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# Effect of Marigold (*Tagetes patula* L.) Aqueous Extract on the Greater Wax Moth *Galleria mellonella* (L., 1758)

Utjecaj vodenog ekstrakta kadifice (*Tagetes patula* L.) na velikog voskovog moljca *Galleria mellonella* (L., 1758)

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## Abstract

The greater wax moth *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) is one of the most significant pests in beekeeping. *G. mellonella* larvae cause serious damage by boring into the honeycombs, leaving behind silken tunnels and feces that can transmit pathogens. This study evaluated the chemotactic response and effects of marigold (*Tagetes patula* L.) aqueous extracts on larval mortality, pupation and adult emergence of *G. mellonella*. In the first experiment, the chemotactic behavior of *G. mellonella* larvae was examined in response to marigold aqueous extracts at concentrations of 5%, 7.5%, and 10%. The results indicate that only the 10% marigold aqueous extract, two hours after treatment, acted as an attractant towards *G. mellonella* larvae. The second experiment investigated the effect of the same concentrations of marigold aqueous extracts on larval mortality, pupation and adult emergence of *G. mellonella*. The results revealed a statistically significant increase in larval mortality in the neem oil and 5% marigold aqueous extract treatments compared to the control treatment. Although 5% marigold aqueous extract exhibited insecticidal activity against *G. mellonella*, further studies are needed to assess practical efficiency.

***Keywords:*** chemotaxis; *Galleria mellonella*; insecticidal activity; larval mortality; marigold aqueous extract

## Sažetak

Veliki voskov moljac (*Galleria mellonella* L., 1758) (Lepidoptera: Pyralidae) je jedan od najznačajnijih štetnika u pčelarstvu. Ličinke velikog voskovog moljca uzrokuju ozbiljne štete na saću, ostavljajući za sobom svilene tunele i ekskremente koji mogu prenositi patogene. Cilj istraživanja bio je proučiti kemotaksični odgovor ličinki i učinke vodenih ekstrakata kadifice (*Tagetes patula* L.) na smrtnost ličinki, kukuljenje i izlazak odraslih jedinki *G. mellonella*. U prvom pokusu ispitano je kemotaksično ponašanje ličinki *G. mellonella* prema vodenim ekstraktima kadifice u koncentracijama od 5%, 7.5% i 10%. Rezultati pokazuju da ekstrakti nisu imali učinka ni pri jednoj od ispitivanih koncentracija, osim kod 10% ekstrakta, kod kojeg je nakon 2 sata zabilježena aktivnost djelujući na ličinke kao atraktant. Drugi pokus istraživao je učinak istih koncentracija vodenih ekstrakata kadifice na smrtnost ličinki, kukuljenje i izlazak odraslih jedinki *G. mellonella*. Rezultati su pokazali statistički značajno veći broj uginulih ličinki u tretmanima neem uljem i 5 %-tnim vodenim ekstraktom kadifice u odnosu na kontrolni tretman. Iako je 5% vodeni ekstrakt kadifice pokazao insekticidnu aktivnost protiv *G. mellonella*, potrebna su dodatna istraživanja kako bi se procijenila praktična učinkovitost.

***Ključne riječi:*** *Galleria mellonella*; insekticidno djelovanje; kemotaksija; mortalitet ličinki; vodeni ekstrakt kadifice

## Introduction – *Uvod*

Insect attacks in agricultural production may cause considerable losses, often exceeding damage caused by phytopathogens and weeds, since these attacks can occur before, during, and after the crop cycle (Santos et al., 2016; Santos et al., 2022). In recent decades, insect pests were mostly managed with synthetic insecticides, but intensive use of chemical pesticides for plant protection can lead to serious environmental pollution and health risks for humans and animals. Furthermore, the unnecessary use of pesticides reduces their effectiveness due to the development of resistant populations of harmful organisms (Tavares et al., 2021). The greater wax moth *Galleria mell-*

*onella* (L., 1758) is an insect from the family Pyralidae within the order Lepidoptera (Kwadha et al., 2017). It was first described in the 4th century BC by Aristotle as a honeybee pest (Serrano et al., 2023). *Galleria mellonella* larvae are one of the most significant pests in beekeeping, causing damage known as galleriasis by boring into the honeycomb and developing silken tunnels, trapping emergent bees, which therefore die of starvation. Other than that, *G. mellonella* larval feces can contain pathogens such as *Paenibacillus larvae* (White, 1906) that cause American foulbrood (Kwadha et al., 2017; Prđun and Virić Gašparić, 2023; Serrano et al., 2023,). Additionally, *G. mellonella* has received more attention as a model host than as a honeybee pest. Their larvae are a popular choice in academia because of numerous advantages, such as ease of inoculation, freedom from legal or ethical constraints and low cost (Piatek et al., 2020; Ménard et al., 2021). While there are measures available on the market for controlling *G. mellonella*, only some are permitted for use in beekeeping, which highlights the need for development of safer and more ecologically acceptable alternatives such as biopesticides. The genus *Tagetes* belongs to the Asteraceae family and is among the four most researched plants that could be used as botanical insecticides (Tavares et al., 2021; Cicevan et al., 2022). Currently, about 126 secondary metabolites have been identified in *Tagetes* species, including phenolic derivatives, thiophene derivatives, phenylpropanoids, triterpenoids, steroids, alkaloids, flavonoids, carotenoids, and others (Salinas-Sanchez et al., 2012; Santos et al., 2022). It has been established that marigold extracts, including essential oils, possess antioxidative, fungicidal, antimicrobial, nematicidal, and insecticidal properties, and those properties vary depending on the marigold species and the type of extract (Weaver et al., 1994; Krishna et al., 2005; Santos et al., 2016). The objective of the research is to determine the effect of aqueous extract of marigold on larval mortality, pupation and adult emergence of the greater wax moth, as well as to examine the orientation behavior of larvae in the presence of these extracts.

