








Courtship and mating traits of *Tenebrio molitor* and *Sitophilus zeamais* are impacted by sublethal concentrations of carlina oxide

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ABSTRACT

Tenebrio molitor L. (Coleoptera: Tenebrionidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) are harmful pests in storages. *Carlina acaulis* L. (Asteraceae) is a medicinal plant used for its beneficial effects throughout Central and Eastern Europe. The essential oil (EO) of this plant, almost entirely made up of carlina oxide, exhibited broad-spectrum properties, including notable pesticidal activity against vectors and agricultural or stored-product pests. This research examined the impact of sublethal concentrations of carlina oxide on the mating behavior of *T. molitor* and *S. zeamais*. *Sitophilus zeamais* adults were more sensitive to carlina oxide than *T. molitor* adults, as LC₁₀, LC₃₀, and LC₅₀ values were lower. The mating success of *T. molitor* and *S. zeamais* was impacted negatively by the exposure of both beetles to sublethal concentrations (LC₁₀ and LC₃₀) of carlina oxide. For both species, the mean duration of each mating phase was significantly affected by exposure to sublethal concentrations of carlina oxide vs. control. The highest mean duration of copulation was recorded in control pairs, which was significantly longer than pairs treated with LC₁₀ and LC₃₀. Concerning lateralization, males of both species showed a left-biased trend, which led to higher successful copulations than right-biased, front or back-side males, regardless of treatment or control. Overall, carlina oxide alters the behavioral traits of *T. molitor* and *S. zeamais*, thus, it should be further considered in Integrated Pest Management (IPM) approaches in the storage environment.

1. Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a noxious insect of stored maize, causing considerable postharvest losses globally (Jiménez-Galindo et al., 2023). Due to the feeding activity of *S. zeamais* larvae and adults on sound grains, a weight loss of approximately 40% can be caused during storage (Ojo and Omoloye, 2012). The yellow mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), infests a wide range of products of animal and plant origin (Hagstrum and Subramanyam, 2009; Vigneron et al., 2019). It impacts the infested foodstuffs with a strong odor through deposition of eggs and frass (CABI, 2019). The management of both pests is challenging due to the developed resistance to various insecticides (Isman, 2006; Haddi et al., 2018). Therefore, there is a need to investigate environmentally-friendly insecticides, such as botanicals, with a wide range of insecticidal activity to control both species.

The stemless carline thistle, *Carlina acaulis* L. (Asteraceae), is an edible perennial herbaceous plant found in Southern and Central Europe. This species is traditionally used in medicine because it exhibits several actions, e.g., antiicteric and diuretic (Gilca et al., 2018; Kozłowska et al., 2018; Balijagić et al., 2021). *Carlina acaulis* essential oil (EO) and its main component carlina oxide are recognized for their broad-spectrum properties, including antinematodal, antifungal, and antibacterial effects (Gras et al., 2017; Strzemski et al., 2017; Rosato et al., 2021). They have also demonstrated remarkable pesticidal activity after being tested against stored-product or agricultural pests (Kavallieratos et al., 2022; Rizzo et al., 2021, 2024; Benelli et al., 2022; Novák et al., 2024) and vectors (Pavela et al., 2021; Tortorici et al., 2024). For instance, *C. acaulis* EO nanoemulsion exhibited elevated mortality rates against *T. molitor* adults (85%) or larvae of *Tribolium confusum* Jacquelin du Val (99%) and *Tribolium castaneum* (Herbst) (94%) (Coleoptera: Tenebrionidae) at 1000 ppm (Kavallieratos et al.,

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2022). The microemulsion, that contained 0.5% *C. acaulis* EO, showed high larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae) (Pavela et al., 2021). Furthermore, carlina oxide killed 97% of the exposed *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) adults (Novák et al., 2024).

The estimation of sublethal effects of green insecticides to noxious insects is an essential tool in Integrated Pest Management (IPM) programs (Zhang et al., 2020; Guedes et al., 2022) because they affect development, mating and related behavioral traits, locomotion, and fecundity (Desneux et al., 2007). For instance, Benelli et al. (2022) examined how the exposure of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) adults to LC₁₀ and LC₃₀ of carlina oxide impacted insect intraspecific aggression dynamics.

