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Life cycle and effectiveness of selective insecticides against *Chrysolina herbacea* (Duftschmid, 1825) under laboratory conditions

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ABSTRACT

The life cycle and effectiveness of insecticides against *Chrysolina herbacea* adults under laboratory conditions were determined in this study. *C. herbacea* was chosen for this study because it is a major pest of Lamiaceae plants, particularly those in the genus *Mentha*. Significant damage is caused to plants by them consuming their leaves. The results of the life cycle revealed that the total egg, larval, pupal, and pre-oviposition periods were determined to be 8.5 ± 1.9, 21.7 ± 1.3, 32.6 ± 2.6, and 11.8 ± 1.2 days, respectively. The life cycle (from egg to pre-oviposition) was 73.3 ± 7.9 days. The toxic effects of alpha-cypermethrin, thiamethoxam, and acetamiprid on the mortality rate of *C. herbacea* adults were tested in laboratory conditions. Alpha-cypermethrin showed a maximum mortality rate in *C. herbacea* adults after 24, 48 and 72 h (26.7, 33.3 and 40 %) respectively. Meanwhile, Acetamiprid's minimum mortality rate (16.7 and 20 %) was observed after 24 and 48 hours of treatment. Results showed that alpha-cypermethrin was the most toxic to *C. herbacea* adults among the treated synthetic pesticides.

دراسة دورة الحياة و فعالية المبيدات الحشريه المختاره ضد الحشرة*Chrysolina herbacea* (Duftschmid, 1825)تحت الضروف المختبرية

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الخلاصه

دراسة دورة حياة وفعالية بعض المبيدات الحشرية ضد الحشرة الكاملة *Chrysolina herbacea* تحت ظروف المختبر. تم اختيار *C. herbacea* لهذه الدراسة لأنها من أكثر الحشرات ضررًا على النباتات عائلةLamiaceae ، وخاصة جنس Mentha يحدث الحشرة ضررا كبيرا للنباتات من خلال استهلاكها اللاوراق. أظهرت نتائج دورة الحياة أن متوسط فترات إجمالي للبيض واليرقات والعذراء و الفترة ما قبل وضع البيض كانت 8.5 ± 1.9 و 21.7 ± 1.3 و 32.6 ± 2.6 و 11.8 ± 1.2 يومًا على التوالي. وجد أن متوسط الفترة الإجمالية لدورة الحياة الحشرة (من البيضة إلى ما قبل وضع البيض) كانت 73.3 ± 7.9 يومًا. تم اختبار التأثيرات السامة لألفا سايبرمثرين وثياميثوكسام وأسيتاميبريد على معدل الوفيات لدى البالغين من *C. herbacea* في ظروف المختبر. أظهر ألفا سايبرمثرين اعلى معدل وفيات لدى البالغين من *C. herbacea* بعد 24 و 48 و 72 ساعة (26.7 و 33.3 و 40٪) على التوالي. في غضون ذلك، لوحظ أدنى معدل وفيات لأسيتاميبريد (16.7 و 20٪) بعد 24 و 48 ساعة من المعامله. أظهرت النتائج أن ألفا سايبرمثرين كان الأكثر سمية للبالغين من *C. herbacea* بين المبيدات الحشرية الاصطناعية االمستحدمة.

الكلمات المفتاحية: خنفساء أوراق النعناع, مدة الاطوار الغير الكاملة ,فعالية المبيدات الحشرية ,الوفيات.