## Materials and Methods – *Materijali i metode*

**Insect Rearing – *Uzgoj kukaca***

The research was conducted in October 2024 under laboratory conditions. Final instar larvae of *G. mellonella* from laboratory culture at the Faculty of Agrobiotechnical Sciences Osijek were used. The larvae were grown under room conditions on artificial food according to a modified method (Wiesner, 1993).

**Collection of Plant Material and Preparation of Aqueous Extract – *Prikupljanje biljnog materijala i priprema vodenog ekstrakta***

The aerial biomass of marigold (*Tagetes patula* L.) were collected in the flowering phase from a private garden in Pakrac, Croatia. The fresh plant material was air-dried in a warm, dark, and dry location for 14 days. The dried plant mass was then ground into powder using an electric mill. Aqueous marigold extract was prepared according to Norsworthy (2003), with several modifications. An amount of 10 g of dry plant mass was mixed with 100 ml of distilled water and left to extract for 24 hours. The mixture was after that strained through muslin cloth to remove larger particles and then filtered through filter paper. The resulting 10% extract was subsequently diluted to obtain concentrations of 5% and 7.5%.

**Chemotaxis Bioassay – *Biološki test kemotaksije***

Petri dishes with a 9 cm diameter were used for the experiment. Each dish was divided into three equal zones marked with numbers 1, 2, and 3. In each zone, a circular mark was drawn on the bottom of the plate. To the circle in zone 1, 10 µl of marigold aqueous extract was applied, while distilled water was applied as a control group to the circle in zone 3. One *G. mellonella* larvae was placed in the middle zone of the dish marked with number 2. The experiment was conducted with three different concentrations of marigold aqueous extract (5%, 7.5%, and 10%). Per treatment, 30 of the final instar larvae were used. After the larvae were placed in the center of zone 2, the dishes were covered and left in the dark. Larvae were examined after 30 min, 1 h, 2 h, and 24 h; their position within Petri dishes was recorded and assigned a prescribed index: 0 – larvae in the control zone, 1 – larvae in the middle zone, and 2 – larvae in the treated zone. The chemotaxis index (CI) was calculated according to the formula by Laznik and Trdan (2016): (Number of larvae in treated zone - number of larvae in control zone)/Total number of larvae per treatment. The chemotaxis index was interpreted as follows: ≥ 0.2 was considered an attractant, 0.2 to 0.1 a weak attractant, 0.1 to −0.1 neutral, −0.1 to −0.2 a weak repellent, and ≤ −0.2 a repellent.

**Bioassay: larval mortality, pupation and adult emergence – *Biološki test: smrtnost ličinki, kukuljenje i izlazak odraslih jedinki***

The assay plates used were 9 cm Petri dishes, lined with circularly cut filter paper, with larvae food placed on top. Three concentrations of marigold aqueous extract (5%, 7.5%, and 10%) were used, neem oil (trade name: Neem Oil, cold-pressed neem (*Azadirachta indica*, A.Juss.) seed oil applied at the manufacturer's recommended concentration of 1%) as a positive control, and distilled water as a negative control. The experiment was set up in three replications, and in each dish, 10 last instar larvae were placed, totaling 30 larvae per treatment. Treatments were performed by applying 1 µl of extracts to the dorsal side of the larvae. After treatment, larvae were left to dry and then transferred to previously prepared Petri dishes, which were closed with lids, and kept at room temperature (24 ± 2 °C) in darkness. After treatment, food was added as usual. The experiment was monitored for 37 days until adults emerged. Turning activity, developmental abnormalities, mortality, number of pupae, number of adults, and other observations were recorded. Larval mortality was recorded on the 3rd and 8th day after the start of the experiment.

**Statistical Analysis – *Statistička analiza***

All collected data was analysed using the Statistica 14.1.0.8 (2023) program. A analysis of variance (ANOVA) with single variable factor was performed, and differences between mean treatment values were tested with the post hoc Tukey HSD test. Statistical analysis of larval mortality was performed on log-transformed data to meet the assumptions of normality and homogeneity of variance, while figures present total mortality percentages ± SE.

## Results and Discussion – *Rezultati i rasprava*

**Chemotactic Response of *G. mellonella* Larvae to Marigold Aqueous Extract - *Kemotaktički odgovor ličinki G. mellonella na vodeni ekstrakt kadifice***

The movement of greater wax moth larvae under the influence of chemical stimuli varied across different time intervals. At 30 min post-treatment, the 5% extract showed no effect on larval chemotaxis, with 57% of larvae remaining in the central zone. Over the 24 h observation period, larval preference shifted slightly, but mostly stayed in the middle zone. Additionally, by the end of the experiment, two dead larvae were found in the centre zone. The 7.5% marigold aqueous extract induced the highest rate of larval movement toward the treated area at 1 h after treatment. After 24 h in this treatment, six dead larvae were found. Similar to the 5% treatment, larvae did not show directional movement during the first 30 min at 10% concentration, with 60% remaining in the central zone. After 1 h, affinity was almost equal toward all zones, and after 2 h, the greatest attraction toward the treated zone was recorded, with a total of 53% of larvae in that zone. After 24 h, preference toward the treated zone decreased to 27% of larvae. In this treatment, after 24 h, dead larvae were also recorded, totaling 10, which represents 33.33% of the total number of larvae in that treatment.