Although there are reports about the laterality and mating behavior of *T. molitor* (Boukouvala et al., 2021), for *S. zeamais*, two early studies have described its mating behavior without focusing on the lateralization (Walgenbach and Burkholder, 1987; Guedes et al., 2017). Regarding the mode of action of carlina oxide, specific reports are lacking to date. However, some hypotheses can be formulated based on literature data related to polyacetylenes. For instance, carlina oxide can be phototoxic, being activated under UV light giving rise to toxic carbocations producing serious damages to insect tissues and organs after ingestion (Benelli et al., 2022; Tortorici et al., 2024). Some phenylacetylenes with similar structure to that of carlina oxide were reported as non-competitive antagonistic inhibitors of the GABA-gated chloride channel in the insect central nervous system, causing neuronal cell hyperexcitability (Lamberth, 2009). Moreover, a possible interaction with the P450 cytochrome detoxification system has also already been proposed for polyacetylenes (War et al., 2020). In addition, there is no data available on the sublethal effects of carlina oxide against *T. molitor* and *S. zeamais*, including courtship and mating behavior. Thus, the objective of the current research was to investigate the influence of low concentrations (i.e., LC₁₀ and LC₃₀) of carlina oxide on courtship, mating, and lateralization of *T. molitor* and *S. zeamais*.

2. Materials and methods

2.1. Isolation of carlina oxide

Carlina oxide, being 96% of the essential oil (GC-MS), was obtained by hydrodistillation of the roots of *C. acaulis* (purchased from the herbal company Minardi A. & Figli, Bagnacavallo, Italy, batch C-070524-33). Notably, 1 kg of coarsely crushed roots was placed into a 20 L flask filled with 10 L of distilled water. The process was stopped once no more EO was condensed. The yield of carlina oxide from the dry plant material was 0.82%. Chemical characterization of carlina oxide was achieved by GC-MS and NMR analyses that were consistent with procedures reported in previous studies (Pavela et al., 2021; Spinozzi et al., 2023a).

2.2. Colonies of beetles

All beetles were obtained from cultures maintained under constant conditions, i.e., 30°C, 65% relative humidity (RH), and total dark (Suleiman et al., 2012; Boukouvala et al., 2021) at the Laboratory of Agricultural Zoology and Entomology (LAZE), Agricultural University of Athens. The initial populations of both species were collected from Greek storage facilities and kept in LAZE since 2003 (*S. zeamais*) and 2014 (*T. molitor*) (Boukouvala et al., 2021; Kavallieratos et al., 2022). Oat bran and potato slivers (source of moisture) were the rearing media for *T. molitor* (Boukouvala et al., 2021), while *S. zeamais* was reared on whole maize kernels (Kavallieratos et al., 2024). *Tenebrio molitor* colonies were maintained in plastic containers measuring 30 cm wide, 30 cm deep, and 14 cm high, while *S. zeamais* colonies were maintained in 5 L glass jars. The tops of the plastic containers and glass jars were covered with gauze for adequate ventilation. Oat bran and maize kernels, before being used in colony maintenance, were disinfected at

sub-zero temperatures and then warmed to room temperature (Athanasidou et al., 2017).

2.3. Evaluation of LC values of carlina oxide

The evaluation of sublethal concentrations of carlina oxide was performed following the experimental procedure utilized by Boukouvala et al. (2023). Specifically, different Petri dishes (50.27 cm² in surface) with filter paper attached to their bottoms were impregnated separately with 7 concentrations of carlina oxide (i.e., 156.25, 312.5, 625, 1250, 2500, 5000, and 10000 ppm) and left to dry. Then, groups of 20 adults (unsexed) < 2 weeks old of *S. zeamais* or *T. molitor* (Kavallieratos et al., 2022, 2024) were released into each dish with the corresponding concentration of carlina oxide and placed in incubators under the aforementioned conditions. Dead *S. zeamais* and *T. molitor* individuals were recorded after 72 and 120 h of exposure, respectively. For each concentration of carlina oxide, five replications were prepared.

2.4. Sex identification of the beetles

Tenebrio molitor pupae were sexed following the description of Bhattacharya et al. (1970); i.e., males have a small swelling extending below the seventh abdominal sternite bearing two short, blunter papillae that are placed close together along the mesal line, while the corresponding swelling of females is bigger and has a pair of more widely spaced papillae, located at its latero-caudal angles. Then pupae were put separately (by sex) in Petri dishes (Ø 5 cm × 1 cm height) under the same conditions and food source as previously mentioned until they reached adulthood and sexual maturity (Boukouvala et al., 2021). Thus, 6-day-old females and males of *T. molitor* were used in the experiments, as it mates 5 days post eclosion (Drnevich et al., 2001). Regarding *S. zeamais*, the newly emerged individuals were sexed according to the characteristics of their rostrum (i.e., male's rostrum is wider and shorter than female's) reported by Halstead (1965). Males and females were kept separately in glass vials (Ø 2.5 cm × 10 cm height) with maize kernels for 3 d until they became sexually mature (Guedes et al., 2017).

