 2022](#)). For *C. obstrictus* analyses, Generalized Linear Models (GLMs) with Poisson distribution and log link function and Wald statistics Type III empirical standard error were used. For the *post-hoc* comparisons, the Tukey test was used. To analyze *N. vitripennis* data, as the residuals of the model were not normally distributed, we used the nonparametric Kruskal-Wallis test, followed by Bonferroni-Dunn’s test for *post-hoc* pairwise comparisons.

3 Results

3.1 Effect of essential oil residues on *C. obstrictus* mortality and immobility

After 3 h of contact with dry residues of essential oil treatments, survival rates did not differ from the negative control treatment ([Figure 1A](#)). At 24 h post-treatment, however, we observed a significant effect on survival ($\chi^2 = 132.41$, $df=7$, $p<0.0001$). The highest mortality (82.5 ± 6.8%) was observed in the *C. verum* oil treatment, followed by the positive control (53.75 ± 3.75%) and *C. cyminum* oil (50.71 ± 8.77%) treatment, while the mortality of weevils exposed to *T. vulgaris*, *C. carvi* and *C. sativa* essential oil treatments did not differ significantly from the negative control ([Figure 1A](#)). Negative control treatment had no effect on survival.

Treatment with essential oils had a significant impact on *C. obstrictus* mortality and immobility rates after 3 h ($\chi^2 = 90.32$, $df=7$, $p < 0.0001$; [Figure 1B](#)). The greatest effect on *C. obstrictus* mortality and immobility was observed in the positive control treatment (76.3 ± 6.3%), followed by *C. carvi* oil (33.8 ± 11.9%) and *C. cyminum* oil (22.5 ± 7.9%). The mortality and immobility rate increased at 24 h post-exposure to dry residues, in all treatments except *C. carvi*. The greatest losses of mortality and immobility in *C. obstrictus* were observed in the *C. cyminum* (87.3 ± 4.9%) and *C. verum* (88.8 ± 4.7%) treatments, which did not differ significantly from the positive control treatment (98.8 ± 1.3%) with lambda-cyhalothrin. Treatments with *T. vulgaris* and *C. sativa* did not

