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ANIMAL AND HUMAN PHYSIOLOGY

The Effect of Four Plant Extracts on Life Expectancy, the Number of Eggs Laid and the Reproductive Physiology of *Acanthoscelides obtectus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Abstract—This study evaluated the mortality rates of eucalyptus (*Eucalyptus globulus*), thyme (*Thymus vulgaris*), laurel (*Laurus nobilis*) and walnut (*Juglans regia*) extracts on *Acanthoscelides obtectus* and *Callosobruchus maculatus* adults and also longevity, the number of eggs laid and the number of adults hatching from these eggs. The plant extracts with the highest mortality on the first day in the contact effect trials were *J. regia* (86.66%) in *A. obtectus*, *J. regia* (33.33%) and *T. vulgaris* (18.33%) in *C. maculatus*. In the repellent effect trials, *T. vulgaris* (80.00%) in *A. obtectus* and *L. nobilis* (96.66%) in *C. maculatus* were detected in the group aerated for 1 hour. In contact effect trials, the shortest longevity was *L. nobilis* (12.87 days) in *A. obtectus*, while *J. regia* (6.00 days) was found in *C. maculatus*. The number of eggs laid and the number of adults emerged were determined at least in *J. regia* in both insect species. On the other hand, in the repellent effect trials the shortest longevity in *A. obtectus* was determined in *T. vulgaris*, the number of eggs laid and the number of adults emerged from the eggs were the lowest in *T. vulgaris* and *L. nobilis* in 1 hour aerated group. In *C. maculatus*, the shortest longevity was found in *L. nobilis*, the lowest number of eggs laid in *T. vulgaris* and *L. nobilis*, and the number of adults emerged from eggs in *T. vulgaris, E. globulus* and *L. nobilis*. According to the results, in addition to the toxic effects of these plants, it was found that all plant extracts had an inhibitory effect on egg laying and the number of adults hatching from these eggs was much lower than the control group. It can be argued that in addition to their insecticidal effects on insects, the plant extracts used also affect ovulation physiology and reduce adult emergence.

Keywords: insect, emerged adults, insect physiology, *Eucalyptus globulus*, *Thymus vulgaris*, *Laurus nobilis*, *Juglans regia*

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INTRODUCTION

The leading stored product pests are *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) and *Callobruchus maculatus* F. (Coleoptera: Bruchidae), which cause great damage to beans and cowpeas (Emekçi and Ferizli, 2000; Şen et al., 2020). The larvae of these insects feed and develop inside the seeds and emerge as adults by making holes in seeds (Edwin and Jakob, 2017). They cause many negativities in stored products such as weight loss, decrease in germination power, decrease in nutritional value in terms of the amount of protein, and decrease in market value (Arshad et al., 2014; Yaman and Şimşek, 2019). One of the most widely used methods in fighting stored product pests is chemical methods. They are preferred due to ease of application, fast results and cheaper cost (Anonymus, 2004). However, insecticide use increases the resistance of insects to insecticides and harms many non-target organisms. Recently conducted studies have shown that insecticides cause serious harms to both the environment and mammal health (Babayiğit et al., 2014; Kayhan, 2020). All these side effects of insecticides force researchers to develop alternative methods. One of these methods is control methods with plant essential oils or extracts in the fight against pests. There are a large number of studies on the insecticidal activities of plant extracts against stored product pests (Arshad et al., 2014; Bahrami et al., 2016; Barros et al., 2015; Oulebsir-Mohandkaci et al., 2015; Selimoğlu et al., 2015).

It has been reported that the essential oil obtained from *Chenopodium ambrosioides* L. leaves show high amount of fumigant toxicity against *Prostephanus truncatus* (Horn), *C. chinensis* L., *C. maculatus*, *A. obtectus*, *Sitophilus granarius* L. (Tapondjou et al., 2002). Bittner et al. (2008) showed that the essential oils they obtained from *Thymus vulgaris, Gomortega keule*, *Laurelia sempervirens*, *Eucalyptus globulus* and *Origanum vulgare* plants had fumigant activity on *A. obtectus.* Çetin and Elma (2017) found that extracts obtained from cinnamon (*Cinnamomum cassia*), laurel (*Laurus nobilis*), clove (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) plants had effects to prevent contact and egg laying against *C. maculatus* adults. In their study, they found that with the increasing concentration in contact effect trials (0.625%, 1.25, 2.50, 5, 10 w/w), mortality rates also increased at the end of 24 hours. The rate of inhibiting egg laying also increased with the increase in concentration. They found mortality rate in laurel as 74.58 in 10% concentration and egg laying inhibition rate as 42.63% in 2.5% concentration. A large number of studies highlight the methods of plant extracts and essential oils that can be alternatives to chemical insecticides used in pest control (Küçük et al., 2006; Gökçe et al., 2009; Saylam and Çetin, 2016; Andy and Edema, 2019). In addition to the toxic, fumigant and repellent effects of plant extracts, nutritional inhibitory, growth regulating and egg laying inhibitory effects are also among the subjects that have been examined in recent years (Elma and Alaoğlu, 2014; Taş et al., 2015; Ghassemi et al., 2016; Erdemir and Erler, 2017). This study examined the mortality rates of four different plant extracts on *A. obtectus* and *C. maculatus* in addition to insects' longevity, the number of eggs laid and the number of adults hatching from these eggs. This way, the effects of plant extracts on the reproduction and ovulation physiology of insects were evaluated in addition to their mortality effects.

MATERIALS AND METHODS

This study was conducted in the laboratory of Sinop University, Faculty of Education, Department of Mathematics and Science Teaching, Biology laboratory in 2021.