2.5. Influence of carlina oxide on mating behavior of *T. molitor* and *S. zeamais*

Females and males (virgins) of *S. zeamais* and *T. molitor* were utilized in the tests. Before the beginning of the observations, males and females of each species were treated separately for 24 h at LC₁₀ and LC₃₀ of carlina oxide (Boukouvala et al., 2023). They were then transferred separately to untreated dishes and left under the natural light conditions of the laboratory for 180 min to adapt to the experimental environment (Benelli et al., 2017a). An arena (Ø 8 cm × 1 cm height) was used, which was surrounded by a filter paper wall to avoid any external cues, such as shadows, bright colors, or objects, that could influence mating pairs under evaluation (Romano et al., 2016). One pair from each species/treatment was tested at each time. Untreated mating pairs from each species (controls) were also observed. Each couple was visually observed for 60 min, or until copulation was completed, if existent (Benelli et al., 2017a). For both species, the side of the body of female (i.e., left, right, front, or back), that was preferred by the male for approach and mounting, was recorded (Boukouvala et al., 2021, 2023).

The counting of mating pairs concluded once 50 successful matings per species/treatment were observed. Concerning *T. molitor*, the total number of couples observed was 97, 125, and 179 for control, carlina oxide LC₁₀, and carlina oxide LC₃₀, respectively. However, 15 out of 97 (control), 17 out of 125 (carlina oxide LC₁₀), and 20 out of 179 (carlina oxide LC₃₀) mating pairs were rejected since no sexual interaction occurred, couples remained still, or females were near the walls of the arena affecting the direction of approach of male individuals (Benelli et al., 2017a). Consequently, 82 control pairs, 108 carlina oxide LC₁₀-treated pairs, and 159 carlina oxide LC₃₀-treated pairs were

analyzed. The duration of the three mating phases of *T. molitor* described by Boukouvala et al. (2021) (i.e., mate detection, precopula including antennal tapping and rubbing behavior, and copula) and the entire sexual interaction was recorded. For *S. zeamais*, 81 couples from control, 127 treated couples with LC₁₀ carlina oxide, and 156 treated couples with LC₃₀ carlina oxide were observed in total. However, 14 out of 81 pairs in the control group, 18 out of 127 in the carlina oxide LC₁₀-treated group, and 23 out of 156 in the carlina oxide LC₃₀-treated group were rejected as previously described. Therefore, 67 (control), 109 (carlina oxide LC₁₀), and 133 (carlina oxide LC₃₀) couples were utilized for the analysis. The duration of the mating phases of *S. zeamais* was recorded as follows: mate detection (i.e., the time needed for ♂ to locate ♀), precopula (i.e., ♂ + ♀ interaction, ♂ mounts ♀, and walking), and copula (i.e., from ♂ insertion of aedeagus into ♀ genital chamber till their separation) (Benelli et al., 2017a; Guedes et al., 2017). The duration of the entire mating sequence was also recorded.

2.6. Statistical analysis

The sublethal concentrations of carlina oxide corresponding to LC values (LC₁₀, LC₃₀, LC₅₀) against *S. zeamais* and *T. molitor*, with the associated 95% confidence limit (CL), were calculated by probit analysis (R statistical package, version 2.15.1) (Finney, 1978; R Core Team, 2017). Mating success of *T. molitor* or *S. zeamais* males treated with carlina oxide (LC₁₀ or LC₃₀) or control was estimated following the equation $y = X\beta + \varepsilon$ (weighted generalized linear model with binomial distribution). Vector of observations is y (i.e., unsuccessful or successful mating). Incidence matrix is X . Matrix fixed effect vector (i.e., carlina oxide exposed to LC₁₀, exposed to LC₃₀ and control *T. molitor* or *S. zeamais*) is expressed by β , and vector of the random residual effect is represented by ε . To identify significant differences between values, a probability level of 0.05 was used. The influence of the exposure to the sublethal concentrations of carlina oxide and control on duration of key behavioral traits during mating sequence for *T. molitor* (i.e., mate detection, antenna tapping, rubbing behavior, copulation, and whole mating sequence) and *S. zeamais* (i.e., mate detection, precopula, copula, and whole mating sequence) did not follow a normal distribution. Thus, data analysis was performed using the Steel-Dwass test at $\alpha = 0.05$ (Romano and Stefanini, 2022) after having been transformed to $\log(x + 1)$ to achieve normal variances (Zar, 2014; Scheff and Arthur, 2018). Data from both species were analyzed with the software JMP 16.2 (SAS Institute Inc, 2021).

