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Activity of Some Essential Oils as Adulticides for The Control of The Red Flour Beetle Adults, *Tribolium castaneum* (Herbst)

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ABSTRACT

Three EOs from the Lamiaceae family were tested for toxicity and repellence (basil, *Ocimum basilium* L; thyme, *Thymus vulgaris* L and sage, *Salvia officinalis* L) were evaluated against to adults of the red flour beetles, *Tribolium castaneum* (Herbst) in this research.

The results showed that basil oil was the most toxic among the oils tested when its effectiveness was tested using the thin film layer and treating grain, where the values of LC_{50} were 0.549% and 2. 831%, in comparison to 0.729% and 5.075 % for thyme oil and 0.711% and 6.319% for sage oil in both toxic methods, respectively. It also had the highest slope = (5.72) in the thin layer treatment, while sage oil had the highest slope = (7.81) in the treating grains method. As a result, basil had the highest relative potency for both toxicity treatments, reaching more than twice the relative effectiveness of sage oil in the thin layer treatment.

All the concentrations of the tested oils, (basil, thyme and sage) were recorded with a maximum percentage of repellence for *T. castaneum* adult during the tested exposure intervals from one hour to 24 hours of exposure so that the lowest percentage of repellence was recorded at 2% concentration with 92.32, 80 and 60 for oils of basil, thyme and sage, respectively after 1 h, it up to 76.67 and 93.33 and 93.33%, respectively after 24hrs from exposure. While the concentrations of 8 & 4% of the three tested oils maintained the highest repellence class V during the different exposure intervals from 1 to 24 hours, with repellence percentages ranging between 86.49 - 100%. It is worth noting that the repellence percentages increased with the increase in concentration, as well as with the increase in the exposure interval.

INTRODUCTION

Stored grains are infested by many pests, resulting in significant losses in both quantity and quality of grain (Phillips and Throne, 2010). The red flour beetle, *Tribolium castaneum* is among the most prevalent pests of stored grains and global distribution. It is a secondary pest of stored grains and a principal pest of many powdered and processed grains, dried fruits, nuts, and chocolate, milk powder, cereal, noodles, and pasta, among other products ((Karunakaran *et al., 2004). It causes economic loss in the amount and goodness of the infected* product, as well as having an impact on grain germination and gluten breakdown in flour. As a result, huge financial losses occur (Tefera *et al.*, 2011).

Essential oils have been demonstrated to be effective against a range of grain pests in storage (Kim *et al.,* 2003; Cardiet *et al.,* 2011; Bilal *et al.,* 2015). The effective chemicals contained in botanical oils play a major role in pest control, as they act as toxic, repellents and affect the development of insects. They can be used as potent fumigants as well as contact toxins (Lee *et al.,* 2004; Mishra *et al.,* 2012; Pavela, 2014). In addition to its significant effects on proteins, fats, carbohydrates, as well as some enzymes (Nathan *et al.,* 2005; El-Gizawy *et al.,* 2019). The Lamiaceae family contains active ingredients that function as repellents, toxicants, fumigants and antifeedants (Pavela, 2004; Mondal and Khalequzzaman, 2010; Saroukolai *et al.,* 2009).

In addition to their tremendous efficacy and versatility of actions, botanical extracts and essential oils have recently gained popularity as an alternative to synthetic pesticides due to their ease of use by small farmers, low toxicity to animal species and natural enemies, as well as food safety and lack of pollutants (Erler, 2005; Cetin and Yanikoglu, 2006; Fabres *et al.,* 2014; Bossou *et al.,* 2015; Upadhyay *et al.,* 2018)

The essential oils with effectiveness on coleopterans insects in stored grains belong to 30 plant families; 22 different species from the family Lamiaceae (Pérez *et al.,* 2010). Essential oils can be used to safely and effectively control red flour beetles, *Tribolium castaneum*, as well as other stored grain insects (Ayvaz *et al.,* 2009; Iram *et al.,* 2013).

The purpose of the study is to see how successful three essential oils (basil, thyme, and sage) are at controlling red flour beetles, *Tribolium castaneum*, in three different ways. This is to show the most effective concentrations for each treatment procedure and the most effective oil among the oils tested.