Insect Cultures

The bean and cowpea seed insect cultures used in the study were obtained from the cultures produced in our laboratory since 2017. The trials were started by establishing the main cultures of bean and cowpea seed insects. The insects taken from the main cultures were placed in 500 mL glass jars containing bean and cowpea seeds (250 g). 3 days-old adult insects that emerged from these cultures were used in trials (5 females and 5 males, a total of 10 adult insects). *A. obtectus* was kept in the refrigerator for 5 minutes for sex discrimination. After the insects became dormant, they were sexed under a binocular. *C. maculatus* sex discrimination was made according to the size of the insects.

Plant Extraction

The plant leaves used in the study were obtained from herbalists. After the leaf parts of eucalyptus (*Eucalyptus globulus*) (Karden brand), thyme (*Thymus vulgaris*) (Defne brand), laurel (*Laurus nobilis*) (Balen brand) and walnut (*Juglans regia*) (Karden brand) plants were ground in a homogenizer (Pro-2000), 5 g of samples were weighed and 50 mL ethanol (Merck 99.5%) was added on them. The solutions were transferred to glass tubes, covered with aluminium foil and left at room temperature for 7 days. Then they were filtered with Whatman filter paper (no. 1) and the organic solvent was separated with the help of evaporator. 10% (in ethanol, w/v) solutions were prepared from each plant extract and used in trials.

Contact Effect Trials

10 *A. obtectus* (5 females and 5 males) adults and 10 *C. maculatus* (5 females and 5 males) adults were taken and placed in separate plastic containers with blotting paper. Next, 1 μL (microliter) of each plant extract was applied to the ventral part of each insect with the help of a micropipette. The insects which were applied plant extract were then transferred to separate Petri dishes containing 10 sterile beans (for *A. obtectus*) and 10 sterile cowpea (for *C. maculatus*). Petri dishes were then incubated at 27 ± 2°C, 60 ± 5% continuous dark conditions. Each Petri dish was checked at intervals of 1, 2, and 3 days, the dead insects were removed from Petri dishes and recorded.

Repellent Effect Trials

10 sterile beans and 10 sterile cowpeas were placed in each Petri dish. The plant extracts were sprayed three times (approximately 2 mL) to each Petri dish from a distance of 30 cm with a spraying bottle. Next, the beans and cowpeas in Petri dishes were aerated for 1, 24, and 48 hours. After the seeds were aerated, 10 *A. obtectus* (5 females and 5 males) were placed in Petri dishes containing beans, 10 *C. macultaus* (5 females and 5 males) were placed in Petri dishes containing cowpea. The aim here is to determine the repellent effects of Plant extract concentrations, which decrease as a result of aeration at the end of 1, 24 and 48 hours. Then the all petri dishes were incubated at 27 ± 2°C, 60 ± 5% continuous dark conditions. The Petri dishes were checked with intervals of 1, 2, and 3 days and the dead insects were taken out of Petri dishes and recorded.

Insects that survived both contact and repellent effect trials after 3 days were taken in longevity trials (the day the insects died was recorded. The difference between the day they emerge from the seeds and the day they die was calculated as the longevity). The eggs on bean and cowpea and petri surface were counted under binocular microscope (Lecia Z4). They were left to incubate again under specified conditions. After 30 days, the adults that emerged from beans and cowpeas were counted and recorded.

Ethanol was used for control groups. Contact effect trials were done in 6 repetitions (6 replication × 10 insects = 60). 60 insects from 3 replicates were used for each

Table 1. Mortality rates (%) in *A. obtectus* and *C. maculatus* at the end of day 1st, 2nd and 3rd as a result of contact effect trials

Plant extracts	*Acanthoscelides obtectus* Mean ± S.E.			*Callosobruchus maculatus* Mean ± S.E.		
	day 1	day 2	day 3	day 1	day 2	day 3
Control	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	3.33 ± 2.10 Ba	13.33 ± 6.14 Aa	15.00 ± 3.41 Aa
J. regia	86.66 ± 4.21 Ba	0.00 ± 0.00 Ab	0.00 ± 0.00 Ab	33.33 ± 6.66 Aa	26.67 ± 4.21 Aa	26.66 ± 11.15 Aa
T. vulgaris	13.33 ± 4.21 Aa	10.00 ± 7.11 Ba	6.66 ± 3.21 Ba	18.33 ± 5.42 ABa	10.00 ± 6.83 Aa	16.66 ± 9.54 Aa
E. globulus	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	6.66 ± 4.21 Ba	20.00 ± 5.16 Aab	30.00 ± 6.83 Ab
L. nobilis	6.66 ± 4.21 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	3.33 ± 3.33 Ba	18.33 ± 5.42 Aab	28.33 ± 4.01 Aa

The same upper case letters in the same column are statistically indifferent. The same lower case letters in the same line are statistically indifferent. S.E.: Standart Error.

plant extract and control groups. A total of 600 insects were used for contact effect trials. Repellent effect trials were done 3 repetitions (3 replications × 10 insects = 30 insects). 30 insects from 3 replications were used for each plant extract and control groups. A total of 900 insect were used for repellent effect trials. Data in all tables (longevity, number of eggs and number of emerged adults) are calculated per individual.

Data Analysis

SPSS 22.0 program was used in data analysis. First of all, Abbott formula was used in determining mortality rates.

$$[(A - B)/A] \times 100,$$

A, number of live insects in control group, %;

B, number of live insects in application group, %;

and corrected mortality rates (%) (Abbott, 1925).

Whether the data were normally distributed was evaluated according to Shapiro–Wilk test ($p > 0.05$). The data were normally distributed and in multiple comparisons of contact effect and repellent effect trials it was found with Generalized Linear model, Two way ANOVA to find out whether there were differences between groups. Tukey HSD Test was used to find out between which plant extracts there were differences and to find out on which day of mortality differentiation started. The significance level was taken as 0.05 for all statistical comparisons in the study.

