



Assessment of Some Essential Oils and Their Mixtures as Material Repellents Against Adults of The Confused Flour Beetle, *Tribolium confusum* (Du Val) (Coleoptera: Tenebrionidae)

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Abstract

Repellency of the six essential oils (EOs), Clove (*Eugenia aromaticum*), Aloe (*Aloe vera*), Lemon (*Citrus limon*), Camphor (*Eucalyptus camaldulensis*), Mint (*Mentha spicata*), Marjoram (*Origanum vulgare*) were evaluated against adult of the confused flour beetle *Tribolium confusum*, by allowing adults to choose between untreated filter paper halves with 1 - 2g white flour or treated with different concentrations of the oils and their mixture at three different exposure times. In individual oils experiments, *Eucalyptus camaldulensis* was found to be the best effective of repellent efficacy, followed by *Mentha spicata* and *Eugenia aromaticum*. *Citrus limon* and *Aloe vera* have moderate repellent efficacy. Results of combination effective showed that mixtures' repellent activity. It has been shown that the most powerful mixture of Clove + Mint repels activity against adults with Lemon + Aloe, Lemon + Marjoram, Lemon + Mint, and Mint + Aloe during the entire exposure period, while Lemon + Camphor and Camphor + Mint lost repellent activity after 24 hours.

Keywords: *Tribolium confusum*, Essential oils, Oils combination, Repellency

1 Introduction

The confused flour beetle, *Tribolium confusum* (du val), known as "bran bugs," is secondary pest commonly found infesting stored products. Milled grain products are primarily under attack, but not the undamaged whole grain (Bennett 2003).

Specimens of beetles have been found in a variety of stored products, such as cereals, oats, dried fruits, peas, oats, milk powder, etc (Mediouni Ben Jemâa et al 2012, Pugazhvendan et al 2009, Bennett 2003). These beetles in infested flour also hitchhike into the home and may multiply into large populations. In warehouses and in factories manufacturing starch goods, it is especially injurious. Larvae-infested flour has an agreeish color and a tendency to become mouldy (Bennett 2003).

T. confusum populations in the storage bins, milling, formulation and pelleting sites were located (Trematerra and Sciarretta 2004). Their develop resistance to some of the few chemical options due to complicates management and Cause significant economic loss (Holloway et al 2020, Isman 2020). The chemical compounds, including both fumigants and contact insecticides, are used on food grains to control storage insects (Jeanmart et al 2016). Due to certain drawbacks related to these chemicals, such as residual effects, the poten-

tial for toxicity and the inherent expense of applying conventional insecticides, successful control measures are highly important, furthermore, pesticides resistance (Daglish 2004, Sylvia et al 2009). Eight strains of red flour beetle, *T. castaneum* all strains were resistant to Malathion and one strain was resistant to phosphine (Zetter and Cuperus 1990).

Might be using aromatic plants as intercrops in agroecosystems to biologically control insect pests (Tang et al 2013). For stored products, plant-obtained materials such as essential oils (EOs) are sources of protection (Shimomura et al 2019). As an alternative to synthetic repellents, plant essential oils (EOs) and botanical insecticides have been used widely (Lee 2018 and Isman 2006). Essential oils are consisted the various monoterpenoids and other molecules shown properties for pests control agents, it work as insecticidal, repellent and antifeedant (Jeyasankar et al 2016, Ukeh et al 2009, López et al 2008).

(Olivero-Verbel et al 2013, Nerio et al 2009) repellency effective properties of Colombian aromatic plants and their molecules contained in EOs have also demonstrated better action than the commercial repellent IR3535 against *T. castaneum*. Strong effects against *T. Castaneum* of the repellent and larvicidal efficacy of essential oils. (Jeyasankar et al 2016) very strong repellent activity of four ingredients as contact and fumigant agents were obtained from *Laurelia sempervirens* and *Drimys winteri* the bark and leaves against *T. castaneum* (Zapata and Smagghe 2010). Seven compounds were extracted from essential oils of *Alpinia zerumbet* (Pers.), (camphor, m-cymene, eucalyptol, o-cymene, borneol, camphene, eucalyptol and eucalyptol) were shown different levels as insecticidal and repellent efficacy towards *T. Castaneum* (Feng et al 2021). The essential oils and components of *Aframomum megueta* (K. Schum) and *Zingiber officinale* (Roscoe) may be ideal as safety fumigants and repellents (Ukeh and Sylvia 2011).