3. Results

3.1. LC values of carlina oxide against *T. molitor* and *S. zeamais*

Sitophilus zeamais adults were more susceptible to carlina oxide compared to *T. molitor* adults since LC₁₀, LC₃₀, and LC₅₀ values were achieved at lower concentrations. Specifically, for *S. zeamais*, carlina oxide LC₁₀ was 251 ppm with 95 % CL (162–374), LC₃₀ was 820 (635–1020) ppm, and LC₅₀ was 1862 (1512–2323) ppm ($\chi^2 = 47.9$, $df = 33$, $p = 0.156$). For *T. molitor*, LC₅₀, LC₃₀, and LC₁₀ of carlina oxide were 2894 (2272–3823) ppm, 1290 (983–1640) ppm, and 401 (251–564) ppm ($\chi^2 = 32.3$, $df = 33$, $p = 0.503$), respectively.

3.2. Sublethal effects of carlina oxide on mating behavior of *T. molitor* and *S. zeamais*

Successful mating of *T. molitor* and *S. zeamais* exposed to carlina oxide (LC₁₀ and LC₃₀) or control is shown in Figs. 1 and 2, respectively. Exposure to sublethal concentrations (i.e., LC₁₀ and LC₃₀) of carlina oxide influenced mating success of *T. molitor* ($\chi^2 = 20.099$, $df = 2$, $p < 0.001$) and *S. zeamais* ($\chi^2 = 25.740$, $df = 2$, $p < 0.001$) males. When exposed to LC₃₀ carlina oxide, males of both species had fewer successful than unsuccessful copulations ($\chi^2 = 21.899$, $df = 1$, $p < 0.001$ for

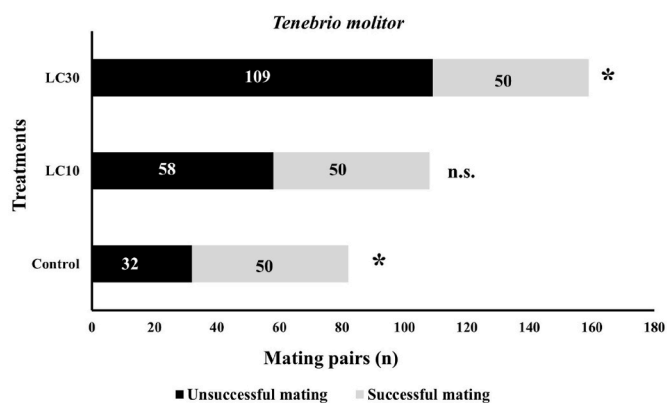


Fig. 1. Successful mating of males of *Tenebrio molitor* treated with LC₁₀ or LC₃₀ of carlina oxide or water (control). Significant differences are indicated by an asterisk (generalized linear model, binomial distribution, $p < 0.01$), while non-significant differences are indicated by the abbreviation n.s.

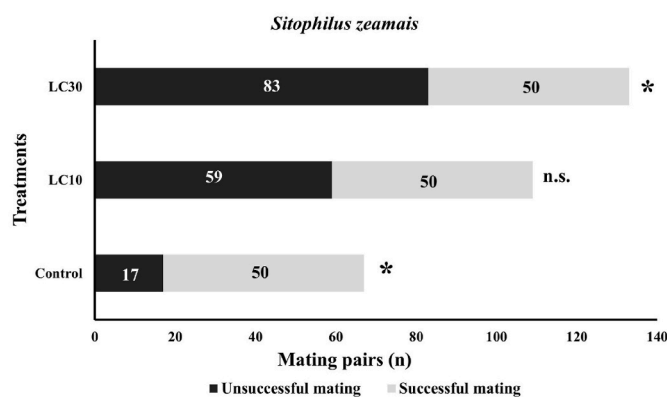


Fig. 2. Successful mating of males of *Sitophilus zeamais* treated with LC₁₀ or LC₃₀ of carlina oxide or water (control). Significant differences are indicated by an asterisk (generalized linear model, binomial distribution, $p < 0.01$), while non-significant differences are indicated by the abbreviation n.s.

T. molitor and $\chi^2 = 8.195$, $df = 1$, $p < 0.001$ for *S. zeamais*). In contrast, untreated males (control) performed a higher number of successful versus unsuccessful matings ($\chi^2 = 3.963$, $df = 1$, $p < 0.001$ for *T. molitor* and $\chi^2 = 16.269$, $df = 1$, $p < 0.001$ for *S. zeamais*). Concerning LC₁₀-exposed males of both species, no significant effect on mating success was observed ($\chi^2 = 0.601$, $df = 1$, $p > 0.05$ for *T. molitor* and $\chi^2 = 0.752$, $df = 1$, $p > 0.05$ for *S. zeamais*) (Figs. 1 and 2).