RESULTS

Contact Effect Trials

In Table 1, in contact effect trials, the highest mortality rates within the first 24 hours in each insect was found with *J. regia* (the same line, the same lower case letter) (*A. obtectus*, $F_{2.15} = 422.500$, $p < 0.001$; *C. maculatus*, $F_{2.15} = 0.238$, $p = 0.791$).

Interestingly, while no mortality effect was found in *J. regia* after 48 hours in *A. obtectus*, the mortality effect was found to continue in *C. maculatus*. The plant extract with the second highest mortality effect was *T. vulgaris* in the first 24 hours (*A. obtectus*, $F_{2.15} = 0.205$, $p = 0.817$; *C. maculatus*, $F_{2.15} = 0.253$, $p = 0.780$). While no mortality effect of *E. globulus* was observed in *A. obtectus* for 72 hours, this effect was observed in *C. maculatus* ($F_{2.15} = 4.512$, $p = 0.029$). In *L. nobilis*, a mortality effect was observed in *A. obtectus* only within the first 24 hours ($F_{2.15} = 2.500$, $p = 0.116$), the mortality effect was found to increase in *C. maculatus,* depending on time ($F_{2.15} = 8.382$, $p = 0.04$). When the results were examined, the highest mortality rate was found in *J. regia* within the first 24 hours, while *E. globulus* and *L. nobilis* were found to be successful in continuing the mortality rate. When compared among plant extracts (same column, same upper case letter), the highest mortality effect in *A. obtectus* was found in *J. regia* in the only first 24 hours ($F_{4.25} = 127.917$, $p < 0.01$). This mortality rate was not found to be statistically significant at hours 48 and 72. In *C. maculatus*, the highest mortality rate within the first 24 hours was found in *J. regia* ($F_{4.25} = 7.798$, $p < 0.001$) and this mortality rate continued at hours 48 and 72 ($F_{4.25} = 1.298$, $p = 0.298$; $F_{4.25} = 0.838$, $p = 0.514$, respectively).

In terms of the longevity of surviving adults as a result of contact effect trials, in *A. obtectus,* the group that had the highest longevity was found with *J. regia* and the group that had the lowest longevity was found with *T. vulgaris*, *E. globulus* and *L. nobilis* ($F_{4.217} = 38.824$, $p < 0.001$). In *C. maculatus,* the group that had the highest longevity was found with *E. globulus* and *T. vulgaris*, while the group that had the lowest longevity was found with *J. regia* and *L. nobilis* ($F_{4.124} = 122.070$, $p < 0.001$). In both insects, the number of eggs laid in all plant extracts were found to be lower when compared with the control groups and statistical difference was found. In *A. obtectus* ($F_{4.16} = 16.890$, $p < 0.001$) and *C. maculatus,* the plant extract with the least number of eggs laid was *J. regia*. When the number of adults emerging from these eggs was examined,

Table 2. Longevity of the surviving adults as a result of contact effect trials, the number of eggs laid, the number of adults hatching from eggs (per individual)

Longevity (d) Mean ± S.E.				Number of egg Mean ± S.E.				Number of emerged adults Mean ± S.E.			
A. obtectus		*C. maculatus*		*A. obtectus*		*C. maculatus*		*A. obtectus*		*C. maculatus*	
Control	17.81 ± 0.21 C	Control	16.32 ± 0.98 C	114.33 ± 12.75 C	Control	100.50 ± 8.34 B	Control	70.66 ± 6.20 B	Control	52.83 ± 4.20 B	Control
J. regia	22.00 ± 0.57 A	*J. regia*	6.00 ± 0.46 A	6.50 ± 1.50 A	*J. regia*	3.00 ± 0.00 A	*J. regia*	0.00 ± 0.00 A	*J. regia*	19.40 ± 8.18 A	*J. regia*
T. vulgaris	13.73 ± 0.53 B	*T. vulgaris*	8.85 ± 1.00 B	49.66 ± 23.16 B	*T. vulgaris*	4.20 ± 0.48 A	*T. vulgaris*	9.00 ± 0.00 A	*T. vulgaris*	35.60 ± 5.53 A	*T. vulgaris*
E. globulus	13.70 ± 2.52 B	*E. globulus*	9.42 ± 1.00 B	12.00 ± 3.18 A	*E. globulus*	17.00 ± 4.04 A	*E. globulus*	4.00 ± 0.00 A	*E. globulus*	29.83 ± 3.63 A	*E. globulus*
L. nobilis	12.87 ± 0.38 B	*L. nobilis*	7.93 ± 0.99 AB	27.00 ± 4.73 B	*L. nobilis*	4.00 ± 1.15 A	*L. nobilis*	6.50 ± 1.38 A	*L. nobilis*	23.83 ± 7.80 A	*L. nobilis*

The same upper case letters in the same column are statistically indifferent. S.E.: Standart Error. d = day.