MATERIALS AND METHODS

Insects:

The adults of the red flour beetles, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). They were sieved from a stock culture of whole wheat flour maintained at laboratory conditions in the Plant Protection Department of Fayoum University's Faculty of Agriculture.

Evaluated Essential Oils:

The EOs are basil (*Ocimum basilium* L), thyme (*Thymus vulgaris* L), and sage (*Salvia officinalis* L), all from the Lamiaceae family were used in this investigation. They are commercial oils produced by El Captain and El Hawag Companies for the extraction of natural oils and cosmetics in Cairo, Egypt, according to the Egyptian standard specifications. The oils are extracted by cold pressing.

Oils Constituent: According to the production company

Basil Oil Contains Linalol- Cineol- Eugenol- Vitamin C & Thymine A.

Thyme Oil Contains Thymol- Carvkrol- Phosphoric acid- Seaman- Azotey phenol & Sulphur.

Sage Oil Contains: Vitamins C- Thujone -Rose acid- Marenic saponins & Flamendinat.

Bioassay:

The bioassays were conducted to show the activity of the tested oils on adults of *Tribolium castaneum* by using three separate methods to test its effect: contact, stomach (and/or) contact, and repellent activities.

Contact toxicity was carried out by exposing a thin film layer from tested oils in a petri dish (9 cm). The Petri dishes were treated by evenly distributed doses (1 ml) per dish of oils diluted in acetone at concentrations of 1, 0.75, 0.5 & 0.25 % for basil oil and 2, 1, 0.5 & 0.25 % for both thyme and sage oils.

Stomach (and/or) contact toxicity was estimated using grain treatment by infesting the adults with samples of treated sorghum grains (5 gm) in a petri dish (9 cm) treated with a dose of 2 ml of each concentration. The concentrations of oils had diluted in acetone, rates 6, 4 & 2% for basil oil, while the thyme oil was 8, 6 & 4 % and 10, 8 & 6% for sage oil, respectively.

The repellence activity was determined by treating half of a 7 cm diameter Whatman No. 1 filter paper with oil concentrations dissolved in acetone of 8, 4 & 2% for basil, thyme, and sage oils, respectively, while the untreated half of the filter paper was just treated with acetone. Cover one side of a 9 cm Petri dish with half of the treated filter paper and the other half with half of the untreated filter paper. Twenty T. castaneum adults were placed in the centre of each Petri dish.

The number of adults on the two halves of filer paper was counted and the percentage of repellence was estimated using the following equation after 1, 6, 12, and 24 hours of exposure.

Percentage of repellence = [NC-NT/ NC+NT] × 100

Adult No. in the untreated half is NC, whereas Adult No. in the treated half is NT. Class 0 = < 0.1, Class I = 0.1-20, Class II = 20.1-40, Class III = 40.1-60, Class IV = 60.1-80, and Class V = 80.1-100 percent, respectively, were used to split the results into 0-V repellence classes (McGovern *et al.,* 1977).

Twenty adults were examined in three replicates for each concentration in each technique and three replicates were treated with acetone as a control. The percentages of mortality were computed after 24 hours of exposure. All studies were carried out at room temperature (28 ± 2^{o}C) in the Plant Protection Department's Entomology Laboratory.

Analysis of Data:

The probit analysis of a computer programme (Lpd line) was used to estimate lethal concentrations (LC50 and LC90) within their 95% fiducial limits (Finney, 1971).

Toxicity indexes and relative potency were computed using the following equations (Sun, 1950):

Toxicity index= [LC50 of the standard material / LC50 of tested material] × 100

Relative potency = LC50 of lowest toxic material / LC50 of the tested material

Using the SPSS computer software version (21), the Duncan test for significant differences (P 0.05) between concentrations was carried.