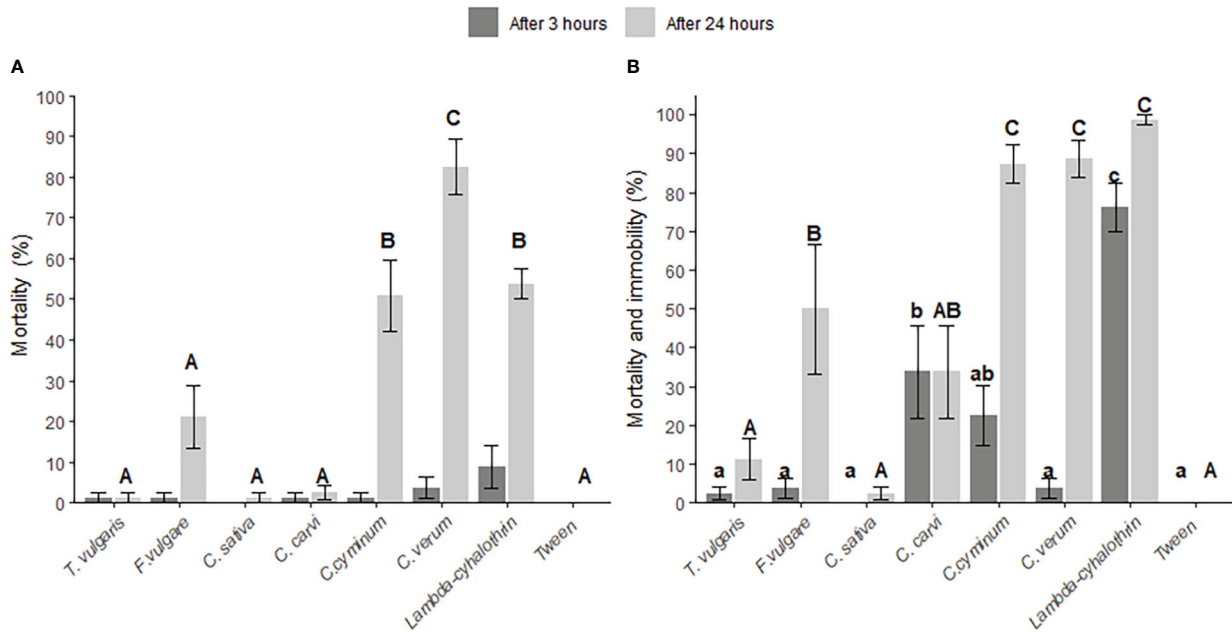


FIGURE 1

Effect of six plant essential oils (*Thymus vulgaris*, *Foeniculum vulgare*, *Cuminum cyminum*, *Cinnamomum verum*, *Carum carvi* and *Cannabis sativa*), lambda-cyhalothrin (positive control) and Tween80 (negative control) on *Ceutorhynchus obstrictus* mortality (A) and mortality and immobility (B), at 3 h and 24 h post-exposure to treated oilseed rape leaves and flowers, N=10 (80 weevils per treatment). All treatments were compared using Generalized Linear Models (GLMs) with Wald statistic Type III, *post-hoc* comparisons with Tukey test, error bars: \pm SE. Different lowercase and uppercase letters indicate significant differences ($p < 0.05$) between treatments at 3 h and 24 h post-exposure, respectively.

differ from the negative control ($p > 0.05$), but *F. vulgare* differed significantly from both the positive and negative control ($p < 0.05$). No mortality nor immobility effects were observed in the negative control treatment.

3.2 Effect of different concentrations of *C. cyminum* and *C. verum* essential oil residues on *C. obstrictus* mortality and immobility

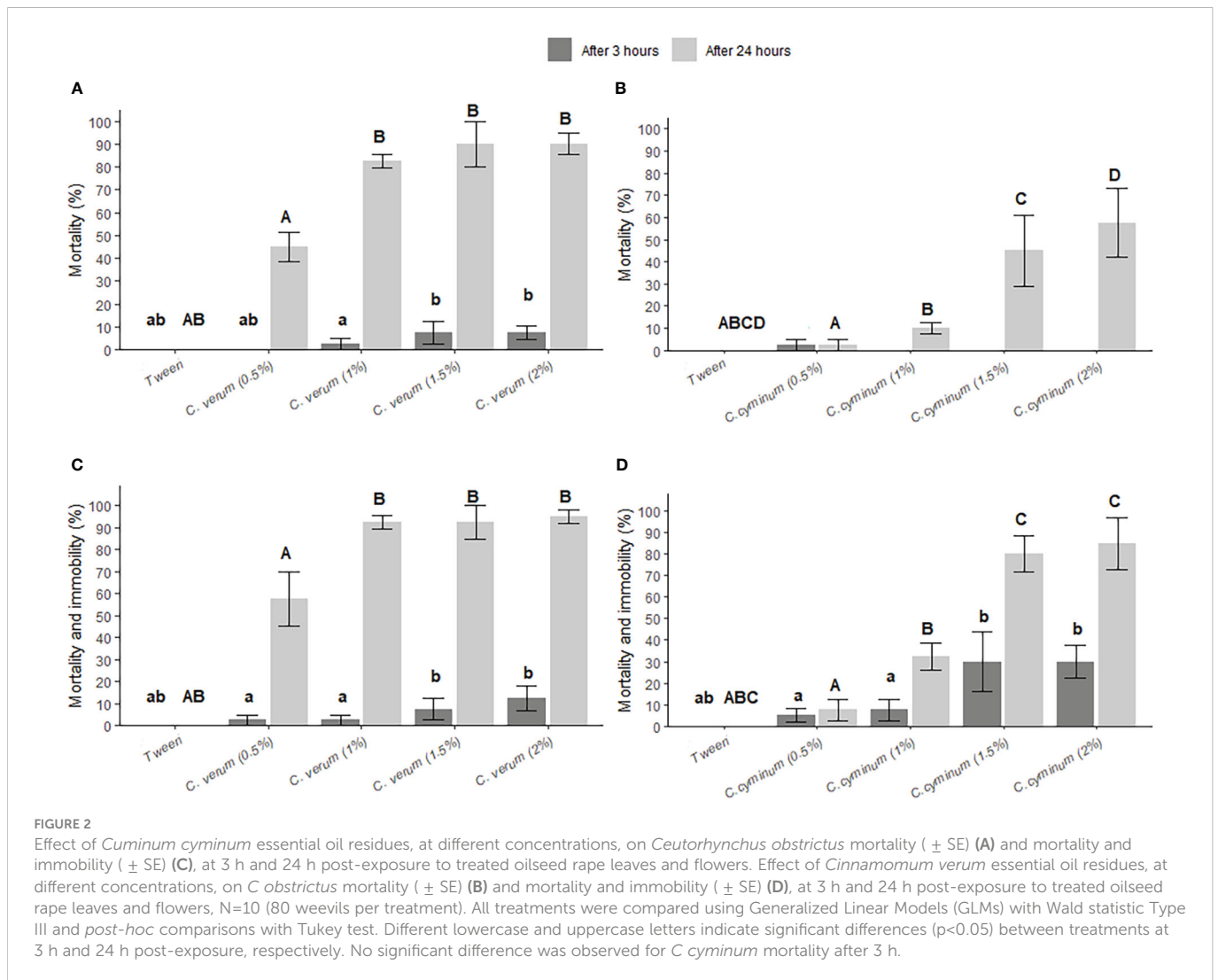
As *C. cyminum* and *C. verum* represented the most effective essential oils against *C. obstrictus*, we examined the effects of their residues against *C. obstrictus* after applying these two essential oils at increasing concentrations (0.5%, 1%, 1.5% and 2%) (Figures 2A-D). At 3 h *C. verum* showed a significant effect on *C. obstrictus* survival ($\chi^2 = 12.93$, $df=4$, $p=0.012$) (Figure 2A), where concentrations of 1% and higher caused mortality; however no significant effect on *C. cyminum* was observed after 3 h (Figure 2B). At 24 h post-treatment, both *C. cyminum* and *C. verum* essential oil residues significantly increased *C. obstrictus* mortality ($\chi^2 = 126.49$, $df=4$, $p < 0.0001$; $\chi^2 = 87.29$, $df=4$, $p < 0.0001$, respectively). *Ceutorhynchus obstrictus* mortality rates in *C. cyminum* treatments were significantly greater in 1.5% and 2% compared to the 0.5% and 1% solutions (Figure 2B). The mortality rates of *C. obstrictus* treated with *C. verum* essential oil at 1%, 1.5% and 2% solutions exceeded 80% ($82.5 \pm 3.1\%$, $90 \pm 10\%$ and $90 \pm 4.7\%$, respectively), each differing significantly from the *C. verum* 0.5% and control treatments. Mortality and immobility rates of *C. obstrictus* reached up to 90% at 24 h post-exposure to dry residues