**Table 1** Chemotaxis Index (CI) of *G. mellonella* larvae at different observation periods after treatment with marigold aqueous extract

**Tablica 1.** Indeks kemotaksije (IK) ličinki G. mellonella tijekom različitih razdoblja promatranja nakon tretmana s vodenim ekstraktom kadifice.

| Concentration<br>*Koncentracija* | 30 minutes<br>*30 minuta* | 1 hour<br>*1 sat* | 2 hours<br>*2 sata* | 24 hours<br>*24 sata* |
|---|---|---|---|---|
| **5 %** | *-0.033* | *-0.033* | *-0.033* | *-0.133* |
| **7.5 %** | *-0.067* | 0.100 | *0.033* | *0.033* |
| **10 %** | -0.067 | -0.067 | 0.267 | *-0.133* |
| *CI interpretation: ≥ 0.2 = attractant; 0.2 to 0.1 = weak attractant; 0.1 to −0.1 = neutral; −0.1 to −0.2 = weak repellent; ≤ −0.2 = repellent.* | | | | |

According to Table 1, the 5% marigold aqueous extract showed no effect (CI = -0.033) at 30 min, 1 h, and 2 h, but

demonstrated weak repellent activity (CI = -0.133) after 24 h. In the treatment with 7.5% marigold aqueous extract, the CI indicates a neutral response at all time intervals, except after 1 h (CI = 0.1), when weak attractant activity was observed. The 10% extract showed no effect at 30 min and 1 h (CI = -0.067 and -0.067, respectively), acted as an attractant after 2 h (CI = 0.267) and demonstrated weak repellent activity after 24 h (CI = -0.133). While field studies have demonstrated the effectiveness of marigold plants as intercrop companions for repelling various Lepidoptera pests (Calumpang and Ohsawa, 2015; Laffon et al., 2022; Shen et al., 2026,), research specifically examining extracts of *Tagetes* species against lepidopteran pests remains limited. The results of this research showed predominantly neutral responses to marigold aqueous extract, with weak repellent activity observed at 5% and 10% concentrations after 24 h. The results of this research showed no consistent repellent effect of marigold aqueous extract, which supports findings of Mmbone et al. (2014), who tested aqueous extract of *Tagetes minuta* L. on adult black bean aphid *Aphis fabae* Scopoli, 1763. Their results showed no significant repellent activity of the extract against black bean aphids. However, their study demonstrated that extract concentration influenced the degree of repellency, with higher repellency observed at higher concentrations, contrary to the present study where greater repellency was recorded at the lowest extract concentration, whereas in the present study, weak repellent activity was observed at both the lowest (5%) and highest (10%) concentrations after 24 h, indicating no clear concentration-dependent response. Murovhi et al. (2020) conducted a field study investigating the effects of aqueous extract of *T. minuta* on okra – damaging aphids, finding that *T. minuta* extract had the greatest effect in reducing aphid populations compared to all other treatments tested. A field study was also conducted by Sakadzo et al. (2020) using aqueous extract of *T. minuta* against rapeseed aphids, which resulted in a statistically significant reduction in aphid populations on rapeseed. Kimutai et al. (2017) investigated the repellent effect of *T. minuta* essential oils against *Phlebotomus duboscqi* Neveu-Lemaire, 1906, finding that the highest repellency was recorded in the first 30 min, then decreased over time. Additionally, repellency increased with increasing essential oil concentration, with 100% repellent activity at 1 mg/ml concentration. These findings are opposed to the present study, in which larvae did not exhibit directional movement in the first 30 min, and weak repellent activity was observed at both 5% and 10% concentration after 24 h. The limited effectiveness of the aqueous extracts in this study could be attributed to multiple factors affecting *Tagetes* composition, including plant species, harvest time, phenological stage, geographic factors, plant parts used, and extraction solvent (Salehi et al., 2018; Murovhi et al., 2020).

**Larval Mortality** – ***Mortalitet ličinki***

After 3 days of exposure, the highest larval mortality was recorded in the neem treatment, where seven larvae died, corresponding to 23.33% mortality. In the 5% marigold aqueous extract treatment, five dead larvae were recorded (16.67%), while three larvae died in the 10% marigold aqueous extract treatment (10.00%). The lowest mortality among treated groups was observed in the 7.5% marigold aqueous extract treatment, with one dead larva (3.33%). No larval mortality was recorded in the control treatment. Although differences in mortality were observed among treatments, statistical analysis showed that there were no statistically significant differences among treatments after 3 days of exposure (Figure 1a). These results indicate that the effect of the tested treatments on insect mortality was already evident within the first days after exposure, particularly in the neem and 5% marigold aqueous extract treatments. After 8 days of exposure, larval mortality increased in all treatments compared to day 3. The highest mortality was recorded in the 5% marigold aqueous extract treatment, where nine larvae died (30.00%), followed by the neem treatment with eight dead larvae (26.67%). Five dead larvae were recorded in the 10% marigold aqueous extract treatment (16.67%), while the lowest mortality among treated groups remained in the 7.5% marigold aqueous extract treatment, with two dead larvae (6.67%). No mortality was observed in the control group throughout the experiment. Statistical analysis revealed statistically significant differences among treatments after 8 days of exposure. The 5% marigold aqueous extract and neem treatments caused significantly higher mortality compared to the control treatment, while the 7.5% and 10% marigold aqueous extract treatments exhibited lower insecticidal efficacy compared to neem and the 5% marigold aqueous extract treatment (Figure 1b). These findings suggest that prolonged exposure enhanced the insecticidal activity of both neem and marigold aqueous extracts against the tested larvae.