RESULTS AND DISCUSSION

The three essential oils examined in this study (basil, thyme, and sage) have high efficacy against the adults of the red flour beetle in terms of contact and stomach toxicity, as well as repellent action. (López *et al.,* 2008; Khani and Asghari, 2012; Chen *et al.,* 2018) they mentioned that toxic and repellent effects of Eos against *T. castaneum* depend on several components, such as caranol, cymol, methyl eugenol,1,8-cineole, estragole, camphene, linalool, terpineol, and a-pinene, limonene**.** Monoterpenes cineole is a component of several essential oils and has strong lethal effects on *Rhyzopertha dominica* and *Tribolium castaneum* via a variety of mechanisms such as fumigant, contact, and stomach (Prates *et al.,* 1998).

Bioassay of Essential Oils for Contact Toxicity Activity Using A Thin Film Layer:

Table 1 illustrates the mortality percentages of *T. castaneum* adults following 24 hours of exposure to various dosages of basil, thyme, and sage oils by the thin film layer method.

Table 1: Mean replicates of the mortality percentages for different concentrations of tested essential oils towards the red flour beetles, *T. castaneum* after 24 hours of exposure using the thin film layer method.

Basil oil	
Concentrations %	**% Mean of Mortality percentages**
1	96.67 ± 3.33 **a**
0.75	81.67 ± 8.33 **a**
0.5	26.67 ± 1.67 b
0.25	6.67 ± 1.67 c
Control	0
Duncan test "F"	**86.23****
Thyme oil	
2	96.67 ± 3.33 **a**
1	60 ± 8.67 **b**
0.5	23.33 ± 3.33 **c**
0.25	13.33 ± 3.33 **c**
Control	0
Duncan test "F"	**53.20****
Sage oil	
2	93.33 ± 4.41 **a**
1	70 ± 2.89 **b**
0.5	40 ± 2.89 **c**
0.25	5 ± 2.89 **d**
Control	0
Duncan test "F"	**131.56****

(a,b,c,d) letters refer to the means significant with the same column. ** refer to significant of ″F-Test″ with the same column**.**

Basil oil at a concentration of 1% and thyme oil at a concentration of 2% had the greatest mortality percentages of 96.67%, followed by sage oil mortality of 93.33% at a concentration of 2 %and basil oil with 81.67% at a concentration of 0.75%. The lowest mortality percentages were 13.33, 6.67, and 5% for thyme, basil, and sage at 0.25% concentrations, respectively. Sage oil has the greatest significant value of F (131.56), followed by basil and thyme oil were 86.23 & 53.20. (Mikhaiel, 2011) revealed that *Ocimum basilicum* (basil oil) caused high mortality in *T. castaneum* adults and larvae after 24 hrs when used as a contact and fumigant method, in addition to being the most persistent. Adult moths, pupae, larvae, and eggs of *Ephestia kuehniella* and *Plodia interpunctella* were shown to have a high mortality rate when treated with basil *Ocimum basilicum* oil (Eliopoulos *et al.*, 2015). (Abd El-Salam, 2010) who reported the *Callosobruchus maculatus* and *Sitophilus oryzae* adults had higher susceptibility towards Eos of *Thymus vulgaris*. Thyme oil and its constituents, especially thymol, were found to be effective against *Tribolium confusum, Rhyzopertha dominica,* and *Ephestia cautella* (Shaaya *et al.*, 1993).

According to the toxicity index table, the lethal concentration LC50 for basil, sage, and thyme oils against adults of T. castaneum after 24 hours of exposure was 0.549, 0.711, and 0.729 percent, respectively, and 0.920, 1.903, and 2.002 percent for LC90 (Table 2). *T. castaneum* adults were highly toxic to basil oil as a contact poison, with an LD50 of 0.045 mg after exposure for 24 hrs. (Kim and Lee, 2014). After 3, 4, and 5 days of treatment, the LC50s of Sitophilus oryzae adults were 8.14, 6.50, and 4.91 l/ml of basil oil, respectively. (Hossain *et al.*, 2014). (Koutsaviti *et al.*, 2018) *Salvia officinalis* oils have been shown to be

toxic to adults of *Sitophylus oryzae*. *Salvia leriifolia* essential oil has acute toxicity in control of *Sitophilus granarius* and *Rhyzopertha dominica* (Hosseini *et al.,* 2013).

Basil oil was also shown to have 1.33 times the relative efficacy of the other oils examined.