(Myrtaceae) usefully applied to control storage pests (Zandi-Sohani et al 2013). About the genus *Aloe* L majority

opinion ratios suggest that the most popular uses of *Aloe* are in medicine and pest management against arthropods and invertebrates, (Asphodelaceae) comprising 548 recognized species (Grace et al 2009). The extracts of the Lamiaceae family have repellents and anti-feedant action (Pavela 2004). Essential oils contain many chemical compounds that cause many metabolic disorders, besides repellent and lethal effects (Deb and Kumar 2020).

The study aims to find safe alternatives to prevent infestation with flour beetles without the need to use the chemical insecticides. Thus, the research paper verified the effectiveness of some essential oils on the confused beetle individually, as well as evaluated after mixing them.

2 Materials and Methods

The experiment was undertaken on the adults of the confused flour beetle, *Tribolium confusum* (du val) (Coleoptera: Tenebrionidae).

2.1 Insects culture

Source of *T. confusum* was collected from infested whole wheat flour from the home kitchen in fayoum governorate, Egypt. It was brought to the laboratory and put it in a plastic container of 26×29 cm containing whole wheat flour under the laboratory temperature 20±2°C. Adults of *T. confusum* were used in subsequent experiments were separated by a sieve.

2.2 Essential oils sources

The six essential oils, Clove *Eugenia aromaticum* (L.) Baill, Myrtaceae, Aloe *Aloe vera* (L.) Burm.f., Asphodelaceae, Lemon *Citrus limon* (L.) Osbeck, Rutaceae, Camphor *Eucalyptus camaldulensis* Dehnh, Myrtaceae, Mint *Mentha spicata* L, Lamiaceae, Marjoram *Origanum vulgare* L, Lamiaceae, were purchased from local market (spice dealer, a store that

sells spices, medicinal plants, and essential oils) at Fayoum Governorate, Egypt, from El-shatby, El-captain and other companies extracting oils, herbs and cosmetics, Cairo, Egypt, which authorized by the Egyptian Ministry of Health for different human uses were experimented, for used in the experiments of repellent activity against adults of *T. confusum*.

2.3 Evaluation of insect repellence

Repellent action in the six essential oils on adult of *T. confusum* was evaluated using the technique certified from (Jo et al 2013) with slight modifications. Using twenty insect adults per dish, a selection bioassay with three replications (dishes) was set up. The plastic dishes (12 cm Length × 9cm width) were divided into three zones.

Test solutions were made by dissolving, 10 ml of distilled water in different (4, 2, 1, 0.5 gm) concentrations of each oil with addition 1-2 drop of triton emulsifier.

Mixtures of oils, The mixture test solution was prepared at a rate of 1:1 by dissolving 0.5 g of the first oil + 0.5 g of another oil in 10 ml of distilled water plus 1-2 drops of tritone emulsifier.

The filter paper for Whatman No. 1 (90 mm) was divided into two halves. At each concentration, half of the filter paper was submerged at the rate of three halves of the filter paper for each concentration. Distilled water + 1-2 drop triton was treated with the other filter paper half. The halves of water untreated and oil treated were fully dried for around 15 min. Untreated halves (control) were placed in one zone. Treated halves of solution of the essential oils were placed in the other zone and put 1g wheat flour on each half. The 20 unsexed adults of *T. confusum* were released in the center of the dish and then the dishes were sealed and incubated at 25±1°C.

For each treated or untreated (control) half and diet, the number of adults present was counted after (1, 3, 24 hours) and calculated the mortality of adult if found that. By using the following equation ("Taulkader, 1944,"

n.d.), the data was expressed as percentage repellency (PR):

$$\text{Percentage Repellency (PR)} = \frac{[NC-NT]}{[NC+NT]} \times 100$$

NC is Adult No. on the control section, NT is Adult No. on the treated section. The values were then categorized as follows 0-V repellency classes, whereas 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%, respectively (McGovern 1977).