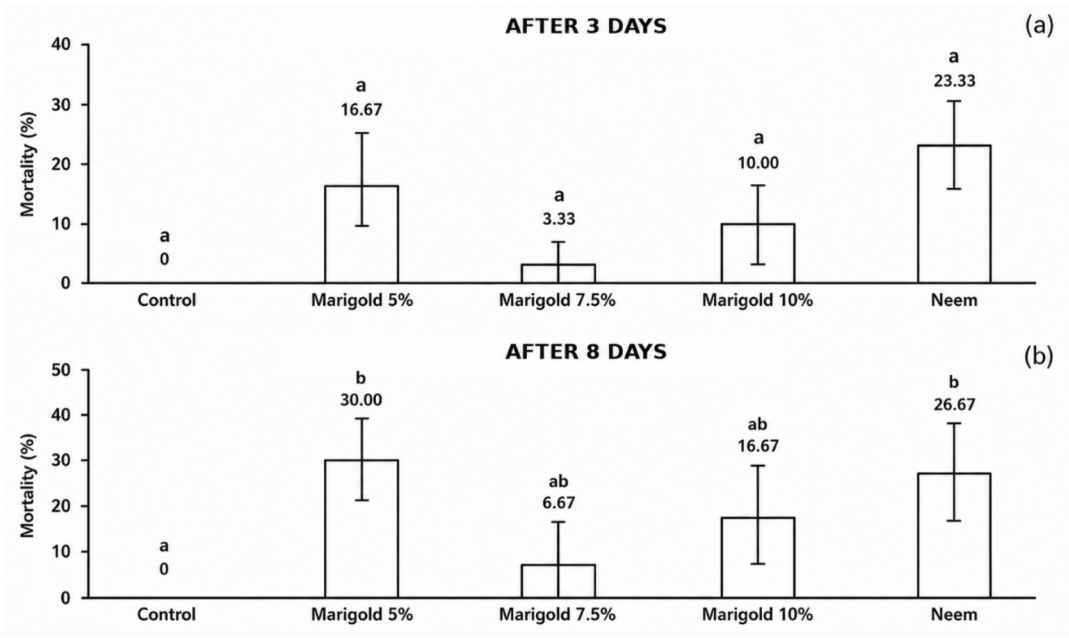



**Figure 1** Mortality of *Galleria mellonella* larvae after exposure to different concentrations of marigold aqueous extract following 3 (a) and 8 (b) days of treatment. Error bars represent standard deviation. Different letters above the bars indicate statistically significant differences between treatments ($p < 0.05$)

**Slika 1.** Mortalitet ličinki po tretmanima nakon 3 (a) i 8 (b) dana.

According to Salinas-Sanchez et al. (2012), a plant extract must cause >40% mortality in test organisms to be classified as a bioinsecticide. In the present study, the highest larval mortality was recorded in the 5% marigold aqueous extract treatment, reaching 30% after 8 days of exposure (Figure 1b). Since this value did not exceed the proposed 40% threshold, the tested marigold aqueous extracts cannot yet be classified as effective bioinsecticides according to this criterion. Interestingly, the lowest extract concentration (5%) induced the highest mortality, while higher concentrations showed lower insecticidal efficacy. This trend contrasts with the dose-dependent relationship commonly reported in the literature, where increasing extract concentrations result in higher larval mortality. For example, Mishra et al. (2023) reported that aqueous extract of *Tagetes erecta* L. caused the highest mortality of *Agrotis ipsilon* (Hufnagel, 1766) larvae at the highest tested concentration, while similar observations were reported by Mmbone et al. (2014) using aqueous extract of *T. minuta* against black bean aphids. Comparable results to the present study were reported by Fabrick et al. (2020), who evaluated methanolic and aqueous extracts of *Tagetes patula* L. against tobacco whitefly, *Bemisia tabaci* (Gennadius, 1889) and *Lygus hesperus* Knight, 1917, where the lowest concentration of methanolic marigold extract resulted in the highest mortality among tested concentrations. Salinas-Sanchez et al. (2012) investigated *T. erecta* flower and leaf extracts against fall armyworm larvae, *Spodoptera frugiperda* Smith, J. E., 1797 and reported 72% larval mortality following oral application. Wells et al. (1993) demonstrated that *T. minuta* extracts exhibited stronger insecticidal effects against *Aedes (Stegomia) aegypti* (L., 1762) and *Anopheles (Cellia) stephensi* Liston, 1901 larvae and adults compared to *T. erecta* and *T. patula* extracts, particularly when extracts were prepared from whole plants. Furthermore, Nikkon et al. (2009) concluded that the insecticidal activity of *T. erecta* extracts against *Tribolium castaneum* (Herbst, 1797) larvae decreased with increasing larval age. Therefore, the relatively low mortality observed in the present study may be associated with the developmental stage of the tested larvae, as younger larval instars could potentially exhibit greater susceptibility to marigold aqueous extracts.

**Pupation – *Kukuljenje***

Pupae were first observed on day seven (Figure 2), with the earliest pupation recorded in the 7.5% marigold aqueous extract treatment. By day nine, pupation was detected in all treatments, although the number of pupae remained relatively low. A substantial increase in pupation was observed from day thirteen onwards, particularly in the control, neem oil, and 10% marigold aqueous extract treatments. The highest number of newly formed pupae across all treatments was recorded on days thirteen and fifteen. The control treatment exhibited the highest overall pupation dynamics throughout the experiment, while the 5% marigold aqueous extract treatment showed delayed and reduced pupation compared to the other treatments. Additionally, reduced larval movement was observed in all treatments on day ten, while approximately 50% of larvae in the 10% marigold aqueous extract and neem oil treatments remained similar in size to larvae at the beginning of the experiment. On day thirteen, one small non-pupated larva was observed in the 5% marigold aqueous extract treatment and died the following day.