Table 3. Mortality rates (%) of repellent effect trials on *Acanthoscelides obtectus* at the end of days 1st, 2nd and 3rd after aeration for 1, 24, and 48 hours

Plant extracts	1 h aerated group Mean ± S.E.			24 h aerated group Mean ± S.E.			48 h aerated group Mean ± S.E.		
	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3
Control	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
J. regia	40.00 ± 20.81 Aab	20.00 ± 5.77 Aa	3.33 ± 3.33 Ba	0.00 ± 0.00 B	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	3.33 ± 3.33 Ba	3.33 ± 3.33 Ba	0.00 ± 0.00 Ba
T. vulgaris	80.00 ± 10.00 Ab	20.00 ± 10.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 B	3.33 ± 3.33 Ba	3.33 ± 3.33 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba
E. globulus	33.33 ± 3.33 Aab	10.00 ± 5.77 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 B	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba
L. nobilis	83.33 ± 6.66 Ab	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 B	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba

The same upper case letters in the same line are statistically indifferent. The same lower case letters in the same column are statistically indifferent. S.E.: Standart Error.

it was found as 0.00 ± 0.00 ($F_{4.13} = 45.891$, $p < 0.001$) in *J. regia* in *A. obtectus*. *J. regia* was followed by *E. globulus, L. nobilis* and *T. vulgaris*. In *C. maculatus*, the plant extract with the least number of adults was found in *J. regia*, again ($F_{4.23} = 1.116$, $p < 0.001$). *J. regia* was followed by *E. globulus, L. nobilis* and *T. vulgaris*. The least number of adults hatching from eggs was found in *J. regia*, while the highest number was found in *T. vulgaris*. As a result, in both insects, the lowest number of eggs and the lowest number of adults that emerged were found in *J. regia*. Although longevity was higher in *J. regia* when compared with the control group, the number of eggs laid and the number of adults that emerged were found to be lower when compared with the control group. Plant extracts other than *J. regia* were found to decrease longevity, the number of eggs laid and the number of adults that emerged from these eggs in both insects.

Repellent Effect Trials

In repellent effect trials, the highest mortality rate was found in *L. nobilis* and *T. vulgaris* in beans aerated for 1 hour ($F_{4.10} = 10.302$, $p = 0.001$). This was followed by *J. regia* and *T. vulgaris*. In seeds aerated for 24 and 48 hours, repellent effect was not as much as expected. In the group aerated for an hour, the mortality rate, which was highest on the first day, decreased on days 2 and 3 in *L. nobilis* ($F_{8.18} = 156.250$, $p <$ 0.001). The same was true for *T. vulgaris* ($F_{8.18} =$ 28.138, $p < 0.001$), *J. regia* ($F_{8.18} = 3.350$, $p = 0.016$) and *E. globulus* ($F_{8.18} = 19.850$, $p < 0.001$).

In Table 4, it can be seen that the plant extract with the highest mortality rate was *L. nobilis* on day 1 ($F_{4.10} = 9.385$, $p = 0.002$). The plant extract with the highest mortality rate was *J. regia* ($F_{4.10} = 9.347$, $p =$ 0.002) on day 1 in the group aerated for 24 hours, while it was found was *J. regia* ($F_{4.10} = 4.250$, $p =$ 0.029) again in the group aerated for 48 hours. The interesting result was that the highest mortality rate was in the group aerated for 24 hours, not in the group aerated for 1 hour. Although mortality rates of *J. regia* extract groups aerated for 1, 24, and 48 hours decreased after day 1, they were high when compared with other plant extracts ($F_{8.18} = 4.692$, $p = 0.003$). Although mortality rate was the highest in *L. nobilis* in the group aerated for 1 hour, mortality rates were found to be very low in groups aerated for 24 and 48 hours ($F_{8.18}= 72.614$, $p < 0.001$). According to the results of repellent effect trials, the group with the highest mortality rate was the group with 1 hour aerated *L. nobilis* extract group and the highest mortality rates were found in groups aerated for 1 hour, except for *J. regia* (Table 4). It can be said that the group aerated for 24 and 48 hours did not have too much repellent effect, at least they did not meet the mortality rates expected for the first day.

As a result of repellent effect trials, in the group aerated for 1 hour, the shortest longevity was found in *T. vulgaris* extract ($F_{4.61} = 81.692$, $p < 0.001$). In the

Table 4. Mortality rates (%) of repellent effect trials on *Callobruchus maculatus* at the end of days 1st, 2nd and 3rd after aeration for 1, 24, and 48 hours

Plant extracts	1 h aerated group Mean ± S.E.			24 h aerated group Mean ± S.E.			48 h aerated group Mean ± S.E.		
	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3
Control	6.66 ± 3.33 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Ab	3.33 ± 3.33 Aa	6.66 ± 6.66 Aa	3.33 ± 3.33 Ab	3.33 ± 3.33 Aa	3.33 ± 3.33 Aa
J. regia	13.33 ± 8.81 Aa	13.33 ± 6.66 Aa	0.00 ± 0.00 Aa	63.33 ± 18.55 Ba	16.66 ± 8.01 Aa	3.33 ± 3.33 Aa	26.66 ± 6.66 ABa	10.00 ± 5.77 Aa	20.00 ± 5.77 Aa
T. vulgaris	36.66 ± 18.55 Aa	6.66 ± 6.66 Ba	0.00 ± 0.00 Ca	3.33 ± 3.33 Bb	0.00 ± 0.00 Ca	10.00 ± 5.77 Ba	10.00 ± 0.00 Bab	3.33 ± 3.33 Ba	13.33 ± 3.33 Ba
E. globulus	30.00 ± 15.27 Aa	20.00 ± 10.00 Aa	3.33 ± 3.33 Aa	0.00 ± 0.00 Ab	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	3.33 ± 3.33 Ab	6.66 ± 5.77 Aa	10.00 ± 6.32 Aa
L.nobilis	96.66 ± 3.33 Ab	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	6.66 ± 6.66 Bb	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	6.66 ± 6.66 Bab	3.33 ± 3.33 Ba	6.66 ± 3.33 Ba

The same upper case letters in the same line are statistically indifferent. The same lower case letters in the same column are statistically indifferent. S.E.: Standart Error.