of *C. cyminum* essential oil applied at 1.5% and 2.0% concentrations ($\chi^2 = 300.77$, $df=4$, $p < 0.0001$; Figure 2D).

3.3 Effect of essential oil residue concentrations on *N. vitripennis* mortality and immobility

After 3 h of exposure to essential oil residues applied at 0.1% concentration, there was a significant effect on *N. vitripennis* mortality and immobility ($\chi^2 = 20.96$, $df=6$, $p=0.0019$), more parasitoids were affected in *T. vulgaris* and *F. vulgare* treatments, $43.5 \pm 11.9\%$ and $15 \pm 8.3\%$, respectively (Figure S11). After 3 h we observed under 10% mortality and immobility for *C. sativa* ($2 \pm 0.42\%$), *C. carvi* (0%), *C. verum* ($7.0 \pm 1.89\%$), *C. cyminum* ($6 \pm 1.07\%$). For insecticide (lambda-cyhalothrin) treated group, a small mortality was observed ($1.5 \pm 0.26\%$), same for negative control (tween) ($4.5 \pm 0.63\%$). At 24 h post-exposure of dry treatment residues, no significant effect on mortality and immobility was observed for any of the essential oil treatments applied at 0.1% concentration ($\chi^2 = 7.49$, $df=6$, $p=0.28$). The lowest mortality and immobility rates were observed in the *C. carvi* treatment ($3 \pm 1.5\%$) and the highest in the *T. vulgaris* treatment ($29 \pm 12.5\%$), but no significant differences between treatments were found. The mortality and immobility rates at 24 h post-exposure to *T. vulgaris* oil residues were lower than at 3 h post-exposure, indicating that some specimens were able to recover from knockdown effects.