2.4 Statistical analysis

Relationships between concentrations of oils and recorded values of percentage repellency (PR in 3 times) were evaluated by using simple correlation (Pearson) (r), SPSS software program was used (Senedecor and Cochran 1967).

3 Results and Discussion

3.1 Individual experiments of essential oils

The repellent effective was studied at four series concentrations of six individual essential oils, against adults of *T. confusum* with three an exposure period (**Table 1**). Positive percentages of adults repellency (in the control section), whereas negative percentages suggest their attraction (in the treated section).

Shown (**Fig 1 & Table 1**) percentage repellency of six essential oils after one hour exposure, observed it is evident that the repellent activity of the two oils of lemon and clove are significant positive correlation to the concentrations (r= 0.98* & 0.23 respectively), as the repellent activity of both increases with increasing concentration recorded 30 and 43.33% PR respectively at 0.4g/1ml. This applies to camphor oil is insignificant positive correlation r=0.002, the higher concentrate, it was found during subsequent periods of exposure that it carries a chronic toxicity reach to 21.7 % mortality, repellent effect was strong with positive class of IV, III, I at 0.2 - 0.1 -0.05 g/1ml.

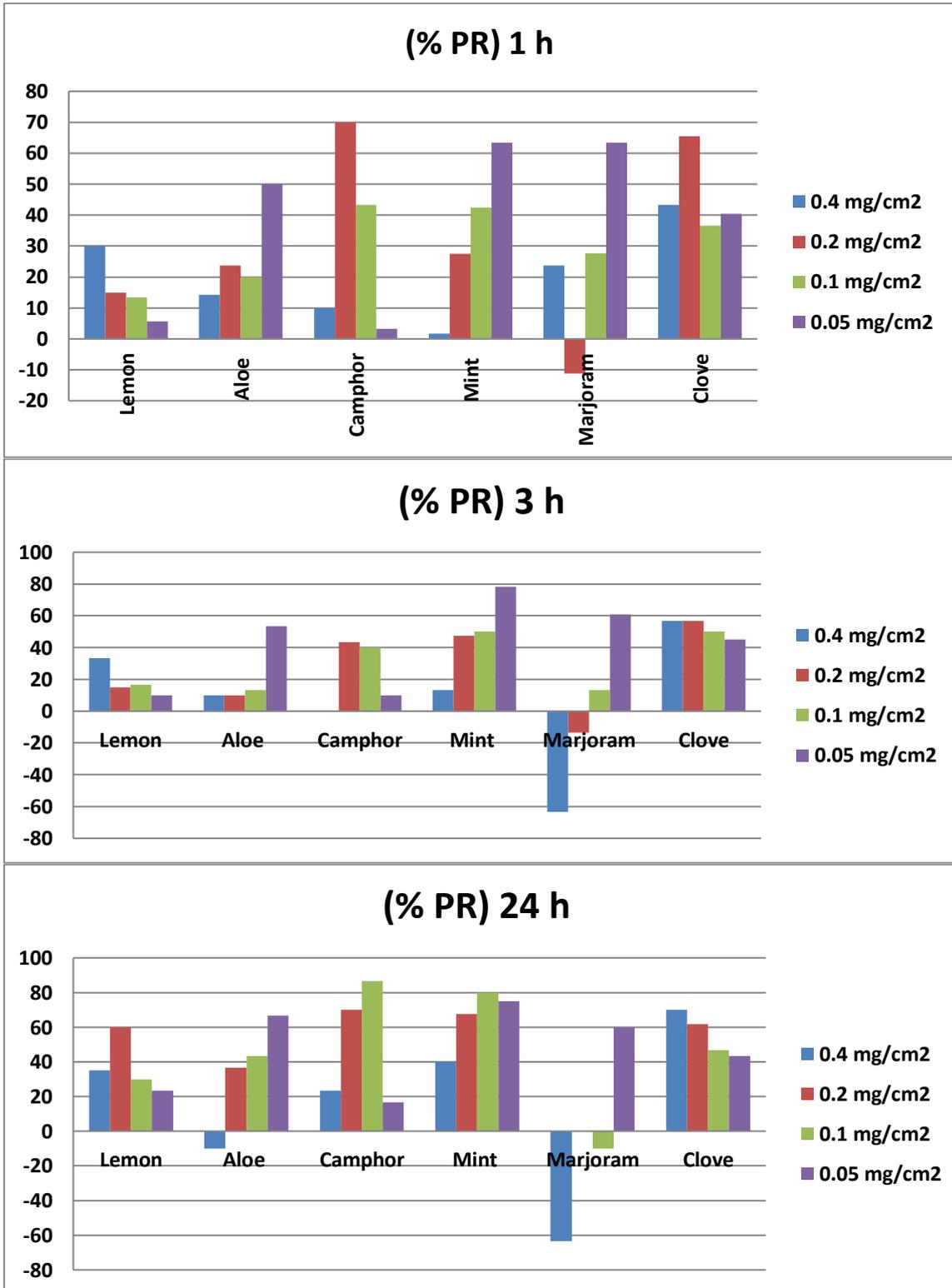


Fig 1. Percentage repellency diagrams of six essential oils against adult of *T. confusum* at different exposure times

Table 1. Percentage repellency of six essential oils against adult of *T. confusum* at three exposure times

Essential oils	Conc mg/1ml	1 Hour			3 Hours			24 Hours		