group aerated for 24 hours, the shortest longevity was found in *E. globulus* ($F_{4.142} = 57.208$, $p < 0.001$), while it was found in *L. nobilis* extract in the group aerated for 48 hours ($F_{4.143} = 26.244$, $p < 0.001$). The number of eggs laid by surviving adults shows that the difference between three aeration times and the control group was significant. In the group aerated for 1 hour, the plant extracts with the lowest number of eggs laid were *T. vulgaris, L. nobilis* and *J. regia* ($F_{4.10} = 17.277$, $p < 0.001$), while it was *L. nobilis* ($F_{4.10} = 8.607$, $p = 0.003$) in the group aerated for 24 hours and *J. regia* ($F_{4.10} = 7.779$, $p = 0.004$) in the group aerated for 48 hours. However, while no eggs were laid in *T. vulgaris* in the group aerated for 1 hour, this number was found to increase to 95.00 ± 16.01 in the group aerated for 48 hours ($F_{2.6} = 10.536$, $p = 0.011$). This increase was also found in *J. regia* ($F_{2.6} = 1.665$, $p = 0.266$), *E. globulus* ($F_{2.6} = 5.971$, $p = 0.037$) and *L. nobilis* ($F_{2.6} = 40.690$, $p < 0.001$). Of these eggs, the ones that were found to have the lowest number of adults emerging were found as *J. regia, T. vulgaris* and *L. nobilis* ($F_{4.10} = 62.769$, $p < 0.001$) in the group aerated for 1 hour. In the groups aerated for 24 hours ($F_{4.10} = 11.329$, $p = 0.001$) and 48 hours ($F_{4.10} = 22.177$, $p < 0.001$), the lowest number of adults emerging were found in only *J. regia*. The number of adults emerging was found to increase as aeration time increased. For example, while the number of adults emerging was found as 9.66 ± 1.20 in the *E. globulus* group aerated for 1 hour, this value was found to increase to 27.66 ± 11.20 in the group aerated for 48 hours ($F_{2.6} = 2.815$, $p = 0.137$). In *E. globulus*, the difference between aeration time and the number of adults emerging was not statistically significant. Same results were also found in *J. regia* ($F_{2.6} = 4.000$, $p = 0.079$). However, when these values were compared with the control group, the statistical difference between was found to be significant. In *T. vulgaris* ($F_{2.6} = 5.719$, $p = 0.041$) and *L. nobilis* ($F_{2.6} = 7.563$, $p = 0.023$), the difference between groups aerated for 1 hour and those aerated for 48 hours was found to be statistically significant and the number of adults emerging was found to increase in parallel with the aeration time (Table 5).

As a result of repellent effect trials, in terms of longevity of surviving adults, it was found that the group that lived the shorts was *L. nobilis* and *T. vulgaris* ($F_{4.65} = 157.117$, $p < 0.001$) in those aerated for 1 hour. It was found as *J. regia* in those aerated for 24 hours ($F_{4.109} = 146.720$, $p < 0.001$) and 48 hours. Longevity was found to decrease when compared with control groups. While *L. nobilis* was the group that lived the shortest in those aerated for 1 hour, longevity was found to increase in groups aerated for 24 and 48 hours ($F_{2.53} = 11.763$, $p < 0.001$). Similar results were found in *T. vulgaris* ($F_{2.48} = 8.151$, $p = 0.001$). No correlation was found between aeration and longevity in *J. regia* ($F_{2.37} = 8.018$, $p = 0.001$) and *E. globulus* ($F_{2.64} = 4.605$, $p = 0.014$) aerated for 1, 24 and 48 hours. However, all groups were found to be significantly different from the control group.

In the group aerated for 1 hour, the plant extracts with the lowest number of eggs laid were *T. vulgaris* and *L. nobilis* ($F_{4.10} = 33.361$, $p < 0.001$). This was followed by *E. globulus* and *J. regia*. In the group aerated for 24 hours, the plant extracts with the lowest number of eggs laid were *T. vulgaris* and *J. regia* ($F_{4.10} = 41.784$, $p < 0.001$), while they were *J. regia* and *E. globulus* ($F_{4.10} = 21.610$, $p < 0.001$) in the group aerated for 48 hours. While a decrease was found in the number of eggs laid although aeration time increased in *J. regia* ($F_{2.6} = 1.06$, $p = 0.403$), there was an increase in other plant extracts. For example, while *L. nobilis* ($F_{2.6} = 5.616$, $p = 0.042$) and *T. vulgaris* ($F_{2.6} = 6.208$, $p = 0.035$) were the plant extracts with the least number of eggs laid, the number of eggs laid was found to increase as the aeration time increased.

When the number of adults that emerged from these eggs was examined, it was found that almost no adults emerged in plant extracts in the group aerated for 1 hour ($F_{4.10} = 23.563$, $p < 0.001$). In the group aerated for 24 hours, the lowest number of adults was in *J. regia* and *T. vulgaris*, while it was in *J. regia* aerated for 48 hours ($F_{4.10} = 8.938$, $p = 0.002$). Interestingly, it was found that almost no adults emerged from the *J. regia* group aerated for 1, 24 and 48 hours ($F_{2.6} = 1.000$, $p = 0.422$). All plant extract groups aerated for

Table 5. Longevity of the surviving *A. obtectus* adults as a result of repellent effect trials, the number of eggs laid, the number of adults hatching from eggs (per individual)