In all essential oil treatments applied at 0.3% concentration, *N. vitripennis* mortality and immobility were significantly decreased at both 3 h and 24 h ($\chi^2 = 24.98$, $df=6$, $p < 0.001$; $\chi^2 = 27.78$, $df=6$,



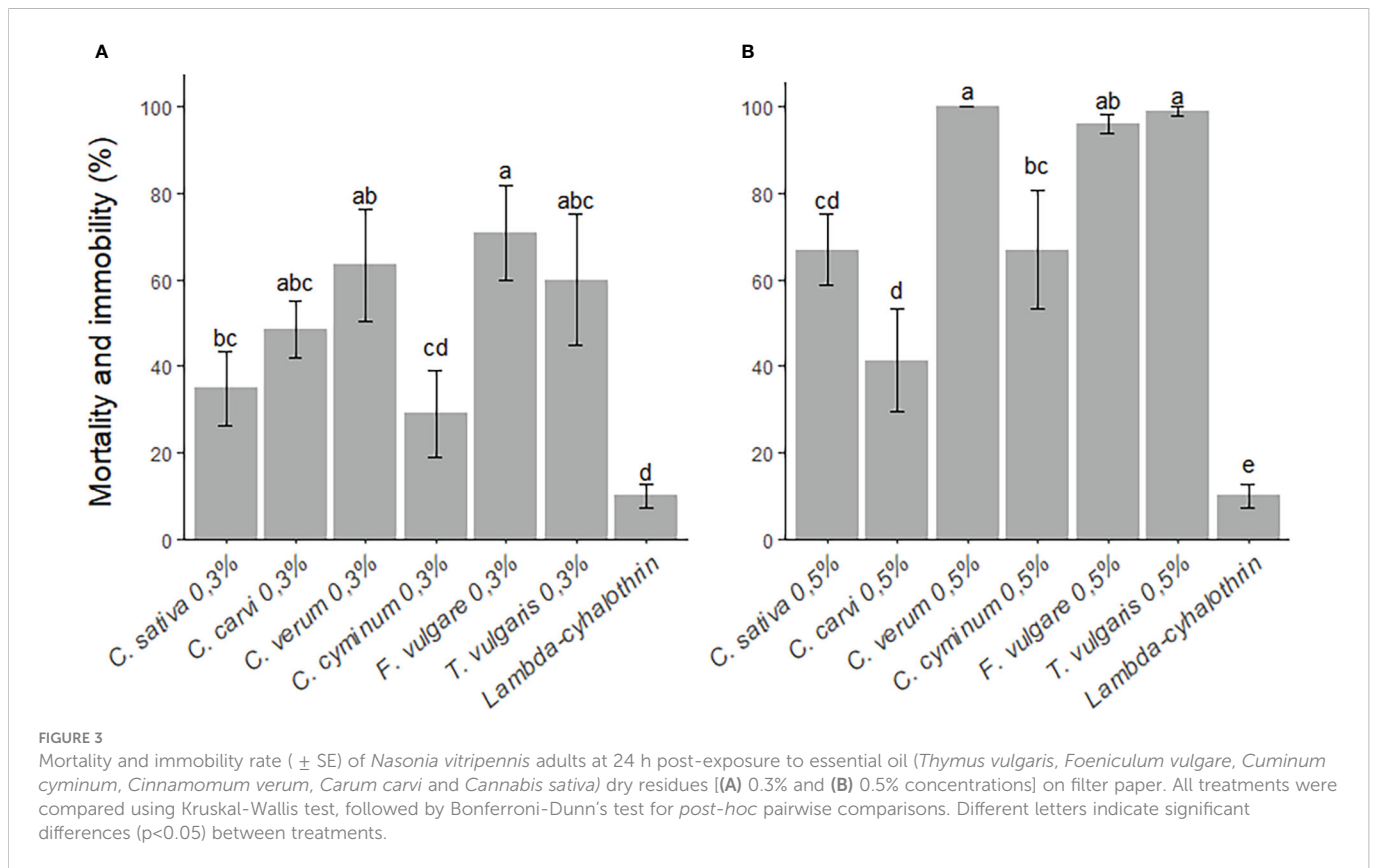
$p < 0.0001$, respectively) post-exposure to essential oil dry residues, compared to the control treatment. At 3 h, the *F. vulgare* treatment at 0.3% concentration resulted in the greatest mortality and immobility ($54 \pm 14.3\%$), followed by the *C. verum* treatment ($53 \pm 15.7\%$), *T. vulgaris* ($50 \pm 5.27\%$), *C. sativa* ($3 \pm 0.48\%$), *C. carvi* ($15 \pm 2.06\%$), *C. cyminum* ($10 \pm 3.16\%$), compared to the insecticide treatment lambda-cyhalothrin ($1.5 \pm 0.26\%$) and negative control (tween) ($4.5 \pm 0.63\%$). At 24 h, *C. verum* oil at 0.3% concentration resulted in the greatest loss of mobility ($63.5 \pm 4.1\%$), followed by *T. vulgaris* oil ($60 \pm 4.8\%$) (Figure 3A).