INTRODUCTION

Chrysomelidae, commonly known as leaf beetles, is one of the largest families of phytophagous Coleoptera (Friedman, 2016), with over 50,000 species in 17 families and more than 2000 genera, and is widely distributed worldwide (Cox, 1976, Booth et al., 1990)This economically important family is considered a serious problem for agriculture worldwide (Aslan *et al.*, 1999) . However, some species of bionomics within this family remain poorly understood, including the mint leaf beetle, *Chrysolina herbacea* (Duftschmid 1825) (Coleoptera: Chrysomelidae). *C. herbacea* is A shiny, metallic green adult with a bronze color, resembling a jewel with an oval body, black legs, and antennae, is observed. The females' color, ranging from green to purplish grey, is noted (Şen, 2015, Ibrahim, 2023), along with black-colored larvae (Friedman, 2016). In the field, *Chrysolina* species oviposit on the above-ground parts of their host plants, and eggs are laid in mass. The eggs are cream-white. First-instar larvae remain aggregated for the first two days before dispersing (Suzuki and Saitoh, 2011). This species was observed on mint leaves in Iraq from January to June (Al-Nadawi, 2019, Ibrahim, 2023). *C. herbacea* feeds on plants in the Lamiaceae family, particularly those in the Mentha genus, which farmers cultivate on a large scale worldwide (Verma, 2006). People use these plants as spices for medicinal purposes (Nayak *et al.*, 2020) and as botanical insecticides to control certain agricultural pests (Kalemba and Synowiec, 2019). The larvae and adult beetles both cause significant damage to plants by consuming their leaves (Bozsik, 2006), resulting in both direct and indirect harm. The larvae and adult beetles chew the leaves, creating numerous holes. The black grubs and beetles cause direct damage, while indirect damage occurs due to leaf loss, which reduces photosynthesis, hinders growth, and may eventually lead to plant death. (Al-Nadawi, 2019). In addition to these damages, mint chemistry is also significantly altered by the beetle (Zebelo and Maffei, 2015, Gonçalves et al., 2019), causing energy to be diverted by the plant toward chemical defenses instead of growth and yield. Heavy infestations can weaken the plant, resulting in reduced biomass and essential oil production, which leads to financial losses for farmers (Zager *et al.*, 2019). Several types of control methods are available, such as cultural, biological, and chemical controls, depending on the specific pest and the approach used (Sandeep, 2024). Farmers have used chemicals for a long time to manage plant and insect pests in their crops (Okosun *et al.*, 2021). Their use becomes essential, particularly when pest populations exceed the economic threshold level (ETL). While insecticides offer benefits, their frequent and excessive application can have detrimental toxic effects on natural enemies, such as predators and parasitoids. Therefore, it is crucial to utilize targeted insecticides in pest control that do not adversely affect beneficial organisms (Ahmed and Majeed, 2016).

Many studies have evaluated the use of Alpha-Aceypermethrin and thiamethoxan against beetles such as *Trogoderma granarium* and *Tenebrio molitor* (Athanassiou *et al.*, 2015). However, scientific literature lacks recent studies on the effect of surfaces treated with pyrethroids against *C. herbacea*. Additionally, there is a lack of available data on the insecticidal efficacy of the neonicotinoid thiamethoxam and Acetamiprid against that species. There are many studies in Iraq about this species in the survey and morphological description, but the life cycle and control of this species are quite limited (Al-Nadawi, 2019, Kalemba and Synowiec, 2019).

This study aimed to elucidate the detailed life cycle and determine the most effective insecticides against *C.herbacea* to establish methods for reducing the damage caused by the beetle.

MATERIALS AND METHODS

Insects: The adults of *C. herbacea* were collected on mint plants from a field in Hiran in the Kurdistan Region using a sweep net and hand during January and February 2024. The sample was maintained at the plant protection laboratories at the College of Agriculture Engineering Science at Salahaddin University Erbil in Iraq under laboratory conditions (26±2ºC and 65±5% R.H).

Life cycle of insects: The following parameters were included in the studies on the life cycle of *C. herbacea* : pre-oviposition period, number of eggs laid, incubation period egg and hatching per day, duration of larval development and pupation, and generation time. In the laboratory, one generation had been raised before they were used. Glass jars (9x6x2 cm) were used to place a single pair of newly emerged adults of *C. herbacea*, with mint leaves also being placed in the jars for adult feeding. The damp filter paper was used to cover the bottom of the box to ensure humidity, while a muslin cloth was used to cover the top for air circulation and the Pre-oviposition period was determined. The female's eggs laid on mint leaves inside a glass jar were collected daily, counted, and transferred to glass Petri dishes (9 cm in diameter). A total of 252 eggs were observed for the number of eggs laid, and the incubation period with the hatching per day was recorded. The larvae were collected after hatching, and they were placed in new glass jars using a fine-point camel hairbrush. A total of 155 larvae were observed for larval duration (days), and 60 pupae were noted for pupal duration days until new adults emerged. The total generation time was estimated.

