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Determination of Repellent Activity of *Carthamus dentatus* Vahl Plant Extracts against Some Storage Pests

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ABSTRACT

The harmful effects of pesticides have led researchers to find feasible, harmless, and economical alternatives. In this regard, plant phenolics have been thoroughly investigated. Storage insects, such as *Sitophilus oryzae*, *Sitophilus granarius*, and *Trogoderma granarium*, significantly impacted seed storage in humid areas. The cauline, phyllary and flower extracts of *Carthamus dentatus* were examined to identify suitable plant extracts. The experiment was conducted under laboratory conditions. Phenolic analysis indicated that chrysin was the most abundant in cauline leaves (39.58 ng/µL), while naringenin was the highest (13.20-15.66 ng/µL) in the phyllaries and flowers. The cauline leaf extract had the highest mortality rate among all the insects. The LT_{50} and LT_{90} values for the cauline leaf extract were found to be 3 and 6 days, respectively. On the other hand, the LT_{50} for the phyllary and flower extracts was approximately twice as long (7.55-8.72 and 8.24-8.78 days for phyllary and flower extracts, respectively). This study also demonstrated that the cauline leaf extract of *Carthamus dentatus* has the potential to obtain organic insecticide that can be used against the evaluated storage insects.

Keywords: *Carthamus*, insect, phenolics, repellent, extract.

INTRODUCTION

Seeds can be stored for extended periods without quality loss if proper storage conditions are maintained. During this storage phase, seeds may be exposed to various factors that cause losses, with pests being the most well-known factor. Cereals are the most preferred group due to their high nutritional value. Several insect species affecting stored cereals including *Sitophilus spp.*, *Tribolium spp.*, *Plodia interpunctella* (Hubner), *Rhyzopertha dominica* (Fabricius), *Oryzaephilus surinamensis* (L.), and *Ephestia kuehniella* (Zell.) (Yaseen et al., 2019; Emekçi and Ferizli, 2000; Hamel et al., 2020; Guru et al., 2022). Cereals must be preserved in good quality until the next sowing period or even longer. As population growth and globalization increase the importance of food accessibility, pests in storage cause significant losses. In the fight against these pests using natural methods, plant extracts and essential oils are commonly employed. These substances can have fumigant, contact insecticidal, repellent, attractant, egg-laying, and insect feeding effects. The use of synthetic insecticides and fumigants in controlling warehouse pests has increasingly led to various problems. In recent years, because synthetic pesticides threaten the health of both consumers and producers, and have negative effects on the environment, research into biologically active, plant-origin bio-insecticides that have minimal impact on non-target organisms and the environment has become a critical approach in agricultural pest management. In many parts of the world, stored grain pests have developed resistance to insecticides, particularly phosphine (Champ, 1985; Bell and Wilson, 1995; Chaudry, 1999; Talukder, 2009). For this reason, researchers are investigating natural phytochemical sources that have effects similar to those of commercial chemicals. The Asteraceae family is the largest source of various phytochemicals in the plant world.

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The genus *Carthamus*, known as safflower, consists of herbaceous, annual plants in the Asteraceae family, typically with a thorny structure. *Carthamus* species grow naturally on slopes, stony lands, and in areas up to an altitude of approximately 2300 meters above sea level (Arslan et al., 2010). Safflower flowers were traditionally used in ceremonies and rituals in ancient Egypt, and were also reported to be the source of red dye used in similar traditional applications in India. Safflower flowers have also been used for medicinal purposes in various cultures. Researchers studying their medicinal properties have identified various phenolic compounds in the composition of the flowers. Previous studies have reported that these phenolic compounds possess antimicrobial properties similar to commercial chemicals, acting against various organisms (Rafiq et al., 2021; Abuova et al., 2022).