The mean duration of each phase during courtship and mating of both species was significantly affected by their exposure to sublethal concentrations of carlina oxide in comparison to control beetles (Figs. 3 and 4). The mean duration (s) of mate detection ($\chi^2 = 225.3$, $df = 2$, and $p < 0.001$), antenna tapping ($\chi^2 = 180.9$, $df = 2$, and $p < 0.001$), rubbing behavior ($\chi^2 = 134.5$, $df = 2$, and $p < 0.001$), and whole mating sequence ($\chi^2 = 36.6$, $df = 2$, and $p < 0.001$) was longer in treated pairs than in the control pairs of *T. molitor* (Fig. 3). In contrast, mean duration of copulation was significantly longer in control pairs (81.4 s) followed by LC₁₀-treated pairs (72.6 s), and LC₃₀-treated pairs (57.4 s) ($\chi^2 = 87.8$, $df = 2$, and $p < 0.001$).

A similar pattern was observed for *S. zeamais* (Fig. 4). The mean duration (min) of copula was significantly shorter in treated couples with carlina oxide LC₃₀ (223.4 min), than carlina oxide LC₁₀-treated couples (230.0 min) and control couples (240.7 min) ($\chi^2 = 102.5$, $df = 2$, and $p < 0.001$) (Fig. 4). Mean duration of mate detection ($\chi^2 = 193.2$, $df = 2$, and $p < 0.001$) and precopula ($\chi^2 = 99.2$, $df = 2$, and $p < 0.001$) was significantly increased after exposure to sublethal concentrations of

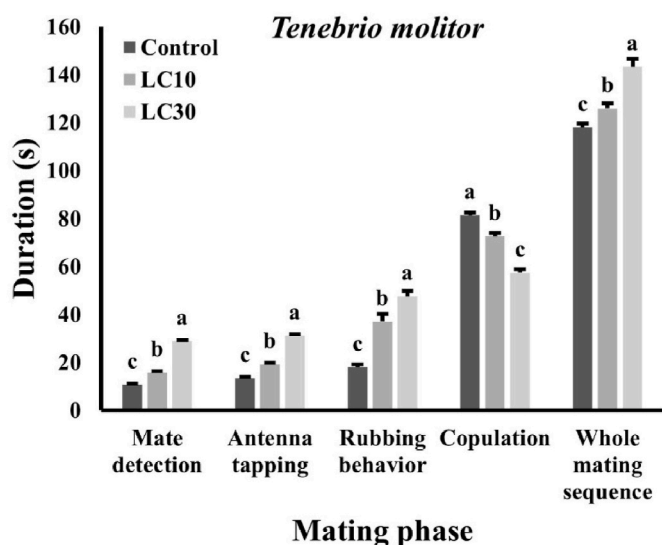


Fig. 3. Effect on the duration (mean followed by SE) of mating phases in *Tenebrio molitor* treated with LC₁₀ or LC₃₀ of carlina oxide or water (control). Within each phase, different letters indicate significant differences (Steel-Dwass test, $p < 0.05$).

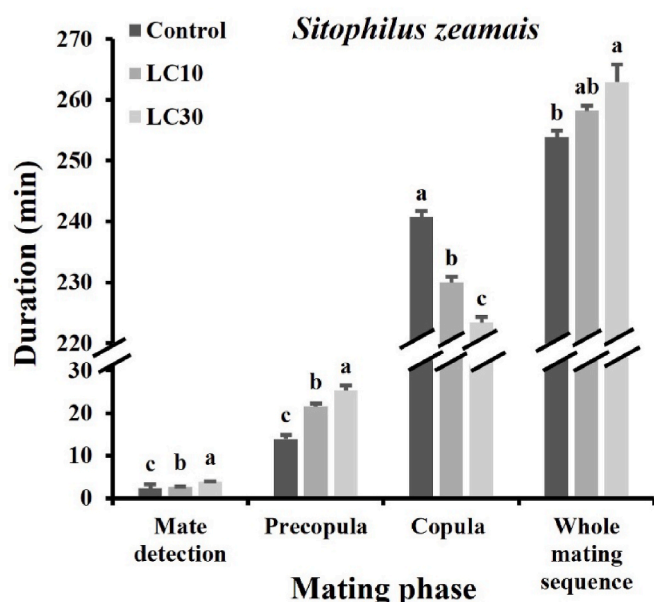


Fig. 4. Effect on the duration (mean followed by SE) of mating phases in *Sitophilus zeamais* treated with LC₁₀ or LC₃₀ of carlina oxide or water (control). Within each phase, different letters indicate significant differences (Steel-Dwass test, $p < 0.05$).

carlina oxide compared to control (i.e., control < LC₁₀ < LC₃₀). Regarding the whole mating sequence, the mean duration was significantly longer in carlina oxide LC₃₀-treated pairs than the control pairs, while the mean duration of the carlina oxide LC₁₀-treated pairs did not differ significantly from the aforementioned pairs ($\chi^2 = 27.4$, $df = 2$, and $p < 0.001$).