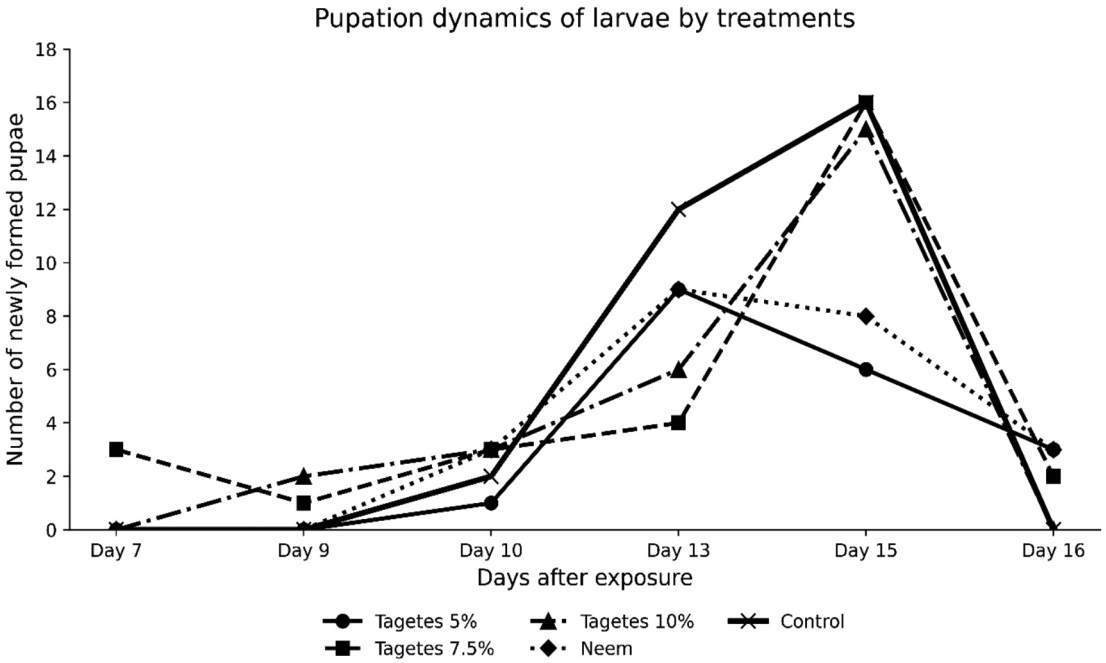



**Figure 2** Dynamics of pupation in *Galleria mellonella* larvae following exposure to different treatments
**Slika 2.** Dinamika kukuljenja ličinki *Galleria mellonella* po tretmanima tijekom pokusa.

The control group exhibited the highest number of pupae with 96.66%, followed by the 10% marigold aqueous treatment with 86.66%, the 7.5% treatment with 83.33%, and the neem oil treatment with 70%. The lowest number of pupae was recorded in the 5% marigold aqueous extract treatment with 60% (Figure 3). Although small differences in mean pupation values were observed among treatments, the applied treatments did not significantly affect pupation rates. The absence of significant effects on pupation in the present study is consistent with the findings of Modise and Ashafta (2015), who reported no significant pupal mortality after the application of aqueous *Tagetes minuta* extracts, regardless of concentration. However, statistically significant effects were observed when hexane extracts were used.

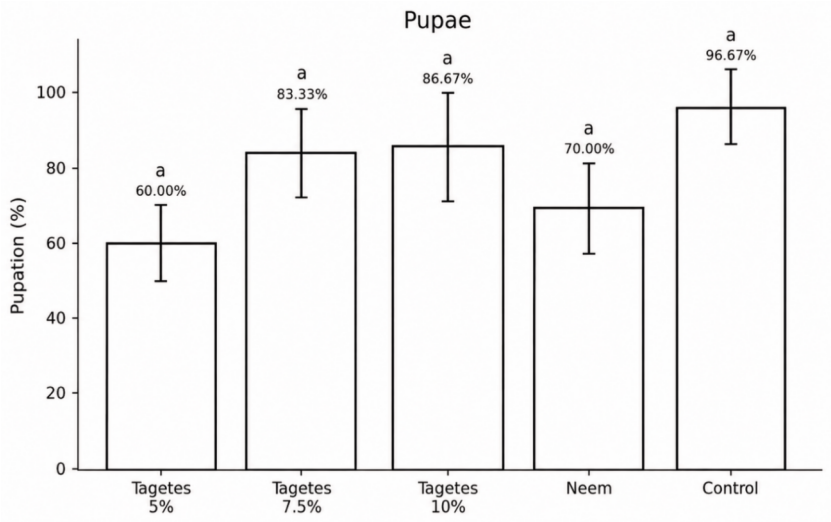



**Figure 3** Pupation percentage of larvae by treatments after 12 days of exposure
**Slika 3.** Postotak kukuljenja ličinki po tretmanima nakon 12 dana.

Similarly, Salinas-Sanchez et al. (2012) achieved significant pupal mortality using *T. erecta* extracts prepared with n-hexane, acetone, and ethanol, resulting in pupal mortality rates of 48%, 60%, and 72%, respectively. Non-polar extracts, particularly those obtained using n-hexane, often contain lipophilic bioactive compounds such as terpenoids, which can more easily penetrate the insect cuticle and enhance insecticidal efficacy (Purnama et al. 2025).

**Adult Emergence – *Pojava odraslih***

The first adult emerged in the neem oil treatment on day 14 post-treatment, while the latest adult emergence was recorded in the 5% marigold aqueous extract treatment on day 27. The highest number of newly emerged adults across all treatments was observed on day 27 post-treatment, after which adult emergence gradually declined (Figure 4).

