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# Repellent, Fumigant and Contact Toxicity of *Salvia Officinalis*, *Rosmarinus Officinalis* and *Coriandrum Sativum* Against *Callosobruchus Maculatus* (Fab.) (Coleoptera:Bruchidae)

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***Abstract:*** *The repellent, fumigant and contact toxicity effects of three essential oils of sage (Salvia officinalis), rosemary (Rosmarinus officinalis) and coriander (Coriandrum sativum) were investigated against Callosobruchus maculatus. The order of decreasing repellent activity was sage > coriander > rosemary. Sage maintained higher repellency after 48h and at lower concentrations compared with coriander and rosemary. Rosemary exhibited the highest degree of oviposition deterrent ability among the three essential oils even at low concentrations of 6.25µl/ml with Discrimination Quotient (DQ) values of +1 indicating that C. maculatus preferred to oviposit all eggs on untreated rather than on treated seeds. C. sativum was the most toxic ($LC_{50}$ = 3.90µl/ml) oil compared to R. officinalis and S. officinalis although the time to achieve 50% mortality ($LT_{50}$) was not significantly different from the other two oils. Both sage and rosemary essential oils were equally potent fumigants to C. maculatus causing 50% mortality ($LT_{50}$) in 14.47 and 12.14h respectively.*

***Keywords:*** *Contact toxicity, fumigant, repellent, essential oils, Callosobruchus maculatus.*

## INTRODUCTION

*Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) originated from Asia and Africa, however is now widely distributed throughout the world due to the export of the legumes from Africa and Asia [1]. It is a major pest of storage legumes, such as cowpea (*Vigna unguiculata*), soybean (*Glycine max*), chickpea (*Cicer arietinum*) and other types of peas [1]. The beetle is multivoltine and has a generation time of 45-48 days [2]. Females can oviposit up to 200 eggs during their lifetime of 9-15 days with the oval shaped eggs being oviposited singly on the seed surface [3, 4]. Damage to seeds is done by the feeding activity of larvae and renders the seed unfit for either consumption or planting. *C. maculatus* causes substantial losses for farmers and have been recorded to destroy 50 to 100% of the seeds in storage thus resulting in a significant decrease in the germination potential of the seed [5]. In the West Indies, damage of 100% of stored cowpea has been recorded, thus emphasizing the importance of protection of stored legumes against this beetle [6]. One major control strategy for *C. maculatus* is the use of fumigants such as methyl bromide or phosphine. These chemicals do result in a decrease in infestation; however, the use of methyl bromide has been restricted due to its damaging effects on the ozone layer [7]. The use of synthetic insecticides in pest control is also toxic to humans if ingested.

Botanical pesticides are great replacements for synthetic insecticides because there is no toxic residues to affect the environment, lower toxicity to mammals and may have medicinal properties for humans [8]. In recent years the essential oils extracted from different plants are being researched

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as potential botanical insecticides for food storage insects. The insecticidal activity of essential oils is as a result of their chemical composition [9, 10, 11]. The number of studies using essential oils against *C. maculatus* has increased, all in an attempt to find the best solution to prevent infestation and loss of legumes. The present study investigates the contact toxicity, repellent and fumigant effects of *Salvia officinalis, Rosmarinus officinalis* and *Coriandrum sativum* against *Callosobruchus maculatus*.

## MATERIALS AND METHODS

### Rearing of *Callosobruchus maculatus*

*Callosobruchus maculatus* was cultured at room temperature (27°C) in darkness from parent insects which were obtained from a store bought pack of pigeonpea (*Cajanus cajan*). Fifty unsexed adults were placed into each of three glass bottles (16cm x 10cm) with tightly fitted mesh covered lids and each containing 500g of *C. cajan* as the food source. Each bottle was placed in brown paper bags (to simulate darkness) and kept at room temperature for 35 days to allow for population growth. These insects were used for conducting bioassays.

### Essential Oil Extraction

Essential oils of sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*) and coriander seeds (*Coriandrum sativum*) were made *via* steam distillation using a Clevenger type apparatus. Sage and rosemary were cut into smaller pieces and separately milled into paste using a Waring® stainless steel blender while coriander seeds were ground to a fine powder. Five hundred grams of each sample was weighed and separately placed in 5L Pyrex® round bottom flasks with 300ml of distilled water. Three steam distillation apparatus were set up for the extractions which lasted 4h per sample. The distillate was collected until the solution went clear. Each distillate was placed into separating funnels and oils extracted using dichloromethane (2 washes of 200ml dichloromethane each time). All dichloromethane was evaporated leaving pure essential oil of each sample. The oils collected were dried over anhydrous $Na_2SO_4$, filtered and stored in aluminum foil covered glass vials. The vials were labelled, weighed and the mass of each oil recorded.