Insecticides: The experiment study tested three synthetic insecticides from two different chemical groups for efficacy against *C. herbacea,* as shown in Table 1. Details regarding the active ingredients, formulation types, trade names, chemical groups, and recommended field rates. Before conducting the bioassays, we prepared the synthetic insecticide concentrations by diluting them with tap water as recommended on the product labels. The choice of these insecticides was based on the fact that these chemicals have not been tested against *C. herbacea* in Iraq.

Table 1. List of pesticides, their active ingredients, formulation types, trade names, chemical groups and recommended field rates.

SN	Active ingredient	Formulation	Trade name	Chemical group	Rate/lit water
1	Alpha-cypermethrin	10% EC	Alpha Royal	Pyrethroids	0.5 ml
2	Thiamethoxam	25%WG	Actara Pro	Neonicotinoids	0.35 g
3	Acetamiprid	20%WP	CITA	Neonicotinoids	0.35 g

EC: Emulsifiable Concentrate, WG: Water-dispersible granules, WP: Wettable powder

Bioassay: The study estimated the toxicity of three pesticides (Alpha-cypermethrin, Thiamethoxam, and Acetamiprid) to *C. herbacea* adults using the Dipping technique. They prepared test solutions with concentrations of 0.5 ml, 0.35 gm, and 0.35 gm per L by diluting the pesticides with tap water. Fifteen individuals were divided into three replicates (5 each), placed in small pieces of gauze, and dipped in insecticide concentrations for ten seconds, including a contro1 group. The treated adults were then transferred to glass Petri dishes nine cm in diameter, containing filter papers, and provided with a leaf of mint as food (Abd-Ella *et al.*, 2022). The researchers recorded the percentage of adult mortality after 24, 48, and 72 hours of exposure. Adults were considered dead if they failed to move when touched with a fine camel brush. All treatments were conducted under controlled conditions: room temperature at 26±2 ℃, RH at 65±5%, and 14:10 (L:D) light-dark cycle. Statistical analysis: Statistical analysis was performed using the SAS software (Godfrey and Loots, 2014), and treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955) at a significance level of 0.05. Regression analysis for certain parameters was conducted using Excel.

RESULT AND DISCUSSIONS

Life cycle of the *C. herbacea*: Oviposition was initiated on the tenth day after the emergence of new adults and concluded at two months based on the data (Figure 1). In two months, a total of 252 eggs were laid. The maximum mean number of eggs deposited by a female was 27 eggs on the 10th day after newly emerged adults, while the minimum mean number of eggs deposited was 1.3 eggs on the 64th day after the new adult's emergence under laboratory conditions.