Today, many chemicals are used to reduce the effects of microorganisms on human health. However, the negative impact of intensive chemical use on health has drawn increasing attention from consumers. As a result, there has been an increasing trend towards natural products, especially in organic and good agricultural practices in recent years. Interest in herbal remedies, plant extracts, and preparations obtained without intensive industrial processes is rising. Health-conscious consumers opt for organic products to maintain a healthy lifestyle and reduce their exposure to synthetic chemicals. This study investigated the potential of extracts from the stem leaves, phyllary leaves, and flower leaves of *Carthamus dentatus* as insect repellents or lethal agents.

MATERIAL AND METHODS

Insect material

The experimental material was obtained from insects found in storage facilities, and their identification was initially carried out by Prof. Dr. İzzet Akça from the Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayıs University. The cultures were purified and propagated, and stock cultures were established. Experiments were carried out using *S. oryzae*, *S. granarius* adults and *Trogoderma granarium* beetle larvae selected from the temperature, humidity and light controlled Entomology Laboratory at the Plant Protection Department of the Faculty of Agriculture of Ondokuz Mayıs University. All tested insects were of the same size and maturity.

Test Environment

For the cultivation of *Sitophilus oryzae* and *Sitophilus granarius*, soft bread whole wheat containing 11-13% product moisture is utilized as a nutrient; grains combined with sugars were used as food for the Kappra beetle. The materials were sieved to remove foreign contaminants and stored at -18°C for around a week to prevent contamination by other insects. About 200-300 mixed-sex adult individuals from female and male persons were added to the one-litter glass jars of clean items after reaching room temperature in the form of 250 gr. In order to ensure egg lying, the jar openings were sealed with gauze to enable air entry and outflow. They were then kept for 3-7 days in a dark environment in an insect breeding chamber set to a temperature of 26±2°C and a relative humidity of 65±5%. After that, daily observations of the new generation's adult emergence were made. The adults were removed from the product with the help of a sieve once the egg-laying process had finished, which had been taken around 35-40 days. These were then added to the uncontaminated product to maintain the culture, and this process was continued throughout the study. New generation adults aged 7 to 10 days were employed in biological experiments.

Plant Material

All plant materials used for extraction were collected from *Carthamus dentatus* plants identified in the natural area of Ondokuz Mayıs University campus, located in Samsun, in the Black Sea region of Turkey. The collection site is situated at 41°21'44.8"N, 36°11'16.5"E, approximately 3.3 km from the coastline at an altitude of 190 meters. The local flora primarily consists of annual and perennial grasses, along with a few tree species.

Healthy and uncontaminated cauline, phyllary, and flower leaves were collected with sharp tweezers and scissors. The collected leaf samples were dried on Wattman papers at room temperature (24°C) for 2 weeks. Daily measurements were made to ensure that the material was dehydrated.

Extract Preparation

Pieces of dry leaves were well ground by an electric blender. The powdered samples were kept in methanol for 24 hours in 3 repeats. The liquid part is filtered, separated, placed in the pipette tank, and weighed. Methanol in the solution was evaporated with the rotary evaporator. The extract weight was calculated by weighing the remaining part. A stock was created by adding 10 times pure water to the dry extract. Additionally, the doses used in this study were prepared by diluting dry extracts 200, 400 and 800 times with pure distilled water.

Phenolic Content Analysis

Phenolic analysis was conducted using an LC-MS instrument, with the conditions according to Hacıkamiloğlu et al. (2022). Flow rate 0.8 ml/min, wavelength 300/200 nm, column temperature 30°C, detector type DAD, injection volume 10 uL. After analysis, 16 different compounds were identified in the chromatogram (Figure 1).

Repellent Impact Study

The experiment was carried out in 4 replications and in each replication, 10 insect materials consisting of adults of the insect species *S. oryzae* and *S. granarius* and larvae of the insect species *T. granarium* were used as experimental material. Each repetition was placed in the 10x10 cm plastic containers. The experiment was conducted under controlled conditions (20±5^{o}C and 60±5% humidity). Each extract was sprayed for exactly 2.00 ml for each replication. Observations recorded for 7 days and the results were analysed.