Mating success of both species was affected by male lateralization when approaching ($\chi^2 = 35.382$, $df = 5$, $p < 0.001$ for *T. molitor* and $\chi^2 = 36.061$, $df = 11$, $p < 0.001$ for *S. oryzae*) and mounting ($\chi^2 = 94.071$, $df = 11$, $p < 0.001$ for *T. molitor* and $\chi^2 = 43.194$, $df = 8$, $p < 0.001$ for *S. oryzae*) the females, regardless the treatment or control (Figs. 5 and 6). Males of both species showed a left-biased trend which resulted in

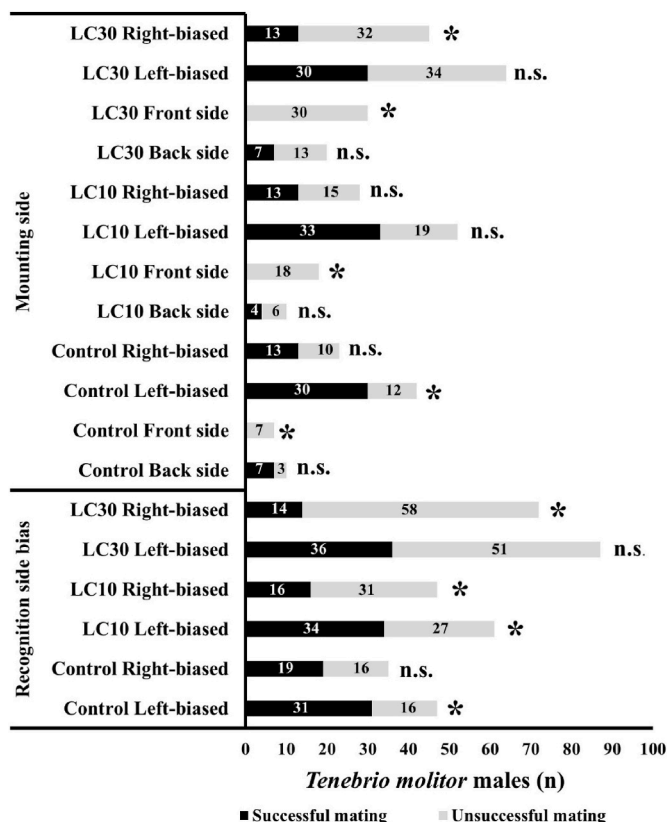


Fig. 5. Successful mating of *Tenebrio molitor* males treated with LC₁₀ or LC₃₀ of carlina oxide or water (control) performing or not lateralized approaches and mountings. Significant differences are indicated by an asterisk (generalized linear model, binomial distribution, $p < 0.01$), while the abbreviation n.s. indicates non-significant differences.

higher successful copulations than right-biased, front-side or back-side males. For *T. molitor*, the carlina oxide LC₁₀-treated males ($\chi^2 = 0.819$, $df = 1$, $p < 0.001$) and control males ($\chi^2 = 4.809$, $df = 1$, $p < 0.001$) that approached females from their left side exhibited high mating success (Fig. 5). Similarly, males of LC₁₀ carlina oxide group ($\chi^2 = 0.819$, $df = 1$, $p > 0.05$) and control group ($\chi^2 = 7.738$, $df = 1$, $p < 0.001$) when performed left-biased mountings achieved high successful copulations. In contrast, mating success of carlina oxide LC₃₀-treated males was low when approached ($\chi^2 = 2.597$, $df = 1$, $p > 0.05$) and mounted ($\chi^2 = 0.266$, $df = 1$, $p > 0.05$) females from their left side. Concerning right-biased males, only in the control groups the mating success was high ($\chi^2 = 0.286$, $df = 1$, $p > 0.05$ for recognition side and $\chi^2 = 0.286$, $df = 1$, $p > 0.05$ for mounting side).