Plant extracts	1 h aerated group Mean ± S.E.	24 h aerated group Mean ± S.E.	48 h aerated group Mean ± S.E.
	Longevity (day)		
Control	17.93 ± 0.21 Ac	17.63 ± 0.36 Ac	17.86 ± 0.23Ab
J. regia	9.00 ± 0.87 Aa	11.34 ± 0.34 Ba	12.33 ± 0.49 Ba
T. vulgaris	0.00 ± 0.00 Ab	13.21 ± 0.30 Bb	11.70 ± 0.62 Ba
E. globulus	11.82 ± 0.70 Aa	11.26 ± 0.30 Aa	11.65 ± 0.57 Aa
L. nobilis	12.40 ± 0.50 Aa	11.40 ± 0.46 Aa	11.37 ± 0.64 Aa
	Number of egg		
Control	147.33 ± 32.91 Ab	121.66 ± 14.83 Ab	130.66 ± 23.72 Ab
J. regia	9.66 ± 4.48 Aa	40.00 ± 10.40 Aa	26.33 ± 16.95 Aa
T. vulgaris	0.00 ± 0.00 Aa	47.66 ± 19.64 Ba	95.00 ± 16.01 Cab
E. globulus	26.00 ± 6.42 Aa	71.66 ± 11.66 Bab	34.00 ± 11.01 ABa
L. nobilis	0.00 ± 0.00 Aa	21.00 ± 4.72 Ba	51.66 ± 5.23 Ca
	Number of emerged adults		
Control	76.66 ± 9.27 Ab	59.66 ± 9.83 Ab	89.00 ± 9.84 Ab
J. regia	1.00 ± 1.00 Aa	7.00 ± 2.08 Aa	1.66 ± 1.66 Aa
T. vulgaris	0.00 ± 0.00 Aa	20.00 ± 7.76 Ba	23.66 ± 4.97 Ba
E. globulus	9.66 ± 1.20 Aa	31.66 ± 4.40 Aab	27.66 ± 11.20 Aa
L. nobilis	0.00 ± 0.00 Aa	11.00 ± 4.04 Ba	14.66 ± 2.60 Ba

The same upper case letters in the same line are statistically indifferent. The same lower case letters in the same column are statistically indifferent. S.E.: Standart Error.

Table 6. Longevity of the surviving *C. maculatus* adults as a result of repellent effect trials, the number of eggs laid, the number of adults hatching from eggs (per individual)

Plant extracts	1 h aerated group Mean ± S.E.	24 h aerated group Mean ± S.E.	48 h aerated group Mean ± S.E.
	Longevity (day)		
Control	17.39 ± 0.26 Ac	17.80 ± 0.19 Ab	17.40 ± 0.27 Ab
J. regia	9.04 ± 0.27 Aa	6.20 ± 1.20 Ba	6.76 ± 0.35 Ba
T. vulgaris	1.00 ± 1.00 Ab	7.56 ± 0.44 Ba	7.00 ± 0.42 Ba
E. globulus	9.14 ± 0.75 Aa	6.73 ± 0.36 Ba	7.21 ± 0.58 Ba
L. nobilis	0.00 ± 0.00 Ab	7.35 ± 0.43 Ba	8.40 ± 0.68 Ba
	Number of egg		
Control	111.33 ± 16.27 Ab	131.66 ± 11.39 Ac	120.66 ± 8.00 Ac
J. regia	10.00 ± 9.01 Aa	1.00 ± 1.00 Aa	0.33 ± 0.33 Aa
T. vulgaris	0.00 ± 0.00 Aa	1.66 ± 1.20 Aa	42.66 ± 16.75 Bb
E. globulus	2.66 ± 2.66 Aa	44.66 ± 14.83 Bb	29.66 ± 3.84 Bb
L. nobilis	0.00 ± 0.00 Aa	18.33 ± 1.76 Bab	44.66 ± 9.93 Cb
	Number of emerged adults		
Control	48.00 ± 9.86 Ab	41.66 ± 4.40 Ab	51.66 ± 4.48 Ad
J. regia	0.33 ± 0.33 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
T. vulgaris	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	43.33 ± 8.81 Bcd
E. globulus	0.00 ± 0.00 Aa	42.33 ± 4.33 Bb	17.00 ± 2.64 Cbc
L. nobilis	0.00 ± 0.00 Aa	1.33 ± 0.33 Aa	30.00 ± 11.54 Bbcd

The same upper case letters in the same line are statistically indifferent. The same lower case letters in the same column are statistically indifferent. S.E.: Standart Error.

1 hour were significantly different from the control group. Although no adults emerged in *T. vulgaris* in the group aerated for 1 and 24 hours, there were too many adults aerated for 48 hours ($F_{2.6} = 24.143$, $p = 0.001$). The same thing was also true for *L. nobilis* ($F_{2.6} = 0.458$, $p = 0.032$) and *E. globulus*.