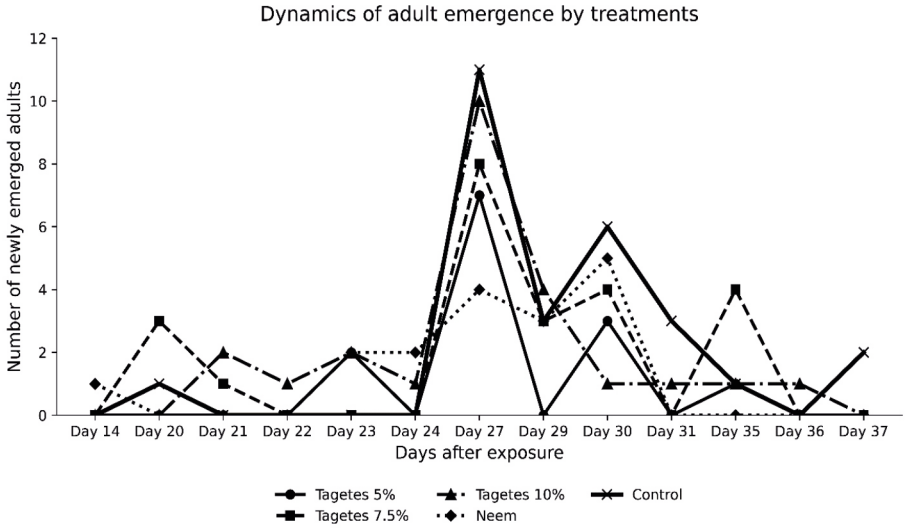



**Figure 4** Dynamics of adult emergence of *Galleria mellonella* under different treatments during the experimental period
**Slika 4.** Dinamika pojave odraslih jedinki *Galleria mellonella* po tretmanima tijekom pokusa.

Adult emergence differed among treatments. The control group resulted in the highest number of emerged adults, with 27 individuals (90%; first emergence on day 20), followed by the 7.5% marigold aqueous extract treat-

ment with 25 emerged adults (83.33%; first emergence on day 20). The 10% marigold aqueous extract treatment resulted in 23 emerged adults (76.67%; first emergence on day 21), whereas the neem oil treatment resulted in 17 emerged adults (56.67%; first emergence on day 14). The lowest number of emerged adults was recorded in the 5% marigold aqueous extract treatment, with 14 emerged adults (46.67%; first emergence on day 27). Statistical analysis revealed a significantly lower adult emergence in the 5% marigold aqueous extract treatment compared with the control treatment, whereas the remaining treatments did not differ significantly from the control group (Figure 5). Dead adults were recorded in all treatments, including five in the 7.5% marigold aqueous extract treatment, four in the 10% marigold aqueous extract treatment, six in the neem oil treatment, and one in the 5% marigold aqueous extract treatment. In the 5% marigold aqueous extract treatment, one dead adult exhibited severe wing damage associated with pathogenic symptoms. Similarly, in the 10% marigold aqueous extract treatment, three adults displayed pathogenic symptoms, including malformed or damaged wings. In the neem oil treatment, the first emerged adult already exhibited clear wing deformation symptoms. At the end of the observation period, the control treatment exhibited the highest number of living adults, whereas the neem oil treatment showed the lowest. Although numerical differences among treatments were observed, statistical analysis revealed no significant differences among treatments compared with the control group.

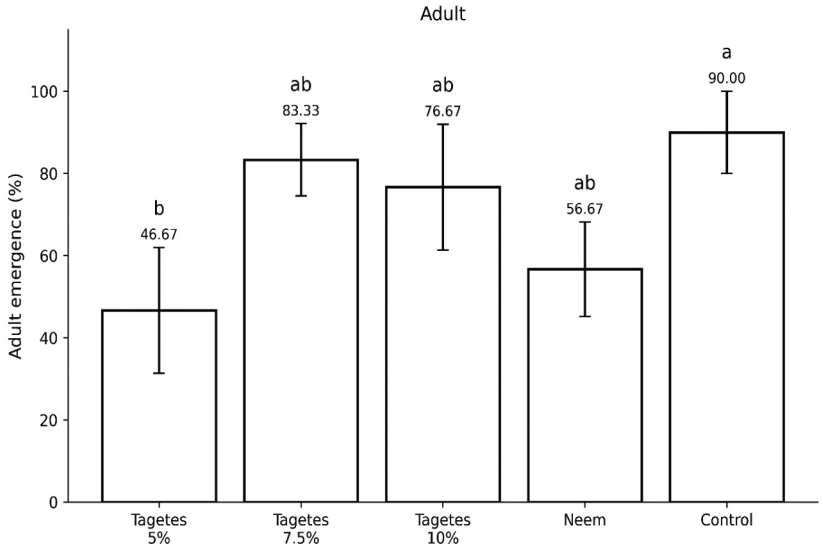



**Figure 5** Effect of treatments of marigold aqueous extracts on adult *Galleria mellonella* emergence
**Slika 5.** Učinak tretmana vodenim ekstraktima kadifice na pojavu odraslih jedinki *Galleria mellonella*.

Islam and Talukder (2005) reported an inhibition rate (IR%) of 57.09% with 5% *T. erecta* extract against *T. castaneum*, resulting in 42.91% adult emergence, without statistically significant differences. Our results show a similar pattern, where 5% marigold aqueous extract achieved the lowest adult emergence (46.67%), also without significant differences among treatments. Dunkel et al. (2010) similarly demonstrated that *T. minuta* extract exhibits concentration dependent effects, where the lowest concentrations prevented adult eclosion of *Tetanops (Eurycephalomyia) myopaeformis* (Röder, 1881).