Regarding *S. zeamais*, the same pattern was noted for males exhibiting left-biased approaches and mountings, resulting in higher mating success than the right-biased males (Fig. 6). Concerning the recognition side, the highest mating success was recorded in control groups in left-biased ($\chi^2 = 11.600$, $df = 1$, $p < 0.001$) and right-biased males ($\chi^2 = 7.391$, $df = 1$, $p < 0.001$) followed by the carlina oxide LC₁₀-treated left-biased males ($\chi^2 = 0.880$, $df = 1$, $p > 0.05$). Males of control that mounted females from their left side ($\chi^2 = 17.816$, $df = 1$, $p < 0.001$) achieved higher successful copulations over males that preferred the right side to mount ($\chi^2 = 1.541$, $df = 1$, $p > 0.05$). In carlina oxide LC₁₀ group, the left-biased males ($\chi^2 = 2.103$, $df = 1$, $p > 0.05$) achieved high mating success. Right-biased males had low mating success ($\chi^2 = 1.542$, $df = 1$, $p > 0.05$). Mating success of carlina oxide LC₃₀-treated males was low when approached ($\chi^2 = 2.597$, $df = 1$, $p > 0.05$ for left-biased males and $\chi^2 = 0.531$, $df = 1$, $p > 0.05$ for right-biased males) and mounted ($\chi^2 = 0.980$, $df = 1$, $p > 0.05$ for left-biased males and $\chi^2 = 3.617$, $df = 1$, $p > 0.05$ for right-biased males) females from their left or right side.

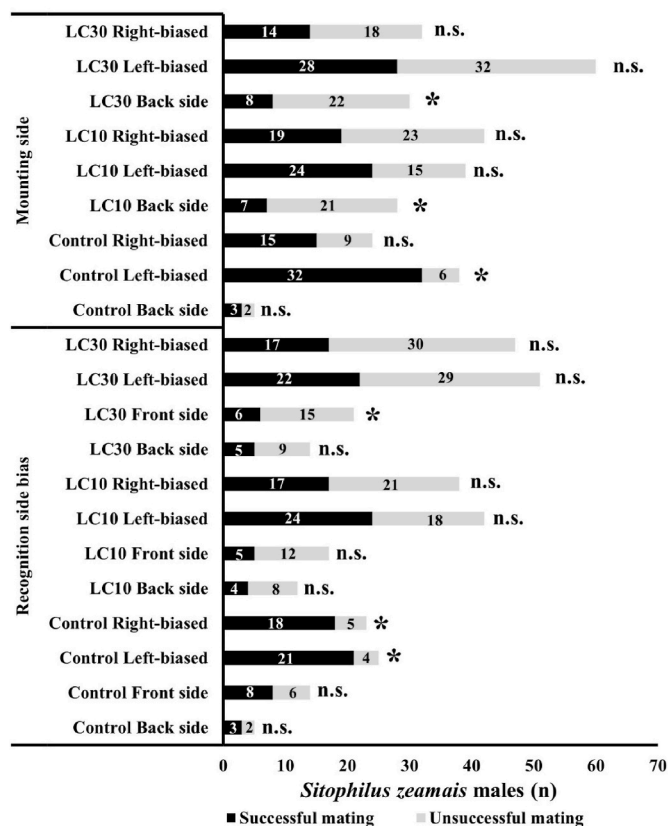


Fig. 6. Successful mating of *Sitophilus zeamais* males treated with LC₁₀ or LC₃₀ of carlina oxide or water (control) performing or not lateralized approaches and mountings. Significant differences are indicated by an asterisk (generalized linear model, binomial distribution, $p < 0.01$), while the abbreviation n.s. indicates non-significant differences.

4. Discussion

Carlina oxide is a polyacetylene contained mainly in the roots of species belonging to the genus *Carlina* L. with an interesting potential as a green insecticide towards several targets including mites, mosquitoes, and beetles among others (Pavela et al., 2021; Kavallieratos et al., 2022; Rizzo et al., 2024; Novák et al., 2024). Its high lipophilicity allows it to penetrate the cuticle of pests producing important morphological and physiological alterations (Spinozzi et al., 2023b; Tortorici et al., 2024). On the other hand, it is still unclear whether this compound may affect the central nervous system or the detoxifying system of insects.

The results of the contact toxicity tests showed that *S. zeamais* adults were more susceptible to carlina oxide than *T. molitor* adults as LC values were achieved using lower concentrations. This variation in susceptibility between the two species could be explained by the different body sizes. According to Lewis (1980), small insects are particularly sensitive when exposed to a toxicant because their size confers an elevated surface area to volume ratio, and the routes to the nervous system are short. Indeed, *S. zeamais* (length of about 3–3.5 mm), is about 6 times smaller than *T. molitor* (length of about 20 mm) (Mason and McDonough, 2012; Hagstrum et al., 2013).