When comparing the slopes of dose-effect lines for the tested essential oils, observed that basil and thyme oils had the highest slopes of 10.69 and 7.69, respectively, while sage oil had the lowest slope of 4.72 (Table 2). (Aisha *et al.,* 2018) maintained that *Thymus vulgaris* caused high mortality and repellent rate in the adult and larval stages of *Tribolium castaneum***.**

The steep slope indicates that just a slight increase in oil dose causes a high mortality rate, whereas the steep slope increases toxicity.

Table 2: Toxicity index of essential oils against adults of *T. castaneum* after 24 hours post-exposure using thin film residue**.**

Essential oils	$\chi 2$	Slope	LC_{50} ppm	Confidence limits 95%		LC_{90} ppm	Confidence limits 95%		Toxicity index %	Relative potency
				Lower ppm	Upper ppm		Lower ppm	Upper ppm		
Basil	10.69	5.72	0.549	-	-	0.920	-	-	100	1.33
Sage	4.72	2.85	0.711	0.606	0.837	2.002	1.557	2.928	77.22	1.03
Thyme	7.69	3.074	0.729	-	-	1.903	-	-	75.31	1

Bioassay of Essential Oils for Contact And/Or Stomach Activity Using Treating Grains:

The highest mortality percentage of *T castaneum* adults using treating grains was recorded by sage oil with 98.33% at 10% concentrations and basil oil, with 96.67% mortality at a concentration of 6%, with a dose of 2 ml/5 gm of sorghum grains. The 4 % conc, on the other hand, resulted in 75 % and 28.33 % mortality for basil and thyme oil, respectively. Basil oil got the highest F-value of 22.54 (Table 3). (Popović *et al.,* 2006) noticed contact toxicity and treating grain for mortality and repellence by *Ocimum basilicum* and *Salvia officinalis* at 2% concentration caused considerable mortality, repellence, and reduced fecundity to *Sitophilus oryzae.* Treating wheat with 15 % thyme oil is a good enhancer for keeping wheat at intervals (Badawi *et al.,* 2017). Wheat treatment with thyme and sage EOs caused 90 percent mortality of *T. confusum* adults after 72 hrs (Sener *et al.,* 2009). The encapsulation by EOs of basil and thyme has been demonstrated to be effective against the rice weevil, *Sitophilus oryzae* (Hossain *et al.,* 2019). Adult *Sitophilus oryzae* was shown to be very susceptible to basil oil fumigation at a concentration of 10% with a dosage of 3 mL (Follett *et al.,* 2014).

The values of LCs for different oils obtained by treating grains are shown in **Table 4**. Basil, thyme, and sage oils have LC_{50}s of 2.514, 4.585, and 5.721, respectively. In addition, the LC_{90}s values for basil, thyme, and sage oils were 5.095, 8.561, and 9.222, respectively. (Karabörklü *et al.,* 2010) pointed to LC_{50} mortality of sage oil was 372.550 (µl/l air) on *T. castaneum*. The acetylcholinesterase and butyrylcholinestearase in *Tribolium spp* and *Rhyzopertha dominica* were inhibited by Eos of *Salvia veneris* with a rate of 85.9% and 12.2%, respectively (Polatoğlua *et al.,* 2017).

The maximum slope was 7.81 for sage oil. While the basil oil had more relative potency than thyme and sage oils, its potency was twice that of sage oil.

Table 3: Mean replicates of the mortality percentages for different concentrations of tested essential oils towards the red flour beetles, *T. castaneum* using grain treating method post 24 hours of exposure.

Basil oil	
Concentrations %	**% Mean of Mortality percentages**
6	96.67 ± 1.67 **a**
4	75 ± 11.55 **a**
2	23.33 ± 7.27 **b**
Control	0
Duncan test "F"	**22.54****
Thyme oil	
8	87.5 ± 2.5 **a**
6	65 ± 10.41 **a**
4	28.33 ± 10.14 **b**
Control	0
Duncan test "F"	**8.83***
Sage oil	
10	98.33 ± 1.67 **a**
8	75 ± 14.43 **ab**
6	45 ± 5.77 **b**
Control	0
Duncan test "F"	**8.77***