Statistical Analysis

Dead individuals were counted under a stereoscopic microscope, and the mortality rate was calculated. Death data were corrected by Abbott's formula (Abbott, 1925). Analysis of variance (ANOVA) and grouping were made in SPSS Statistics (Version 17.0).

RESULTS AND DISCUSSION

Phenolic Analysis

Differences in the phenolic components are shown in the Table 1. The total amount of phenolic components was found in the highest flowers (54.10 ng/uL) and stem (52.23 ng/uL), while the amount in filari was much lower (28.75 ng/uL).

Table 1. Phenolic component composition of cauline, phyllary and flower extracts (ng/uL)

Components		Cauline	Phyllary	Flower
1	Chrysin	**39.58**	6.99	11.30
2	Naringenin	5.78	**13.20**	**15.66**
3	Rosmarinic acid	2.77	2.91	1.91
4	Naringin	1.32	0.15	-
5	Catechine hydrate	0.75	1.93	3.73
6	Quercetin	0.62	0.77	1.19
7	Chlorogenic acid	0.47	0.22	0.57
8	Hydroxy sinamic acid	0.38	0.34	-
9	Resveratrol	0.23	0.24	0.53
10	o-Coumaric acid	0.20	1.07	**10.35**
11	Salicylic acid	0.12	0.79	2.53
12	Caffeic acid	-	-	0.41
13	p-Coumaric acid	-	-	1.69
14	Rutin	-	-	2.12
15	t-Ferulic acid	-	0.14	1.49
16	t-Cinamic acid	-	-	0.62
Total		**52.23**	**28.75**	**54.10**

In the extract obtained from Caulin leaves, Chrysin, a phenolic compound, was found in the highest amount, followed by Naringenin, Rosmarinic acid, and Naringin, and other phenolic compounds were found in lesser amounts (Figure 1a). On the other hand, in the extract extracted from Phyllary leaves, the Narengenin phenolic compound was found in the highest amount, followed by Chrysin, Rosmarinic acid, Catechin hydrate and o-Coumaric acid. In contrast, other phenolic compounds were found in lesser amounts (Figure 1b). In the extract extracted from flower leaves, Narengenin phenolic compound was the most abundant, followed by Chrysin, o-Coumaric acid, Catechine hydrate, Salicylic acid, Rutin, Rosmarinic acid, p-Coumaric acid, t-Ferulic acid and Quercetin, respectively (Figure 1c).