The negative impact of carlina oxide on the male mating success of both species is an interesting finding of the current study. The exposure of *S. zeamais* and *T. molitor* to LC₁₀ and LC₃₀ of carlina oxide resulted in lower mating success when compared to the control beetles. Similar findings have been reported for males of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) after exposure to the LC₁₀ and LC₃₀ of the hexane extract (HE) derived from *Acmella oleracea* (L.) R. K. Jansen (Asteraceae) (Boukouvala et al., 2023). The reduction in the ability of

males of the aforementioned species to mate successfully after their sublethal exposure to carlina oxide (current study) or *A. oleracea* HE (Boukouvala et al., 2023) may lead to the inhibition of their population growth. The duration of the phases during courtship and mating of the tested species was affected after their exposure to carlina oxide. According to previous reports, the chemical communication of insects, such as pheromone production and mate finding, can be influenced by exposure to sublethal concentrations of insecticides (Desneux et al., 2007; Müller, 2018; Wang et al., 2018). Recently, Gidari et al. (2024) found that α -cypermethrin LC₃₀-treated males of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) needed more time to detect females than LC₁₀-treated and control males. In the current study, for both species, the duration of mate recognition was longer in treated pairs than in control pairs. Furthermore, a significant reduction in the duration of copulation was observed in the pairs treated with carlina oxide compared to the controls. In a previous study, de Oliveira et al. (2012) reported a reduction in the mating duration of *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) males treated with azadirachtin (neem) at the maximum recommended dose for tomato crops, which may impair the reproduction capacity of this predator. Treated males of *P. nigrispinus* may exhibit a reduction in the production of sperm, and through shorter copulation, males are not able to transfer sufficient sperm to females (Rodrigues et al., 2009; de Oliveira et al., 2012).

The presence and extent of behavioral asymmetries are another aspect that needs to be investigated. Lateralization, i.e., the differences in the structures and/or functions of the right/left hemispheres of the brain, may boost the ability of brain to accomplish cognitive tasks that involve the two hemispheres concurrently (Rogers et al., 2004). So far, several males of stored-product coleopterans present a left-biased trend in population level, leading to higher numbers of successful copulations than the right-biased males. For instance, males of the tenebrionids *T. castaneum* and *T. confusum* performing left-biased approaches and mountings, succeeded in more matings than males displaying right-biased traits (Benelli et al., 2017a; Boukouvala et al., 2019). Similar observations have been reported for males of the dermestid *Trogoderma granarium* Everts (Coleoptera) (Benelli et al., 2017b). Also, most males of the laemophloeid *Cryptolestes ferrugineus* (Stephens) (Coleoptera) presented left-biased turnings of 180°, accomplishing more successful matings when compared to right-biased males (Boukouvala et al., 2022). In the current study, *T. molitor* and *S. zeamais* were left-biased at the population level during mate approach and mounting, achieving more successful copulations than the right-biased males, regardless of the treatment and control. The exposure of both species to carlina oxide LC₁₀ and LC₃₀ did not affect lateralized traits but negatively affected mating success in comparison to the control. Similarly, Gidari et al. (2024) reported that the lateralized approaches of *A. diaperinus* males to their mates (i.e., right-biased) were not altered after their treatment with α -cypermethrin (LC₁₀ and LC₃₀), but mating success was changed. Whether natural or chemical insecticides modify lateralization features and mating performance of stored-product insects as a general trend remains to be investigated.

This study shows that the use of carlina oxide at low concentrations negatively impacts the mating behavior, the duration of phases during courtship and copulation, and successful matings of *T. molitor* and *S. zeamais*, issues that could influence the reproduction potential (Boukouvala et al., 2023). In other insect species, e.g., *M. dirhodum*, the LC₃₀ of carlina oxide decreased the potential natality by 53% compared to the control and caused a 36% inhibition of fertility (Novák et al., 2024). In addition, when larvae of *Cx. quinquefasciatus* exposed to LC₃₀ of a carlina oxide analogue, 29% successful adult emergence was recorded (Spinozzi et al., 2023a). Nonetheless, more investigation is required to determine whether carlina oxide is linked with successful insemination, fertility and fecundity, egg hatchability, and morphological deformations of progeny of *T. molitor* and *S. zeamais*.

CRediT authorship contribution statement

Maria C. Boukouvala: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nickolas G. Kavallieratos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Filippo Maggi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Marta Ferrati:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Eleonora Spinozzi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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