(a,b,c,d) letters refer to the means significant with the same column. ** refer to significance of ″F-Test″ with the same column

Table 4: Toxicity index of essential oils against adults of *T. castaneum* after 24 hours post-exposure using grain treating method

Essential oils	$\chi 2$	**Slope**	**LC_{50} ppm**	**Confidence limits 95%**		**LC_{90} ppm**	**Confidence limits 95%**		**Toxicity index %**	**Relative potency**
				Lower ppm	**Upper ppm**		**Lower ppm**	**Upper ppm**		
Basil	0.403	5.02	2.831	2.514	3.130	5.095	4.512	6.031	100	2.23
Thyme	0.045	5.65	5.075	4.585	5.536	8.561	7.463	10.984	55.78	1.25
Sage	0.889	7.81	6.319	5.721	6.758	9.222	8.597	10.261	44.80	1

Repellence Activity:

The various responses of *T. castaneum* adults are shown in Table 5 for tested essential oils during four exposure times. PR values ranged from 60 to -100 % for all tested oils concentrations during the time periods studied. Overall, increasing the concentrations of three examined EOs and increasing exposure times increased the repellence percentage in *T. castaneum* adults.

In terms of results of *Ocimum basilium* EOs, *T. castaneum* had the greatest significant repellence at concentrations of 8, 4 and 2% after the first hour of treatment, and the percentage of repellence were 96.67, 96.67, and 92.32%, respectively occupied the highest category of repellence V.

The percentage of repellence gradually increased with the increase in the duration of exposure to concentrations 8 & 4 until it reached 100% repellence.

While the concentration of 2% gradually decreased during the different exposure periods, recording 76.67% with a category of repellence IV at 24h of exposure. After 24 hours of exposure, the strongest impact F values = 8.6 were recorded.

Table 5: Percentage repellence (PR) of essential oils against adults of *T. castaneum* at different exposure periods during one day.

Tested oils	Conc	1 Hour		6 Hours		12 Hour		24 Hour	
		% PR	Class	% PR	Class	% PR	Class	% PR	Class
Basil	8	96.67 ± 3.33 **a**	V	100 ± 0 **a**	V	100 ± 0 **a**	V	100 ± 0 **a**	V
	4	96.67 ± 3.33 **a**	V	100 ± 0 **a**	V	100 ± 0 **a**	V	96.67 ±3.33 **a**	V
	2	92.32 ± 3.88 **a**	V	89.65 ± 6.08 **a**	V	80 ± 10 **a**	IV	76.67 ± 6.67 **a**	IV
	Duncan test "F"	**0.5**		**2.90**		**4***		**8.6***	
Thyme	8	93.33 ± 3.33 **a**	V	96.67 ± 3.33 **a**	V	93.33 ± 6.67 **a**	V	100 ± 0 **a**	V
	4	93.33 ± 6.67 **a**	V	96.67 ± 3.33 **a**	V	100 ± 0 **a**	V	100 ± 0 **a**	V
	2	80 ± 5.77 **a**	IV	90 ± 5.77 **a**	V	93.33 ± 6.67 **a**	V	93.33 ± 3.33 **a**	V
	Duncan test "F"	**2**		**0.8**		**0.5**		**4***	
Sage	8	96.67 ± 3.33 **a**	V	100 ± 0 **a**	V	100 ± 0 **a**	V	100 ± 0 **a**	V
	4	86.49 ± 8.79 **a**	V	96.67 ± 3.33 **a**	V	96.67 ± 3.33 **a**	V	96.67 ± 3.33 **a**	V
	2	60 ± 15.27 **a**	III	96.67 ± 3.33 **a**	V	96.67 ± 3.33 **a**	V	93.33 ± 6.67 **a**	V
	Duncan test "F"	**3.34**		**17.29****		**1.5**		**1.75**	

For *Thymus vulgaris* EOs, Table 5 revealed that the maximum significant repellence of *T. castaneum* adults was 100 % at 8 & 4 % conc after 24 hrs of exposure. According to (Manole and Fătu, 2015), thyme oil has a strong repellent effect against *Sitophilus granarius* when mixed with wheat grains, hence avoid to eat grains may be a good way to avoid this species attacking. Basil and thyme oils have a powerful repellent action (class V) against *T. castaneum* adults during one day of exposure (Mona Abd El-Aziz and El-Sayed, 2009). Also (Eman Ismail, 2018) pointed out thyme oil is a highly potent repellent and toxicant