		% PR ¹	Class	% Mortality	% PR	Class	% Mortality	% PR	Class	% Mortality
Camphor <i>Eucalyptus camaldulensis</i>	0.4	10	I	0	0	0	13.33	23.40	II	21.67
	0.2	69.96	IV	0	43.33	III	0	70	IV	0
	0.1	43.33	III	0	40	II	0	86.67	V	0
	0.05	3.33	I	0	10	I	0	16.67	I	0
Pearson (r)		0.002			-0.34			-0.15		
Clove <i>Eugenia aromaticum</i>	0.4	43.33	III	0	56.67	III	0	70	IV	0
	0.2	65.55	IV	0	56.67	III	0	61.67	IV	0
	0.1	36.66	II	0	50	III	0	46.67	III	0
	0.05	40.35	II	0	45	III	0	43.33	III	0
Pearson (r)		0.23			0.87			0.96*		
Mint <i>Mentha spicata</i>	0.4	1.75	I	0	13.33	I	0	40	II	0
	0.2	27.5	III	0	47.5	III	0	67.5	IV	0
	0.1	42.5	III	0	50	III	0	80	IV	0
	0.05	63.33	IV	0	78.33	IV	0	75	IV	0
Pearson (r)		-0.99**			-0.97*			-0.93		
Lemon <i>Citrus limon</i>	0.4	30	II	0	33.33	II	0	35.15	II	38.33
	0.2	15	I	0	15	I	0	60	III	0
	0.1	13.33	I	0	16.67	I	0	30	II	0
	0.05	5.67	I	0	10	0	0	23.33	I	0
Pearson (r)		0.98*			0.94			0.37		
Aloe <i>Aloe vera</i>	0.4	14.28	I	16.67	10	I	26.67	-10	0	28.33
	0.2	23.63	II	0	10	I	0	36.67	II	0
	0.1	20	II	0	13.33	I	0	43.33	III	0
	0.05	50	III	0	53.33	III	0	66.67	IV	0
Pearson (r)		-0.79			-0.72			-0.99*		
Marjoram <i>Origanum vulgare</i>	0.4	23.64	II	31.67	-63.33	0	83.33	-63.33	0	96.67
	0.2	-11.11	0	33.33	-13.33	0	66.67	0	0	96.67
	0.1	27.59	II	0	13.33	I	11.67	-10	0	43.33
	0.05	63.33	IV	3.33	60.71	IV	3.33	60	III	6.67
Pearson (r)		-0.51			-0.99*			-0.92		

¹PR stands for percentage repellency.

It is high significant negative correlation in both Mint and Aloe oils ($r = -0.99^{**}$ & -0.79 , res), where the highest percentage of repellency at the lowest tested concentration recorded 63.33 and 50% PR respectively at 0.05g/1ml.