In all essential oil treatments applied at 0.5% concentration, *N. vitripennis* mortality and immobility were significantly decreased at both 3 h ($\chi^2 = 59.54$, $df=6$, $p < 0.0001$) and 24 h ($\chi^2 = 53.84$, $df=6$, $p < 0.0001$). At 3 h post-exposure to the *C. verum* treatment at 0.5% concentration, the mortality and immobility rate of *N. vitripennis* was 100%, followed by the *T. vulgaris* and *F. vulgare* treatments (100% and $97 \pm 2.1\%$, respectively), *C. cyminum* ($44 \pm 4.48\%$), *C. carvi* ($21 \pm 3.31\%$) and *C. sativa* ($24 \pm 2.95\%$). At 24 h, there was a significant loss of mortality and immobility in all 0.5% concentration essential oil treatments. The greatest loss of mortality and immobility at 24 h was observed in the *C. verum* treatment (100%) (Figure 3B). Contact with

dry residues of the insecticide lambda-cyhalothrin did not result in a significant loss of *N. vitripennis* mortality and immobility, compared to the negative control treatment. All essential oils, at 1.5% concentration, resulted in 100% mortality of *N. vitripennis* after 3 h.

3.4 Effect of essential oils on the number of next generation *N. vitripennis*

After allowing *N. vitripennis* to parasitize untreated blow fly pupae for 7 days, we treated the parasitized pupae to determine the post-parasitism mortality of developing parasitoids. Compared to the untreated control group, the average number of next generation *N. vitripennis* adults that emerged was greatest in the group consisting of untreated pupae (223 ± 16.3 specimens), followed by pupae treated with *C. verum* oil (176 ± 11.3 specimens) and *F. vulgare* oil (171.8 ± 12.5 specimens), although the differences were not significant (Figure 4). Compared to untreated pupae, significantly less parasitoids emerged from *C. sativa*, *C. cyminum* and *T. vulgaris* treatments decreasing the number of emerging parasitoids similar to lambda-cyhalothrin where only 85.7 ± 16 next generation parasitoids emerged.



4 Discussion

The present study showed that contact with essential oil residues *via* treated oilseed rape leaves and flowers caused both mortality and immobility in *C. obstructus* adults. In addition to *C. obstructus*, essential oil treatments showed an impact on the mortality and immobility of a model pteromalid parasitoid, *N. vitripennis*, and furthermore influenced the number of next generation adult parasitoids that emerged from their hosts. Our results showed that at 24 h post-treatment with *C. cyminum* and *C. verum* essential oils at 1.5% concentration they were as effective as the synthetic insecticide lambda-cyhalothrin, mortality and immobility of *C. obstructus* adults reached 82.5% for *C. verum* and 50.7% for *C. cyminum*. There is an overlap in the occurrence of *B. aeneus* and *C. obstructus* in oilseed rape fields (Veromann et al., 2006; Sulg et al., Under Review); *B. aeneus* arrives a little bit earlier than *C. obstructus*, as its flight threshold temperature is 12°C (Williams, 2010), whereas for *C. obstructus*, it is 13–15°C (Free and Williams, 1979; Lerin, 1991). However, they are both present in oilseed rape fields from the green bud stage (BBCH 51) (Veromann et al., 2006; Veromann et al., 2012). Therefore, it is possible that treatments targeting *B. aeneus* may also contribute to *C. obstructus* control and *vice versa*. Our new findings show the potential of *C. verum* essential oil to manage *C. obstructus*, but as it greatly exceeds that of previously reported for *B. aeneus* (17.5% combined immobility and mortality) (Willow et al., 2020), the two species are unlikely to be managed simultaneously using only *C. verum*. Based on previous results and our new findings, the treatment of oilseed rape with *C. verum* affects two of its main pests to some extent, therefore indicating the need for further investigations. The essential oils used in our study were almost the same (excluding anise) as Willow et al.