For *Salvia officinalis* EOs, in **Table 5**, the increasing concentration of EOs and exposure times resulted in the highest repellence percentage of *T. castaneum* adults. after 1 hour at concentrations 8, 4 & 2 from the *S. officinalis*, the repellence percentage of T. *castaneum* had been recorded at 96.67, 86.49 and 60 % respectively, increased gradually during exposure intervals to reach class V at 24 hrs post-exposure with 100, 96.67, 93.33 PR% respectively, for three tested concentrations. (Yoon and Tak, 2018) maintained that Linalyl acetate, the most abundant ingredient in sage oil, was the most responsible for its repellent action. Salvia officinalis has the greatest mortality and repellence rates against the bean weevil, *Acanthoscelides obtectus* (Scariot *et al.*, 2016).

CONCLUSION

It was concluded that the dose and concentration for each type of oil change according to the method of application, and that basil oil is the most toxic when applied in two methods: a thin layer and treating grains, followed by thyme and sage oil.

While the three oils showed a strong repellence when used at medium concentrations of 8, 4 and 2%, As a result, the three effects can be accomplished simultaneously when utilising oils in medium concentrations.

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ARABIC SUMMARY

نشاط بعض الزيوت الأساسية كمبيدات للبالغين لمكافحة الحشرات الكامله لخنفساء الدقيق الحمراء **Tribolium *castaneum* (Herbst)**

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في هذا البحث تم تقييم أنشطة السمية والطرد لثلاث زيوت أساسيه من العائلة الشفوية Lamiaceae وهي الريحان *Ocimum basilium* , الزعتر Thymus vulgaris والمريمية *Salvia officinalis* ضد البالغين من خنافس الدقيق الحمراء.*Tribolium castaneum*. أظهرت النتائج أن زيت الريحان كان الأكثر سمية بين الزيوت المختبرة عند اختبار فعاليته باستخدام الطبقة الرقيقة ومعالجة الحبوب، حيث كانت قيم التركيز النصفي القاتل (LC_{50}) 0.549و2.831٪ مقارنة بـ 0.729٪ و075.5 ٪ للزعتر. الزيت و0.71٪ و6.319 ٪ لزيت المريمية بكلتا الطريقتين السامتين على التوالي. كما كان له أعلى انحدار = (5.72) في معاملة الطبقة الرقيقة، بينما كان لزيت المريمية أعلى انحدار = (7.81) في طريقة معالجة الحبوب. نتيجة لذلك، كان للريحان أعلى فعالية نسبية لكلا معاملتي السمية، حيث وصل إلى أكثر من ضعف الفعالية النسبية لزيت المريمية في معالجة الطبقة الرقيقة.

سجلت جميع تراكيز الزيوت المختبرة (الريحان والزعتر والمريمية) أعلى نسبة طارده للحشرات الكامله *T. castaneum* خلال فترات التعرض المختبرة من ساعة إلى 24 ساعة، حيث سجلت أقل نسبة طارده عند تركيز 2٪ كانت 92.32 و80 و60% لزيوت الريحان والزعتر والمريمية على التوالي بعد ساعة، وصلت إلى 76.67 و93.33 و93.33٪ على التوالي بعد 24 ساعة من التعرض. بينما حافظت تراكيز 8 و4٪ من الزيوت الثلاثة المختبرة على أعلى درجة طارده للحشرات الفئة V خلال فترات التعرض المختلفة من 1 إلى 24 ساعة، حيث تراوحت نسب الطرد بين 86.49 - 100٪. وتجدر الإشارة إلى أن نسب الطرد تزداد مع زيادة التركيز وكذلك مع زيادة فترة التعرض.

الكلمات المفتاحية: خنفساء الدقيق الحمراء، الزيوت الأساسية، الريحان، المرمية، الزعتر , مواد سامة , طاردة.