## Conclusion – *Zaključak*

Aqueous *Tagetes patula* extracts had limited effects on *Galleria mellonella* larval chemotaxis, except for the 10% extract, which exhibited attractant activity. The 5% extract caused the highest larval mortality and reduced adult emergence, indicating the greatest insecticidal potential among the tested concentrations. Although higher extract concentrations accelerated pupation, they prolonged adult development. Overall, the 5% aqueous *Tagetes* extract showed promising potential for the control of *G. mellonella*, warranting further investigation under practical conditions.

## References – *Literatura*

Calumpang, S.M.F., Ohsawa, K. 2015. Repellency of Marigold, *Tagetes erecta* L. (Asteraceae) Volatile Organic Chemicals to Eggplant Fruit and Shoot Borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Crambidae). *Journal of the International Society for Southeast Asian Agricultural Sciences*, 21: 119-128.

Cicevan, R., Sestras, A.F., Plazas, M., Boscaiu, M., Vilanova, S., Gramazio, P., Vicente, O., Prohens, J., Sestras, R.E. 2022. Biological Traits and Genetic Relationships Amongst Cultivars of Three Species of *Tagetes* (Asteraceae). *Plants,* 11: 760. https://doi.org/10.3390/plants11060760

Dunkel, F.V., Jaronski, S.T., Sedlak, C.V., Meiler, S.U., Veo, K.D. 2010. Effects of Steam-Distilled Shoot Extract of *Tagetes minuta* (Asterales: Asteraceae) and Entomopathogenic Fungi on Larval *Tetanops myopaeformis*. *Environmental Entomology*, 39: 979-988. https://doi.org/10.1603/EN09259

Fabrick, J.A., Yool, A.J., Spurgeon, D.W. 2020. Insecticidal activity of marigold *Tagetes patula* plants and foliar extracts against the hemipteran pests, *Lygus hesperus* and *Bemisia tabaci*. *PLOS One*, 15(5): e0233511. https://doi.org/10.1371/journal.pone.0233511

Islam, M.S., Talukder, F.A. 2005. Toxic and residual effects of *Azadirachta indica*, *Tagetes erecta* and *Cynodon dactylon* seed extracts and leaf powders towards *Tribolium castaneum*. *Journal of Plant Diseases and Protection*, 112: 594-601. https://doi.org/10.1007/BF03356157

Kimutai, A., Ngeiywa, M., Mulaa, M., Njagi, P.G.N., Ingonga, J., Nyawamu, L.B., Ombati, C., Ngumbi, P. 2017. Repellent effects of the essential oils of *Cymbopogon citratus* and *Tagetes minuta* on the sandfly, *Phlebotomus duboscqi*. *BMC Research Notes*, 10: 98. https://doi.org/10.1186/s13104-017-2396-0

Krishna, A., Prajapati, V., Bhasney, S., Tripathi, A. K., Kumar, S. 2005. Potential toxicity of new genotypes of *Tagetes* (Asteraceae) species against stored grain insect pests. *International Journal of Tropical Insect Science,* 25: 122-128. https://doi.org/10.1079/IJT200560

Kwadha, C.A., Ong'amo, G.O., Ndegwa, P.N., Raina, S.K., Fombong, A.T. 2017. The Biology and Control of the Greater Wax Moth, *Galleria mellonella*. *Insects,* 8: 61. https://doi.org/10.3390/insects8020061

Laffon, L., Bischoff, A., Gautier, H., Gilles, F., Gomez, L., Lescourret, F., Franck, P. 2022. Conservation Biological Control of Codling Moth (*Cydia pomonella*): Effects of Two Aromatic Plants, Basil (*Ocimum basilicum*) and French Marigolds (*Tagetes patula*). *Insects*, 13: 908. https://doi.org/10.3390/insects13100908

Laznik, Ž., Trdan, S. 2016. Attraction Behaviors of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) to Synthetic Volatiles Emitted by Insect Damaged Potato Tubers. *Journal of Chemical Ecology*, 42: 314-22. https://doi.org/10.1007/s10886-016-0686-y

Ménard, G., Rouillon, A., Cattoir, V., Donnio, P.Y. 2021. *Galleria mellonella* as a Suitable Model of Bacterial Infection: Past, Present and Future. *Frontiers in Cellular and Infection Microbiology,* 11: 782733. https://doi.org/10.3389/fcimb.2021.782733

Mishra, S., Chitranshi, R., Shekher, R., Saini, M., Goyal, D., Kumar, R., Narayan, S., Srivastava, S., Kumar, A. Katiyar, S. 2023. A study of dry flower extract of *Tagetes erecta* as herbal pesticides on larval mortality and emergence of *Agrotis ipsilon*. *European Chemical Bulletin*, 12: 18698-18705. doi: 10.48047/ecb/2023.12.si4.1661.

Mmbone, S., Mulaa, M., Wanjala, F.M., Nyukuri, R.W., Cheramgoi, E. 2014. Efficacy of *Tagetes minuta* and *Taphrosia vogelii* crude Leaf Extracts on *Tetranychus urticae* (Acari: Tetranychidae) and *Aphis fabae* (Homoptera: Aphididae). *African Journal of Science and Technology*, 5: 168-173. http:/dx.doi.org/10.14303/ajfst.2014.055

Modise, S.A., Ashafa, A.O.T. 2015. Larvicidal, pupicidal and insecticidal activities of *Cosmos bipinnatus*, *Foeniculum vulgare* and *Tagetes minuta* against *Culex quinquefasciatus* mosquitoes. *Tropical Journal of Pharmaceutical Research*, 15: 965-972. http://dx.doi.org/10.4314/tjpr.v15i5.10

Murovhi, J., Phophi, M. M., Mafongoya, P. 2020. Efficacy of plant materials in controlling aphids on Okra (*Abelmoschus esculentus* L. Moench) in Limpopo Province of South Africa. *Agronomy*, 10: 1968. https://doi.org/10.3390/agronomy10121968

Nikkon, F., Habib, M. R., Rezaul, K., Ferdousi, Z., Motiur, R., Ekramul, H. 2009. Insecticidal activity of flower of *Tagetes erecta* L. against *Tribolium castaneum* (Herbst). *Research Journal of Agriculture and Biological Sciences*, 5: 748-753.