DISCUSSION

This study evaluated the mortality rate of *J. regia, T. vulgaris, L. nobilis*, and *E. gobulus* on *A. obtectus* and *C. maculatus*, longevity, the number of eggs laid and the number of adults emerging from these eggs. There are a large number of studies investigating the mortality rate of plant extracts and plant essential oils especially on pests (Nafiseh et al., 2015). Kırpık et al., (2019) examined the fumigant effects of laurel (*L. nobilis* L.) and zahter (*Thymbra spicata* L.) essential oils on *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). They found mortality rates of laurel about 100% at the end of the first 24 hours in both insects species. In a study by Bittner et al. (2008) which examined the mortality rate of *E. globulus* and *T. vulgaris* on *A. obtectus*, they found mortality rate around 80% at the end of 96 hours. Bahrami et al. (2016) found *J. regia* had 100% mortality rate on *R. dominica* (Coleoptera: Bostrichidae) after 48 hours. The present study showed similar insecticidal activity to the aforementioned studies. As a result of contact effect trials, the highest mortality rate was found in *J. regia* in *A. obtectus* (86.66%) and *C. maculatus* (33.33%) at the end of first 24 hours. This was followed by *T. vulgaris* (13.33%) and *L. nobilis* (6.66%) in *A. obtectus, T. vulgaris* (18.33%), *E. globulus* (6.66%) and *L. nobilis* (3.33%) in *C. maculatus* at the end of the first 24 hours. The mortality effect of *T. vulgaris* continued to decrease for 48 (10.00%) and 72 (6.66%) hours in *A. obtectus*. Other plant extracts had no such effect. Interestingly, the mortality effect of *T. vulgaris* and *J. regia* on *C. maculatus* continued for 48 (*T. vulgaris*: 10.00% and *J. regia*: 26.67%) and 72 (*T. vulgaris*: 16.66% and *J. regia*: 26.66%) hours. However *E. globulus* (48 hours: 20.00%, 72 hours: 30.00%) and *L. nobilis* (48 hours: 18.33%, 72 hours: 28.33%) continued to increase. This may be due to the fact that the insect's response to some components in the plant extract is delayed, and the lethal effect of plant extracts on the insect gradually intensifies, as it cannot continue its physiological reactions and homeostasis.

In a study which examined the effects of thyme (*Origanum syriacum* L.), lavender (*Lavandula angustifolia* L.), sage (*Salvia officinalis* L.), fennel (*Foeniculum vulgare* Mill.) and laurel (*L. nobilis* L.) on the fumigant toxicity of *C. maculatus*, it was found that insecticidal activity was 100% at the end of 72 h (Kaya et al., 2018). In a study they examined the fugimant, mortality and repellent effects of rosemary (*R. officinalis*), laurel (*L. nobilis*), echinecea (*Echinacea purpurea*), marjoram (*Origanum majorana*), basil (*Ocimum basilicum*) and fennel (*Foeniculum vulgare*) essential oils on *Sitophilus granarius* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae), Teke and Mutlu, (2020) found that all essential oils showed mortality and repellent effects and decrease was found in F_1 generation when compared with control groups. Ocaliptol and terpinin acetate are the main components of laurel extract. In the *A. obtectus* repellent effect trials, the highest mortality effect was detected in the *L. nobilis* after the first 24 hours (day 1) in the group aerated for 1 hour. This was followed by *T. vulgaris, J. regia* and *E. globulus*. *J. regia, T. vulgaris* and *E. globulus* were the plant extracts whose mortality effect continued for 48 (day 2) hours in the 1 hour aerated group. No mortality was observed after 72 (day 3) hours. The lack of mortality effect of the 24- and 48-h aerated method on these insects is due to the reduced concentration of plant extract. In the *C. maculatus* repellent effect trials, the plant extract with the highest mortality effect in the group aerated for 1 hour was *L. nobilis* at the end of the first 24 (day 1) hours. This was followed by *T. vulgaris, E. globulus* and *J. regia*, respectively. At the end of 48 hours (day 2), the mortality effect continued in *E. globulus, J. regia* and *T. vulgaris*. For 72 hours these could not be said (day 3). What is interesting here is *J. regia*, the plant extract with the highest mortality effect in the 24 and 48 hours aerated group. This mortality effect was highest in the first 24 (day 1) hours in the group aerated for 24 hours, and this effect continued for 48 hours (day 2). In the group aerated for 48 hours, the highest mortality effect in the first 24 hours (day 1) was again found in *J. regia*, although it was less than the other groups. And the mortality effect continued on the 2nd and 3rd days. The main components in plant extract affect the parameters tested. It is possible to increase these examples because in the fight against harmful insects, the priority is the mortality rate of plant extract. However, the long-term effects of these, in other words, whether these plant extracts affect egg laying or the number of adults that emerge from these eggs, have become much more important recently in integrated fight methods. This is because the physiological egg laying inhibitory effect of the plant extract will reduce the destructive effect of F_1 seeds.

In a study they conducted with eucalyptus and thyme on *A. obtectus,* Erol and Birgücü (2020) found a mortality rate of 61.33% with thyme and 56.00% with eucalyptus at the end of day 7 at 5000 ppm L-1 dose. In a study they examined the mortality, repellent and offspring rates of *J. regia* and *A. obtectus,* Jovanovic et al. (2007) found repellent rate as 12%, mortality rate as 4.1% and offspring rate as 41.6%. In the present study, the highest repellent effect was seen in *T. vulgaris* (80.00%) and *L. nobilis* (83.33%) in *A. obtectus* in the group aerated for 1 hour, and in *L. nobilis* (96.66%) in *C. maculatus*. The different results in our study when compared with Erol and Birgücü (2020) and Jovanovic et al. (2007)'s study can be resulting from the doses and methods used. In this study, *J. regia* and *T. vulgaris* were found to have the highest lethal effect in the first 24 hours for both species in contact effect studies. In the repellent effects studies, *L. nobilis* was the most lethal for both species in the 1 hour aerated group. As can be seen, the lethal and repellent effects of plant extracts and volatile fatty acids are quite high. These plants contain substances such as terpeneoids and alcohols. Compounds of plant extracts may not kill especially monoterpenoids directly; however, they may cause decrease in reproduction performances or various developmental disorders. It is also known that monoterpenoids have neurotoxin effects against insects and their egg mortality rate starts only when the nervous system starts to develop (Campolo et al., 2018). The changes in permeability of vitelline membrane may ease the diffusion of essential oils to eggs during embryogenesis. Thus, they can affect the physiological and biochemical process of eggs. However, in addition to their effects during gametogenesis, it is still disputable whether essential oils affect courtship and mating behaviors and indirectly affect egg formation and egg laying (Campolo et al., 2018). In the present study, all plant extracts were found to show different results in different life stages of *A. obtectus* and *C. maculatus* in contact and repellent effect trials. In contact effect trials, the shortest longevity was observed in *A. obtectus* in *T. vulgaris* (13.73 days), *E. globulus* (13.70 days) and *L. nobilis* (12.87 days), while in *C. maculatus* it was observed in *J. regia* (6.00 days). In the repellent effect trials, the shortest longevity was observed in *T. vulgaris* in *A. obtectus* and *L. nobilis* in *C. maculatus* in the group aerated for 1 hour. These differences between the contact effect studies and the repellent effect trials may be due to the fact that the effects of the lethal chemical substances contained in the plants are lost due to the method-induced aeration. As can be seen, in insects treated with plant extracts, longevity of adults, the number of eggs they lay and the number of adults emerging from these eggs were found to be decreased when compared with control groups. According to these results, in addition to the mortality rate of these plants, especially *J. regia*, *T. vulgaris* and *L. nobilis* were found to have inhibitory effects on egg laying. It can be concluded that biochemical and physiological activities of these eggs are affected so that they can be adults.