The results indicated that the mortality of *T. confusum* adults remarkable from the beginning of exposure to Marjoram oil, which made high concentrations of it highly toxic (acute toxicity), and the lowest concentrate was maintained with a high of PR 63.33% (class IV), $r = -0.51$.

Tabulated and illustrated data showed that with the increase in exposure length, the repellent efficacy of lemon, clove, mint, aloe, and camphor oils increased. Continued positive correlation of Lemon oil and Clove during the two subsequent exposure periods, with $r = 0.94$ & 0.37 for Lemon and 0.87 & 0.96^* for Clove, respectively. In contrast, the Camphor oil turn into negative correlation with both Mint and Aloe -0.34 & -0.15 , -0.97^* & -0.92 and -0.72 & -0.99^* after 3 and 24 hours from exposure. In contrast, the repellent efficacies of lowest concentration 0.05g/ ml of Marjoram slightly decreased from 63.3 (class IV) to 60 (class III) with negative significant correlation after 3 hours exposure $r = -0.99^*$ and negative repellency with increased percentages of mortality for the three highest concentration reach to 96.67, 96.67, 43.33, 6.67 respectively. The mortality of *T. confusum* adults was increased to 28.33 for Aloe oil at 0.4 g/ 1ml .

Overall, essential oils were effective against the confused flour beetle *T. confusum*, *Eucalyptus camaldulensis* was found to be the best effective 86.67% repellent efficacy, follow by *Mentha spicata* and *Eugenia aromatum*, which are considered the most effective and stable in all concentrations tested during the entire exposure period. *Citrus limon* and *Aloe vera* have moderate repellent efficacy. The *Origanum vulgare* high lethal at the used concentrations.

3.2 Combination effect of essential oils

The average concentration of 0.1g/1ml was chosen as the mixing concentration at a ratio of 1: 1 for each oil against adults of *T. confusum* with three an exposure period (**Table & chart, 2**).

It was indicated in (**Table, 2**) and (**Chart, 2**) the high repellent efficacy in most mixed plant oils compared to oils alone.

All treatments were Synergism effect against adults of *T. confusum* with negative PR -3.33- -3.33 and -23.33 % for Camphor + Marjoram, Camphor + Clove and Clove + Marjoram after one hour exposure, while recorded combination of Clove + Mint and Mint + Aloe the best effective 76.67% repellent efficacy, follow Clove + Aloe, Lemon + Mint and Lemon + Aloe of 73.33.63.33.63.33 %PR, respectively. Shown moderate repellent efficacy for Camphor + Mint, Lemon + Marjoram, Lemon + Clove and Camphor + Aloe class III and II. Lemon + Camphor and Mint + Marjoram were recorded lowest 20 & 16.67 %PR, respectively.

The second exposure time at 3 hours, the percentage of repellent is decreased for all combination except, Camphor + Clove and Clove + Marjoram and Camphor + Marjoram moved from negative repellency to positive repellency of 40, 3.33 & 0 %PR, addition increased of repellent efficacy for Clove + Mint and Marjoram + Aloe reach to 86.67 & 36.67%.

At last exposure period after 24 hours from exposure, there are mixtures whose repellent activity decreases slightly such as Clove + Aloe, Mint + Aloe, Marjoram + Aloe from class IV, IV & II to II, III and I, while others maintain and increase their high repellent activity, such as Lemon + Mint, Lemon + Marjoram, Lemon + Clove, Lemon + Aloe, and Clove + Mint, While Camphor + Mint has lost its repellency after one day of exposure.

Table 2. Percentage repellency of combination six essential ratios (1:1) against adult of *T. confusum* during three exposure times