Norsworthy, J.K. 2003. Allelopathic Potential of Wild Radish *(Raphanus raphanistrum*). *Weed Technology*, 17: 307-313. https://doi.org/10.1614/0890-037X(2003)017 [0307:APOWRR]2.0.CO;2

Piatek, M., Sheehan, G., Kavanagh, K. 2020. Utilising *Galleria mellonella* larvae for studying *in vivo* activity of conventional and novel antimicrobial agents. *Pathogens and Disease*, 78: ftaa059. https://doi.org/10.1093/femspd/ftaa059

Prđun, S., Virić Gašparić, H. 2023. Veliki (*Galleria mellonella* L.) i mali (*Achroia grisella* Fabricius) voskov moljac – štetnici u pčelarstvu i metode suzbijanja. *Glasilo biljne zaštite*, 23: 360-373.

Purnama, U., Riyanti, S., Faramayuda, F. 2025. Activity Assay and Identification of Larvicidal and Repellent Compound Groups of Marigold Flower (*Tagetes erecta* L.). *International Journal of Applied Pharmaceutics,* 17: 153-161.

Sakadzo, N., Chisvuure, A., Nhara, R. 2020. Evaluating the Repellent Properties of Mexican Marigold (*Tagetes minuta*) Aqueous Extracts on Aphid Population in rape (*Brassica napus*). *Acta Scientific Microbiology*, 3: 65-70.

Salehi, B., Valussi, M., Morais-Braga, M.F.B., Carneiro, J.N.P., Leal, A.L.A.B., Coutinho, H.D.M., Vitalini, S., Kręgiel, D., Antolak, H., Sharifi-Rad, M., Silva, N.C.C., Yousaf, Z., Martorell, M., Iriti, M., Carradori, S., Sharifi-Rad, J. 2018. *Tagetes* spp. Essential Oils and Other Extracts: Chemical Characterization and Biological Activity. *Molecules*, 23: 2847. https://doi.org/10.3390/molecules23112847

Salinas-Sanchez, D.O., Aldana-Llanos, L., Valdes-Estrada, M.E., Gutierrez-Ochoa, M., Valladares-Cisneros, G., Rodriguez-Flores, E. 2012. Insecticidal activity of *Tagetes erecta* extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Florida Entomologist*, 95: 428-432. https://doi.org/10.1653/024.095.0225

Santos, P.C., Santos, V.H., Mecina, G.F., Andrade, A.R., Fegueiredo, P.A., Moraes, V.M., Silv,a R.M.G., Silva, R.M. 2016. Insecticidal activity of *Tagetes* sp. on *Sitophilus zeamais* Mots. *International Journal of Environmental & Agriculture Research*, 2: 31-38.

Santos, P.C., Granero, F.O., Junior, J.L.B., Pavarini, R., Pavarini, G.M.P., Chorilli, M., Zambom, C.R., Silva, L.P., Silva, R.M.G. 2022. Insecticidal activity of *Tagetes erecta* and *Tagetes patula* extracts and fractions free and microencapsulated. *Biocatalysis and Agricultural Biotechnology*, 45: 102511. https://doi.org/10.1016/j.bcab.2022.102511

Serrano, I., Verdial, C., Tavares, L., Oliveira, M. 2023. The Virtuous *Galleria mellonella* Model for Scientific Experimentation. *Antibiotics*, 12: 505. https://doi.org/10.3390/antibiotics12030505

Shen, J., Wang, H., Zhao, M., Zhang, R., Zhang, M., Mao, Y., Chen, S., Zhu, C., Han, T., Shen, X., Li, S. 2026. Efficacy of three *Tagetes* species for the suppression of lepidopteran pests in bagless apple orchards. *Frontiers in Agronomy*, 7: 1670233. https://doi.org/10.3389/fagro.2025.1670233

Tavares, W.R., Barreto, M.d.C., Seca, A.M.L. 2021. Aqueous and Ethanolic Plant Extracts as Bio-Insecticides—Establishing a Bridge between Raw Scientific Data and Practical Reality. *Plants,* 10: 920. https://doi.org/10.3390/plants10050920

Weaver, D.K., Wells, C.D., Dunkel, F.V., Bertsch, W., Sing, S.E., Sriharan, S. 1994. Insecticidal Activity of Floral, Foliar, and Root Extracts of *Tagetes minuta* (Asterales: Asteraceae) Against Adult Mexican Bean Weevils (Coleoptera: Bruchidae). *Journal of Economic Entomology*, 87: 1718-1725. https://doi.org/10.1093/jee/87.6.1718

Wells, C., Bertsch, W., Perich. M. 1993. Insecticidal volatiles from the marigold plant (genus *Tagetes*). Effect of Species and Sample Manipulation. *Chromatographia*, 35: 209-205. https://doi.org/10.1007/BF02269705

Wiesner, A., Gotz, P. 1993. Silica Beads Induce Cellular and Humoral Immune Responses in *Galleria mellonella* Larvae and in Isolated Plasmatocytes, Obtained by a Newly Adapted Nylon Wool Separation Method. *Journal of Insect Physiology*, 39: 865-876. https://doi.org/10.1016/0022-1910(93)90119-C