In their study they examined the effects of St. John's Wort, cumin, thyme and anasone on egg hatching of *C. maculatus*, Taş et al. (2015) found egg hatching was 83.35% in thyme. In a study they examined the toxic and egg laying inhibitory effects of thyme on *A. obtectus,* Lazarević et al. (2020) found that thyme reduced female egg laying and adult emergence. In this study, *T. vulgaris* showed similar effects. Especially in contact effect trials, while the number of eggs laid in *A. obtectus* (49.66) was high, the number of adults hatched from these eggs (9.00) was found to be quite low. This is the opposite in *C. maculatus*. Especially in the repellent effect trials, *T. vulgaris* and *L. nobilis* in both insect species had a negative effect on the number of eggs laid and adult emergence in the group aerated for 1 hour. This shows that these plant extracts not only affect the mortality of these insects, but also affect the egg laying behavior and the number of adults hatching from these eggs. It is possible to multiply these examples. In a study in which *E. globulus* and various plant extracts were combined in the fight with *A. obtectus,* Tegegne (2017) found mortality rate (32.67%), emergence rate of F_1 offspring (2.00%), egg laying inhibitory effect (95.96%) and seed weight loss (0.13%) in 2% *E. globulus* + *Allium sativum* combination. In a study they examined the repellent, toxic and reproduction inhibitory effects of *E. globulus* and *L. nobilis* and 13 essential oils on *A. obtectus,* Papachristos and Stamopoulos (2002) found that *E. globulus* and *L. nobilis* had high repellent and toxic effects and reduced fecundity and adult emergence. They also found that 174 eggs were laid in L. *nobilis*, 38 eggs were laid in *E. globulus* (very low when compared with the control group). In this study, the number of eggs laid in contact effect trials was considerably lower in *L. nobilis* (*A. obtectus*: 27.00, *C. maculatus*: 4.00) and *E. globulus* (*A. obtectus*: 12.00, *C. maculatus*: 17.00) compared to the control group (*A. obtectus*: 114.33, *C. maculatus*: 100.50). All males and females in the control group were kept in Petri dishes for their entire lives (mean: *A. obtectus*: 17.81 days, *C. maculatus*: 16.32 days). In the plant extract groups, the longevity of the other groups was shorter than the control group, except for *J. regia* (especially *A. obtectus*: 22.00 days). For this reason, the number of eggs laid in the control groups may have been quite high. Interestingly, although the longevity was longer in the *J. regia* groups than in the control group, the number of eggs they laid was the lowest. For this reason, it can be thought that *J. regia*, including other plant extract groups, also affects the egg-laying physiology of these insects. Similar results were found in the repellent effects trials. In a study Regnault-Roger and Hamraoui (1994) conducted with plant extracts including *L. nobilis*, *E. globulus* and *T. vulgaris* and *A. obtectus,* longevity of adults, the number of eggs they laid, the number of adults that emerged and adult emergence/egg laying rates differed according to plant type, but all plant extracts were found to decrease in the specified parameters. In a study they examined the fumigant toxicity of *L. hybrida*, *R. officinalis* and *E. globulus* essential oils on *A. obtectus* eggs, Papachristos et al. (2004) found that 3-day-old eggs were more tolerant (when compared with those which were 4-days-old and older). They found that exposure time to essential oils inhibited egg hatching and increased the mortality rate of larvae hatching from the egg. The longevity in contact and repellent effect trials in the present study are in parallel with this study.

CONCLUSIONS

Since storage pest insects cause so much damage to stored products, most studies with plant extracts are generally on their lethal effects on these insects. However, recently, in addition to the lethal effect of plant extracts, studies have been focused on the number of eggs laid by these insects and the adults emerged from these eggs in order to avoid the destructive effect of the offspring they will make in the warehouses. In this study, besides the lethal effect of these plant extracts, it is possible to show the effects on the reproductive behavior and physiology of these insects. The data of this study are valuable for our understanding of the impact that offsprings can have in warehouses. As can be seen from the results, the number of eggs laid and the number of adults hatched by insects and beans treated with plant extracts are quite low compared to the control group. It reveals that especially *J. regia, T. vulgaris* and *L. nobilis* extracts affect the reproductive physiology of these insects and can be developed as a new environmentally friendly control agent against stored product pests. In the repellent effect experiments, the idea of decreasing the concentration was ignored because the Petri dishes were not covered with paraffin and they were air-permeable. In this sense, contact effect trials can give us a better idea. In order to better interpret the results, it is necessary to conduct experiments with decreasing and increasing plant concentrations in future studies and to determine their effects.

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