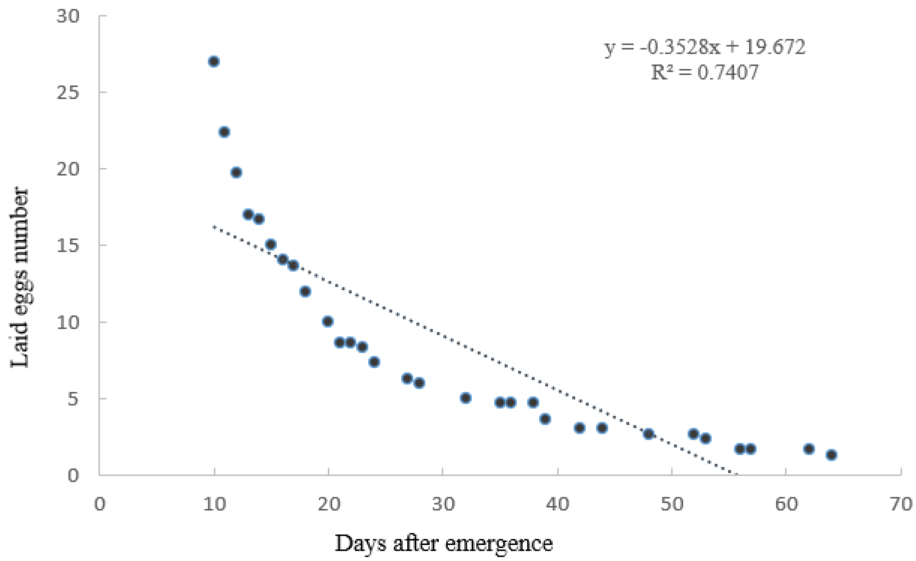

Figure 1. Regression between days of oviposition of *C. herbacea* after emergence and the number of eggs laid

In Figure 2, it was shown that the eggs began hatching after 6 days of being deposited by the female and continued to hatch for a total of 11 days. The highest number of eggs was found to hatch 9 days after being laid, while the lowest number was found to hatch 11 days after being laid.

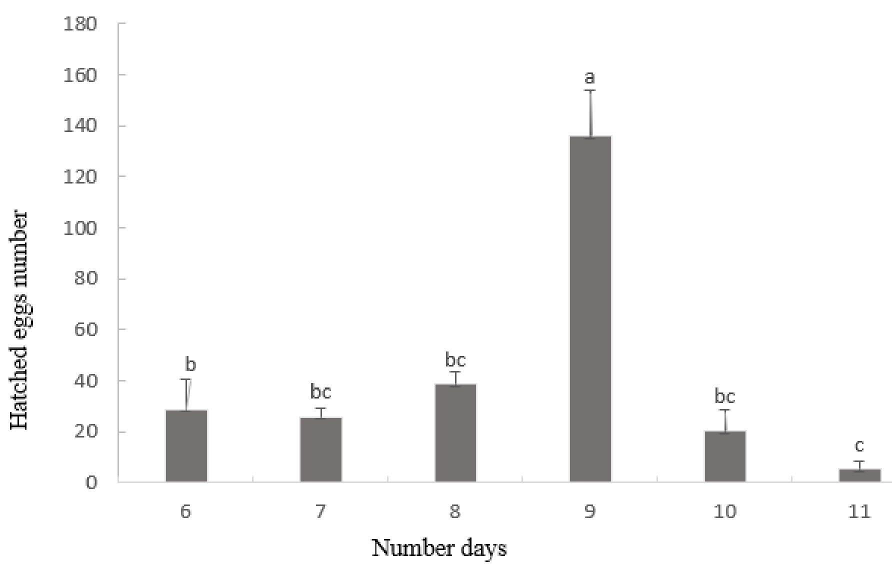

Figure 2. Number of days and number of eggs hatched

Table 2 indicates that the egg incubation period was (8.5 ± 1.9) days, with the highest number of days required for eggs to hatch being (11 days) and the lowest number of days being (6 days) under laboratory conditions. It was observed by Suzuki and Saitoh (2011) The incubation period of *Chrysolina* (*Erythrochrysa*) *polita* (Linnaeus, 1758) was found to be 15 days under laboratory conditions of 16L-8D at 20℃. The total mean duration of larval stages was (21.7 ± 1.3), with the highest total duration of the larval stage being (24 days) and the lowest duration being (20 days). The mean period of the pupal stage was 32.6 ± 2.6 days, with the highest duration of the pupal stage being (35 days) and the lowest duration being (28 days). The mean pre-oviposition period was (11.8 ± 1.2) days. Based on the duration of the different developmental stages (egg to pre-oviposition), the generation period of *C. herbacea* was estimated. The highest generation period of *C. herbacea* was (83 days). The lowest period was (64 days), with a mean period of (73.3 ± 7.9) days under laboratory conditions, as shown in Table 2.