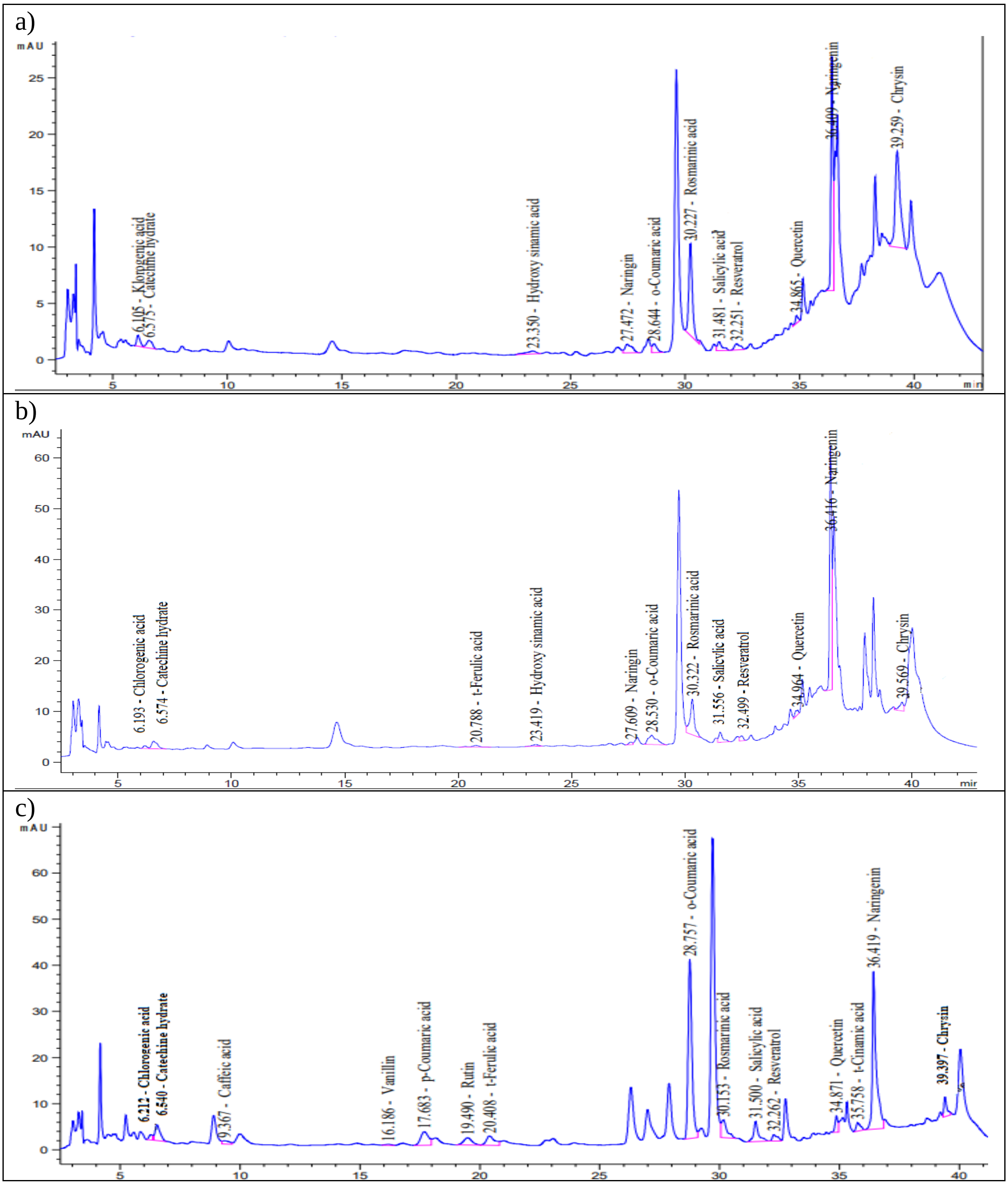

Figure 1. LC-MS chromatograms obtained from analyses of different plant parts.
a) Extract of Cauline leaves; b) Extract of Phyllary leaves; c) Extract of flower leaves.

Repellent Impact Study

Cauline, phyllary and flower extracts have a very significant effect on *S. oryzae*, *S. granarius* adults and *T. granarium* (P<0.01). At the end of the 7 days observation period, the highest number of dead insects (6.20 pcs/petri) was recorded in the extract application obtained from the cauline leaves extract, followed by the applications of extracts obtained from phyllary leaves (2.50 pcs/petri) and flower leaves (2.30 pcs/petri), respectively. It was also determined that there was no significant difference in the number of insects killed between the effects of extracts obtained from phyllary leaves or flower leaves (Figure 2).