Mixture oils 0.5+0.5g /1ml	1 Hour			3 Hours			24 Hours		
	% PR ¹	Class	% Mortality	% PR	Class	% Mortality	% PR	Class	% Mortality
Lemon + Camphor	20	I	1.67	0	0	5	-33.33	0	10
Lemon + Mint	63.33	IV	0	50	III	0	63.33	IV	1.67
Lemon + Marjoram	46.67	III	0	30	II	0	66.67	IV	0
Lemon + Clove	40	II	0	20	I	0	46.67	III	0
Lemon + Aloe	63.33	IV	0	50	III	0	76.67	IV	0
Camphor + Mint	56.67	III	0	23.33	II	0	-6.67	0	0
Camphor + Marjoram	-3.33	0	1.67	0	0	15	23.33	II	20
Camphor + Clove	-3.33	0	0	40	II	0	18.33	I	5
Camphor + Aloe	30	II	0	26.67	II	0	40	II	0
Clove + Mint	76.67	IV	0	86.67	V	0	66.67	IV	0
Clove + Marjoram	-23.33	0	0	3.33	I	1.67	60	III	0
Clove + Aloe	73.33	IV	0	33.33	II	0	40	II	3.33
Mint + Marjoram	16.67	I	0	3.33	I	0	46.67	III	3.33
Mint + Aloe	76.67	IV	0	63.33	IV	0	50	III	0
Marjoram + Aloe	21.67	II	1.67	36.67	II	3.33	20	I	6.67

¹PR stands for percentage repellency

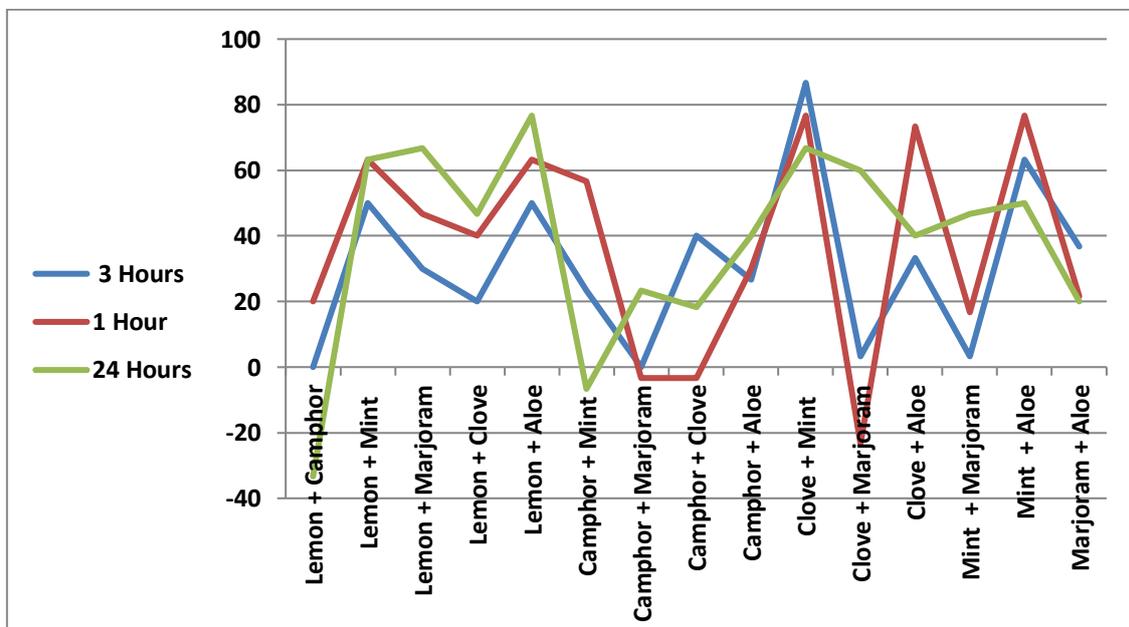


Fig 2. Diagrammatic of percentage repellency of combination six essential against adult of *T. confusum* during three exposure times

Camphor + Marjoram, Camphor + Clove, Clove + Marjoram the repellent activity gradually increased, reaching its maximum repellency rate at 24 hours. It was noticed that the oil mixtures with lemon oil provided its repellent efficiency, which transferred it from a medium to a high class of repellency, exception of mixing camphor with lemon, as mixing them together inhibited the repellent effect of them.

Shown few percentage of mortality for some essential oils mixture the highest values were recorded by Camphor + Marjoram and Lemon + Camphor 20 & 10 % respectively.