Suzuki and Saitoh (2011) reported that less similar duration days were observed, with some notes on the life history of *Chrysolina* (*Erythrochrysa*) *polite* (Linnaeus, 1758). It was recorded that the 1st instar larva takes 4-9 days, the 2nd 4-7 days, the 3rd 6-10 days, and the 4th 16-22 days, and the total larval instar period was 30-40 days. The pupa period was noted to be 10-14 days. The total period from egg to adult was 63-68 days under laboratory conditions of 16L-8D at 20℃. The growth of larvae of the leaf beetle *Chrysolina brunnicornis* in the field in the cage was observed to be rather slow. After ten days, the first instar was seen moulting to the second instar, followed by the third instar ten days later. Pupal development was found to last for ten to fourteen days (Khruleva, 2004).

Table 2: Duration (days) of life cycle parameters for *C. herbacea* under laboratory conditions.

Life stage	N	Duration(days) Mean ± SD.	Range
Egg	252	8.5 ± 1.9	(6-11)
Larvae	155	21.7 ± 1.3	(20-24)
Pupa	60	32.6 ± 2.6	(28-35)
pre -oviposition	32	11.8 ± 1.2	(10-13)
Generation -period	**Egg to egg**	73.3 ± 7.9	(64-83

The effect of different selected insecticides on the Percent mortality of *C. herbacea:* The results are presented in Table 3. The results show that Alpha-cypermethrin recorded the maximum adult mortality with 26.7, 33.3, and 40 % at 24, 48, and 72 hours after treatment, respectively. It was followed by Thiamethoxam, which made 23.3, 26.7, and 33.3 % adult mortality at 24, 48, and 72 hours after treatment, respectively. Acetamiprid showed 16.7, 20 and 36.7% minimum adult mortality at 24, 48 and 72 hours after treatment, respectively. Alpha-cypermethrin is the fastest of the three, providing rapid effects within minutes to hours upon contact with the insect; as a pyrethroid insecticide, it disrupts sodium channels in the insect's nerve cells, causing hyperactivity, paralysis, and death (Bhardwaj *et al.*, 2020). Acetamiprid and thiamethoxam are both intermediate-speed systemic insecticides. These require time to be absorbed by the plant and taken up by the pest, and their effects typically take several hours to days to appear (Simon-Delso *et al*., 2015) (Simon-delso *et al* 2015). These findings were in agreement with those of Kalemba and Synowiec (2019), who reported that alpha-cypermethrin 15% WDG (150 and 300 g/ha) and lambda-cyhalothrin CS10% (75 ml/ha) were the most effective insecticides for the control of canola flea beetles belonging to the Chrysomelidae family after 14 days of insecticide application. Alpha-cypermethrin was more effective than thiamethoxam in controlling *C. herbacea.* This result was similar to previous findings by Athanassiou *et al.* (2015)who reported that alpha-cypermethrin was more toxic than thiamethoxam against *Trogoderma granarium* adults after 1,3, and 7 days of exposure.

Table 3: Effect of different selected insecticides on the Percent cumulative mortality of *C.* *`herbacea* under laboratory conditions:

		% Mortality (±SE)					
Treatment	**n**	**24 h**		**48 h**		**72 h**	
Alpha-cypermethrin	15	26.7±	3.3a	33.3±	3.3a	40.0±	5.8a
Thiamethoxam	15	23.3±	3.3a	26.7±	3.3a	33.3±	8.8a
Acetamiprid	15	16.7±	8.8a	20.0±	5.8b	36.7±	8.8a
Control	15	0.0±	0.0b	0.0±	0.0c	0.0±	0.0b

Means followed by the same letters in each column are not significantly different (P<0.05)

CONCLUSIONS

In conclusion, a study of the life cycle of *C. herbacea* reported several key stages, including the pre-oviposition period, the number of eggs laid, the incubation period of eggs, the hatching rate per day, the duration of larval development and pupation, and the overall generation time. In terms of insecticide effectiveness, Alpha-cypermethrin was found to be highly effective, with significant control of *C. herbacea* achieved after 24, 48, and 72 hours of exposure, outperforming other tested insecticides. These findings have important implications for pest management in mint crops, with targeted control methods being developed by researchers to protect crops and minimize economic losses.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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