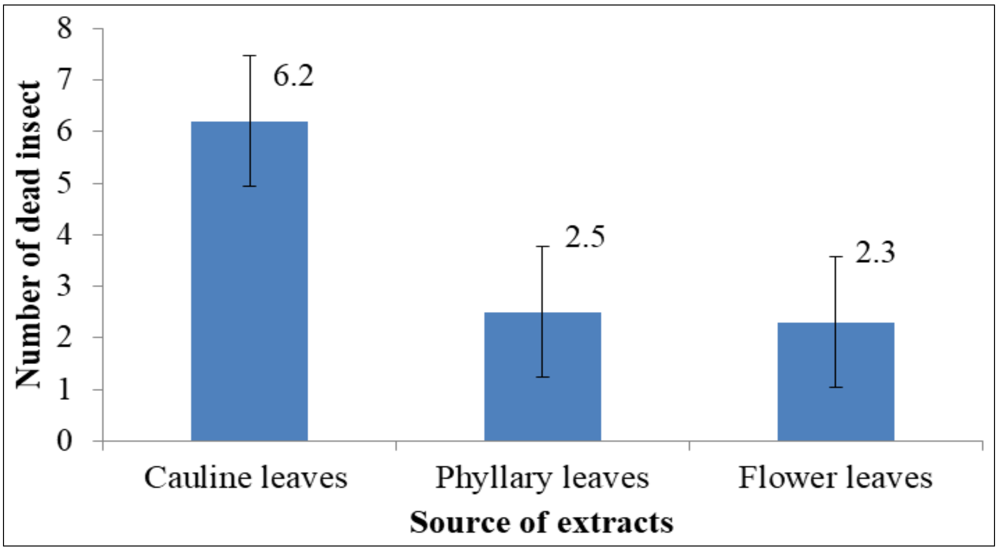

Figure 2. Number of insects killed by different extract sources

It was determined that the effects of the extract types used on the mortality rates of the insect species used in the experiment differed. In fact, it was determined that deaths started on the first day of all insect species in flower and stem extract applications. In the phyllary extract application, it was determined that deaths started from the first day in *S. oryzae* species. In contrast, deaths started from the third day in *S. granarius* and *T. granarium* species. On the other hand, it was determined that the mortality rate on the last day in stem extract application approached 100% (97.5%). In comparison, it remained at 35% and 40% in phyllary and flower extract applications, respectively (Table 2).

Table 2. Daily mortality rates (%) due to caulin leaf extract, phyllary leaf extract and flower extract

Name of Insect	Source of Extract	Observation Period (days)						
		1	2	3	4	5	6	Fınal
S. oryzae	Cauline	17.5	30.0	42.50	62.50	75.00	80.00	**97.50[a]**
	Phyllary	5.00	5.00	10.00	22.50	22.50	30.00	**35.00[b]**
	Flower	12.5	12.5	17.50	25.00	30.00	30.00	**40.00[b]**
S. granarius	Cauline	10.0	35.0	60.00	65.00	77.50	80.00	**97.50[a]**
	Phyllary	0.00	0.00	7.50	22.50	22.50	32.50	**35.00[b]**
	Flower	12.5	12.5	17.50	25.00	30.00	30.00	**45.00[b]**
T. granarium	Cauline	22.5	30.0	42.50	62.50	75.00	80.00	**97.50[a]**
	Phyllary	0.00	0.00	7.50	22.50	22.50	30.00	**35.00[b]**
	Flower	10.0	12.5	17.50	25.00	30.00	30.00	**40.00[b]**

a, b represents statistically different classes.

It can clearly be understood that there is a significant difference between the extracts. When the LT_{50} and LT_{90} values were examined, it took about 3 days for half of the population to die with the cauline leaf extract applied, while it took about 6 days for 90% to die (Figure 3). On the other hand, although there was no statistically significant difference between phyllary and flower extracts, it was determined that the time for half of the population to die (LT_{50}) was approximately 7 days in phyllary leaf extracts. In comparison, this time was approximately 8 days in flower extracts. The time for 90% of the population to die (LT_{90}) was approximately 14 days in phyllary leaf extracts, while it was approximately 17 days in flower extracts. Maximum LT_{50} duration was observed on flower extract (8,78 days) and maximum LT_{90} with flower extract (17,03 days) on *S. oryzae*. Cauline extract had 50% less period of time than other extract sources to reach LT_{50} for 3 insect species.