(2020). Gas chromatography–mass spectrometry results of the *C. verum* oil used in the present study are reported in detail in Willow et al. (2020). The primary active compound in the *C. verum* oil used was reported to be (E)-cinnamaldehyde (46%), followed by caryphyllene (15%), linalool (12%) and D-limonene (8%).

According to our best knowledge, the toxicities of essential oils for *C. obstructus*, or other species in the genus *Ceutorhynchus*, have not previously been assessed. However, there are previous studies examining other members of the family Curculionidae, where essential oil treatment efficiencies have been examined. For instance, essential oils isolated by hydrodistilling the dried fruit of *Trachyspermum ammi* (L.) Sprague ex Turrrill (Apicaceae) and *Nigella sativa* L. (Ranunculaceae) have shown repellent activity and toxic effects against the rice weevil (*Sitophilus oryzae* L.) (Chaubey, 2012). The rice weevil was also examined by Saad et al. (2018), where they found that, from all examined compounds contributing to acetylcholinesterase inhibition, the most promising was trans-cinnamaldehyde. Different essential oils, including *C. verum*, were studied against the stored product pest *Sitophilus zeamais* Motschulsky by Ramlal et al. (2020). They observed repellent and lethal effects of *C. verum* oil, resulting in 78% and 97% mortality at concentrations of 75 and 100 $\mu\text{L/mL}$, respectively. Similar to our study, the main constituent of *C. verum* essential oil in their study was cinnamaldehyde (62%) confirming that this compound can be potentially exploited against weevil pests.

The mortality rates in other essential oil treatments in our study were under 50% at 24 h, suggesting that their efficacy was insufficient for use in controlling *C. obstructus* abundance in oilseed rape crops. In the present study, we did not examine other effects than mortality and immobility of these essential oils on *C. obstructus* adults, e.g. repellence

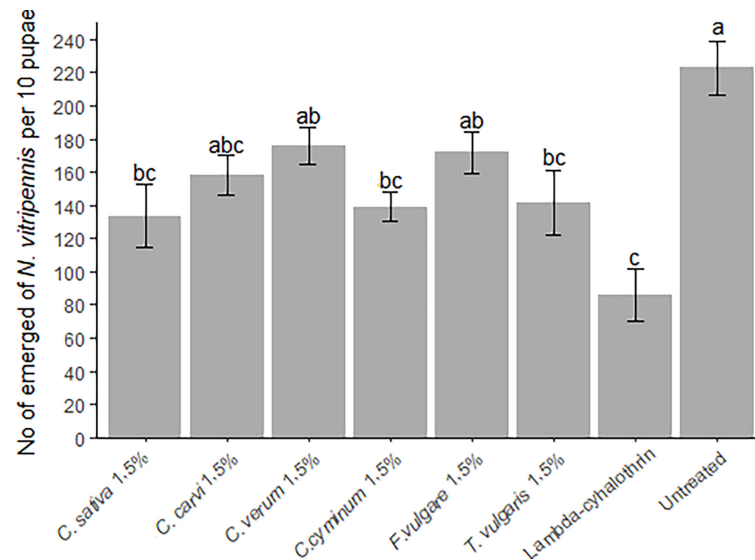


FIGURE 4

Mean (\pm SE) number *Nasonia vitripennis* specimens emerged from ten blow fly (*Calliphora* sp.) pupae treated with different essential oils (*Thymus vulgaris*, *Foeniculum vulgare*, *Cuminum cyminum*, *Cinnamomum verum*, *Carum carvi* and *Cannabis sativa*) (1.5% concentration) and lambda-cyhalothrin (positive control), as well as in untreated pupae, 7 days after first generation *N. vitripennis* were introduced to their hosts. Differences between treatments were compared using Kruskal-Wallis test, followed by Bonferroni-Dunn's test for *post-hoc* pairwise comparisons. Different letters indicate significant differences between treatments ($p < 0.05$).