Finally, the most effective mixture of Clove + Mint was shown to repel action against adults of *T. confusum* from tabulated and illustrated results, during the entire exposure period. Lemon + Aloe, Lemon + Marjoram, Lemon + Mint, and Mint + Aloe followed up. In comparison, after 24 hours, Lemon + Camphor and Camphor + Mint lost their repellent activity.

There are numerous studies about the use of plant oils for pest management, especially flour beetles. In this work found the *Eucalyptus camaldulensis* the best repellent at 1g /1ml against *Tribolium confusum*, *Mentha spicata* and *Eugenia aromaticum* are high repellents towards *Tribolium confusum*. *Citrus limon* and *Aloe vera* have moderate repellent efficacy, while *Origanum vulgare* high lethal at same used concentration. These findings are consistent with several prior researches using essential oils as a repellent for flour beetles, such as (Bhaskar Mi and Tripathi 2011) observed, *Schyzgium aromaticum* was highest repellent activity against *Tribolium castaneum*. (Ramsha et al 2019) investigated the repellent action of clove, coriander, neem and mint extracts towards *Tribolium castaneum*. The effects of these plant extracts have been shown to have effective insect repellent action. Coriander, accompanied by mint, was the most effective extract, while clove was less effective. The most effective concentration was 1.5 percent emphasis.

The fore-mentioned results are consistent with his findings (Salem et al 2018) at concentrations of 0.31 $\mu\text{L}/\text{cm}^2$ and 0.078 $\mu\text{L}/\text{cm}^2$ resp of *Mentha pulegium*, the repellent activity arrived to 80 and 60 percent after 1 and 24 h of *Tribolium castaneum* exposure. And (Khani and Asghari 2012) demonstrated good larvicidal efficiency to *Tribolium Castaneum* with *Mentha longifolia* oils.

Positive effects of repellent activity and larval mortality of clove oil on *T. castaneum* Larvae (Jeyasankar et al 2016).

(Ofori et al 2010) noticed the bioactivity of camphor, extracted from basil shrub essential oil, *Ocimum kilimandscharicum*, against *Tribolium castaneum*, by used contact, grain application, and repellency. Camphor was extremely poisonous in camphor-treated grain, growth of eggs and immature stages inside grains, and progeny emergence, were totally inhibited. Repellency of camphor (Pavela 2004) indicated that *Origanum majorana* extract was highly repellent for adults of the Colorado potato beetle (*Leptinotarsa decemlineata*). Repellent activity for the *Amomum villosum* essential oil and its four components at high concentration but the Limonene, Camphene and Camphor have high fumigant on *T. Castaneum* (Chen et al 2018).

The mixture of essential oils provided a higher repellent efficacy in these articles, which is consistent with the (pavela 2015) tested the acute toxicity effectiveness of 30 aromatic compounds and their reciprocal binary combinations against *Culex quinquefasciatus* larvae. In total, 435 binary combinations, of which 249 combinations had a major synergism, were tested. An important antagonistic impact on mortality was demonstrated by 74 combinations. (Lee et al 2020) found that the repellent efficacy of *Origanum vulgare* and *Tanacetum cinerariifolium* against *Plodia interpunctella* larvae was greatest. *T. Cinerariifolium* demonstrated effective repellence against the adults and larvae of *Tribolium castaneum* and improved repellent efficacy by combining them.

(Benelli et al 2017) found that by preparing simple binary mixtures of essential oils, larvicidal efficacy can be improved against *Culex quinquefasciatus*, (Diptera: Culicidae), such as *Satureja Montana* + *Aloysia citriodora* at (ratio 1:1), which displayed greater larvicidal toxicity.

4 Conclusion

Concluded that it was observed that the confused beetle *Tribolium confusum* camphor, clove and mint displayed the greatest repellent behavior of all six essential oils, while the marjoram oils have a lethal effect, all individual oils have repellent activities of varying degrees against adults. Mention that 0.1g / 1ml the most effective concentration. The combination of oils has synergism repellent efficacy. Lemon + Aloe, Lemon + Mint, Lemon + Marjoram and Clove + Mint are most of repellent efficacy, in contrast Lemon + Camphor is least effective.

Thus, essential oils and their mixtures are an important and safe source of insect repellents, especially flour beetle.

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