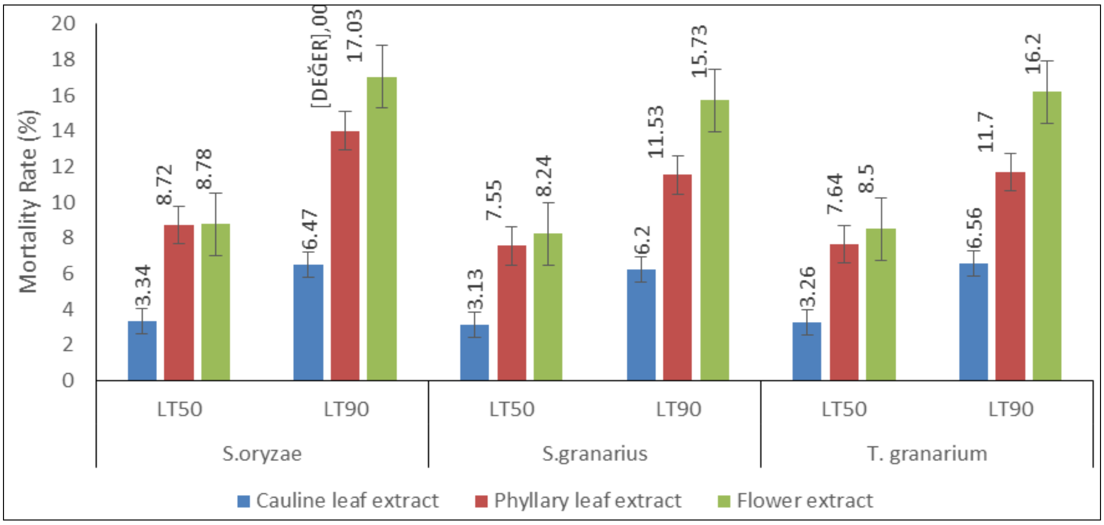

Figure 3. Variation of LT_{50} and LT_{90} periods according to extracts of different origins

While the mortality rates observed as a result of the administration of 200, 400 and 800-times diluted doses of the cauline extract did not differ on a species basis, significant differences were observed between the applications ($P<0.01$). It was determined that the mortality rate decreased when the dilution rate of the applied extracts was increased. Indeed, the highest mortality rate was obtained from the 200-times dilution application (Figure 4).

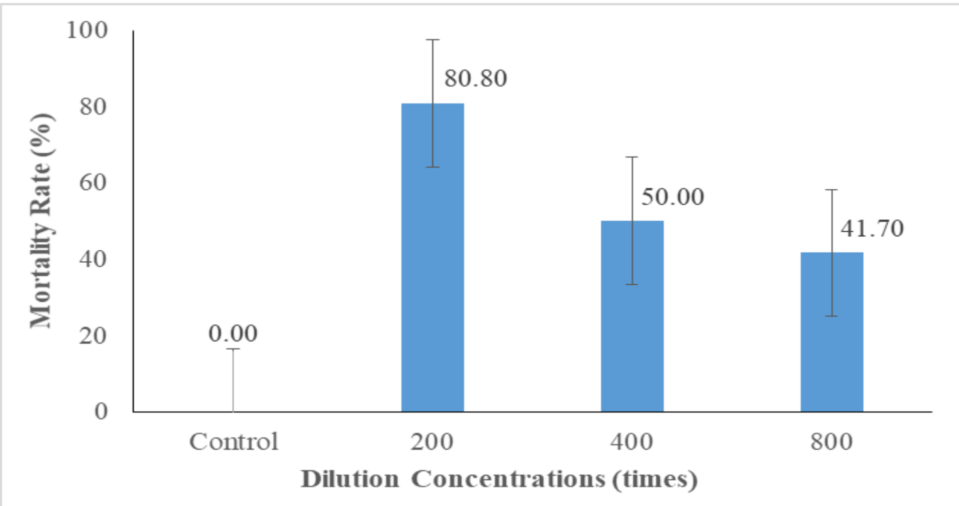

Figure 4. Change in mortality rates depending on dilution intensity

The application of *Carthamus dentatus* extract represents a novel approach in this research, complementing previous studies on plant extracts used against storage insects (Eliopoulos et al., 2015; Rajkumar et al., 2019; Teke and Mutlu, 2020; Pang et al., 2021).