etc. For instance, previous studies have shown the repellent effects of essential oils on insects (reviewed in Lee (2018)) and also their potential to be used in storage facilities as pest management approaches (Cook et al., 2007b; Nerio et al., 2010; Campolo et al., 2018; Xu et al., 2018; Bandeira et al., 2021), but the use for managing agricultural pests have gained less attention. Essential oils can also be used to manage agricultural pests, but in order to use them in agricultural fields, the essential oils need to be more stable, preventing them from evaporating or biodegrading (Oladipupo et al., 2022). Future studies on *C. obstrictus* should examine behavioral effects in addition to the effects examined in the present study as exposure to essential oils, as well as treatment residuals, can result in behavioral changes, for example deterring egg laying or acting as antifeedant (Lazarević et al., 2020; Magierowicz et al., 2020; Stenger et al., 2021). Examining our two most effective (against *C. obstrictus*) essential oils, *C. verum* and *C. cyminum*, at four different concentrations, showed *C. verum* oil at 1% concentration to be the most efficient, resulting in almost 90% mortality and immobility at 24 h post-exposure, whereas *C. cyminum* oil at 2% concentration resulted in 90% mortality and immobility at 24 h post-exposure. We presented mortality and immobility rates, since in nature, immobility is likely to result in mortality. Immobile insects are easier prey, as well as they may also die of dehydration, starvation, cold or heat stress, etc., as a result of being in contact with toxic substances.

Similar to other insecticides, the effects of essential oils on non-target organisms, including economically beneficial insects, should always be assessed. It is necessary to assess the impact of essential oils on the natural enemies of target pest species (e.g. relevant model parasitoids). The present study demonstrated that *C. verum* essential oil has the potential to control abundance of *C. obstrictus*, but at the same time it resulted in almost 100% mortality in the model pteromalid parasitoid *N. vitripennis* at 3 h post-exposure to

treatments at 1.5% concentration of all studied essential oils except *C. sativa*. Testing the oils at 0.1% concentration showed that at 3 h post-exposure, *T. vulgaris* oil residues resulted in the highest mortality and immobility rates in *N. vitripennis*, although some parasitoids were able to recover from immobility by the 24 h time point. Similar to our results, Werdin González et al. (2013) found that one day old residues of *T. vulgaris* essential oil resulted in 100% mortality in parasitoid *Trissolcus basalis* Wollaston (Platygastridae) while one week old residues did not result in any mortality. When targeting a pest species, knowledge of parasitoid distribution in or arrival to the crop is crucial, as pesticide application times can be planned in a manner where the pesticide residues represent an insignificant threat to target pest-relevant biocontrol agents. Essential oil residues of *C. verum* show promising results for controlling *C. obstrictus*. However, *C. verum* oil treatment, at 0.1% concentration, resulted in an immobility rate of 17% in *N. vitripennis*. Increasing the concentration of *C. verum* oil to 0.3% resulted in almost four times this immobility rate in *N. vitripennis*, and residues from a 0.5% concentration application of *C. verum* oil resulted in 100% immobility. Thus, residues of *C. verum* essential oil, even when applied in low concentrations, are not safe for the parasitoid *N. vitripennis*. It remains unclear whether the essential oils could cause side effects in the next generation of *N. vitripennis*.

When treating parasitized pupae with *C. sativa* and *C. cyminum* it lowered hatching of the new generation of *N. vitripennis* to the same level as treatment with insecticide. But as direct exposure to essential oil residues, in the concentration of 1.5%, caused 100% mortality after 3 h among *N. vitripennis*, we can only assume that fly pupae served as protective shield mitigating the toxic effect of treatments. It has been reported that lambda-cyhalothrin can alter the ability of the parasitoids to find and infest their hosts, even when mortality is not observed among next generation female parasitoids (Desneux et al.,

2004). Even though parasitoids developed in our study, their following parasitism efficacy remains unknown, and should be investigated in future studies. Whether the pods concealing parasitoids of *C. obstrictus* provide similar protection from developing parasitoids needs investigating. It also remains unknown whether the essential oil treatments in the present study had sublethal effects on next generation adult parasitoids, representing a crucial knowledge gap that is in need of assessment. Undetected sublethal effects, could result in death or decreased fecundity or jeopardize host location abilities. Our results show the potential of essential oils use in controlling *C. obstrictus*. However, much more research is needed before essential oils could be recommended for pest control.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SS, RK, and EV conceived the study. SS, TK, and RK performed the experiments. SS performed data analyses and visualization. SS and EV wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2023.1107201/full#supplementary-material>

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