Cauline leaf extract had the highest and earliest mortality rate among all applied extracts. As a result of the analysis, it was determined that the most significant difference in the composition of the stem leaf is in the content of chrysin, an important flavonoid (Table 2). In this study, a strong regression (R^2=0.99) was found between chrysin content and mortality rates. Chrysin is a flavone, which is mostly found in passion flowers (*Passiflora* spp.), some medicinal plants, and honey (Mani and Netesan, 2018). Puri et al. (2022) evaluated chrysin against melon fruit (*Z. cucurbitae*) and found strong adverse effect on larval development on increasing doses. While many other biological functions, chrysin has short bioactive period (Renuka and Vijayakumar, 2018).

Naringenin is the second highest metabolite with its ratios in flower and stem leaves. It was determined that the highest naringenin content was in flower, followed by phyllary leaves and stem leaves, respectively (Table 2). Naringenin is a polyphenolic flavonoid, that can interfere insect life cycle and used as insecticide. Golawska et al. (2014) shown the effect of naringenin and quercetin on aphids. Naringenin prolonged the passive ingestion, also higher concentrations stopped salivation. Other inhibitory properties of naringenin on insects have been reported (Stec et al., 2020) and recommended for insecticide utilizing component (Su, et al., 2018) by other researchers.

While plant extracts offer numerous beneficial aspects, challenges such as the inconsistent presence of secondary elements, variability in quality, the influence of environmental conditions on quality, and the formation of undesirable metabolites within the plant pose significant hurdles. According to these disadvantages, companies focus on purifying and utilizing bioactive components. However, this technique is hindered by the short active lifespans of bioactive substances, directing researchers to explore the broader implications of plant extracts. Many researchers have been testing different extracts of plant parts.

Rafiq et al. (2021) tested *Carthamus oxyachanta* leaf and flower extract against soil borne fungus *Rhizoctonia solani* and found significant reduction on fungal growth. Yaman and Şimşek (2019) examined the contact toxicity of rosemary extracts on *S. oryzae* and increasing mortality retested observed in the ethanol extract at 16.67% at 24 hours, 30% at 48 hours and 58.33% at 72 hours.

R. officinalis essential oil evaluated on different storage insects (Shalaby and Khater, 2005; Isikber et al., 2006; Salman et al., 2014; Saeidi et al., 2019). Yıldırım et al. (2011) reported that the essential oil of rosemary caused 93% of deaths on *Sitophilus granarius* L., while Kiran and Prakash (2015) observed 75% of deaths after 72 hours on *S. oryzae*. Rozman et al. (2007) reported 1,8 cineole caused 100% death on *S. oryzae* and 97% on *R. dominica*.

Researchers stated that mortality of rosemary essential oil might depend on high content of 1,8 cineole, α-pinene and βcaryophyllene (Rojht et al., 2012; Kiran and Prakash, 2015).

The adverse effects of chemicals used in chemical control on the environment and human health have led researchers to use alternative control methods. For this purpose, essential oils and extracts of plant origin are widely used. It will also provide a different perspective on combating the active substances extracted from plant parts.

CONCLUSIONS

This study investigated chemical properties of *Carthamus dentatus* Vahl and the findings shown that *Carthamus dentatus* Vahl cauline leaf extract is a potential candidate to be used as natural insecticide in food storages such as warehouses and silos. This study did not investigate deterioration period and environmental impact.

This study also shown possible insecticidal effect of chrysin, which is related with increasing mortality rate, also decline with decreasing concentration. Studies shown pure chrysin may not have lethal effect, but chrysin in leaf extract has lethal effect which shown in this study.

Carthamus dentatus Vahl grows naturally in Europe, Turkey and Middle East. It is wild relative of *Carthamus tinctorius* L., which is rising oil crop. It is known as a weed and does not yield substance in any industrial field. Chrysin content might depend on genetic base, environment conditions or plant adaptation.

Our aim is evaluate the chrysin content and metabolism for its possible use in the future. On the other hand, genetic base of the chrysin accumulation would be investigate for further studies.

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