### Repellency Test

The repellent effect of each essential oil was evaluated against *C. maculatus* adults. Test solutions (3.12, 6.25, 12.5, 25.0 and 50.0 µl/ml) were prepared by diluting each essential oil in 100% ethanol. Whatman® No. 41 ashless 9 cm filter paper was cut in half. Each dose was applied uniformly to one half of the filter discs with a micropipette and labelled appropriately. The other half was treated with 1 ml of 100% ethanol and labelled as the control. The oil treated halves were allowed to air dry for 10 minutes and then a control and treated half remade using clear adhesive tape on the underside. The remade filter paper discs were placed in 9 cm Petri dishes with 10 unsexed adult *C. maculatus* placed in the middle of the filter paper and subsequently enclosed with mesh covered petri dish covers. Each of the five treatments was replicated 5 times. The number of insects on the treated and control halves of the discs were recorded after 12h, 24h and 48h. The Percentage Repellency (PR) was calculated based on the formula [12]:

$$PR = [(N_c - N_t)/(N_c + N_t)] \times 100$$

Where $N_c$ = No. of insects on control half of filter paper after required exposure interval

$N_t$ = No. of insects on treated half of filter paper after required exposure interval

The percentage repellency was categorized based on the classification of [13]. The Repellent Index (*RI*) [14] was also calculated using the formula:

$$RI = 2G/G + P$$

Where $G$ = No. on treatment side and $P$ = No. on control side. The standard deviations of the mean values of the *RI* were also calculated and essential oils at different concentrations classified based on whether ($RI > 1 + SD$) the oil was an attractant, ($RI$ between $1 - SD$ and $1 + SD$) then the oil was indifferent (= neutral) or ($RI < 1 - SD$) then the oil was classified as a repellent.

### Oviposition and Contact Mortality Bioassays

The mortality of *C. maculatus* adults was determined when exposed to essential oils of sage, rosemary and coriander. Each essential oil was diluted with 100%

ethanol to obtain 3.12, 6.25, 12.5, 25.0 and 50.0 µl/ml. Each of test solutions was mixed with 40g of *C. cajan* dried seeds and placed in 375ml glass jars. The jars were tumbled for 5 minutes to allow the test solutions be evenly distributed throughout the surface of the seeds and samples were allowed to air dry for 10 minutes for the solvent to evaporate. Ten unsexed *C. maculatus* adults were introduced to each mesh covered jar. There were 5 replicates for each essential oil treatment.

The number of dead insects and eggs oviposited were recorded and dead adults removed daily over a 7-day period. The $LD_{50}$ and $LT_{50}$ were calculated for each essential oil using probit analysis [15]. The Discrimination quotient (DQ) was also calculated for *C. maculatus* exposed to five concentrations of the three essential oils. The DQ [16] was calculated based on the formula:

(No. of eggs on control seeds) – (No. of eggs on treated seeds)

$$DQ = \frac{\text{(No. of eggs on control seeds)} - \text{(No. of eggs on treated seeds)}}{\text{Total no. of eggs}}$$

The values range from –1 indicating that all eggs were oviposited on treated seeds to +1 where all eggs were oviposited on control seeds.

## Fumigant Bioassay

To determine the Fumigant Concentration of each essential oil causing 50% mortality ($FC_{50}$), five serial dilutions were made from the stock oil. Fumigant concentrations corresponding to 0.33, 0.66, 1.32, 2.63 and 5.26µl/L air were applied to Whatman® No. 41 filter paper discs (5.5 cm diameter). The treated filter paper was taped to the underside screw cover of a 950ml glass jar. The control consisted of filter paper treated with only ethanol. Ten adults were placed in each glass jar and the cap tightly secured. Three replicates were used for each concentration of each essential oil. Mortality counts were taken at 2h intervals for 24h and then at 48h. Beetles were considered dead when no movement was observed. The mortality data was adjusted for control mortality using Abbott's formula [17] and data was then subjected to probit analysis to estimate $FC_{50}$ and $FT_{50}$ (50% fumigant time) values.

## RESULTS

The mean number of eggs oviposited on *C. cajan* seeds treated with *C. sativum, R. officinalis* and *S. officinalis* essential oils at different concentrations and five time periods is presented in Figures 1-3. In all cases, lower numbers of eggs were oviposited at all concentrations for all oils tested compared with the control. *R. officinalis* treated seeds had the least eggs oviposited compared to the other two oils and especially so at higher concentrations (Figure 2). Eggs were only oviposited after 120h and only at 3.12 and 6.25µl/ml indicating that *R. officinalis* oil was the most potent and long-lasting anti-oviposition oil against *C. maculatus* among the three tested. This trend was also observed from the Discrimination Quotient (DQ) in Table 1.

For *R. officinalis* oil concentrations greater than 6.25 ml/ml, *C. maculatus* females preferred to oviposit all eggs on the control rather than treated (DQ = 1), while for *C. sativum* this occurred at concentrations higher than 12.5 ml/ml. *S. officinalis* essential oil appeared ineffective as an oviposition deterrent except at the highest concentration tested (50ml/ml). The DQ values for both *C. sativum* and *R. officinalis* oil remained high as long as 120 days post application compared with *S. officinalis.* The contact mortality for coriander, sage and rosemary essential oils is presented in Figures 4-6 and Tables 2-3. Total mortality (100%) was achieved for coriander (at 12.50ml/ml), sage (at 25ml/ml) and rosemary (at 50ml/ml) only after 72h and not at either 24 or 48h apart from coriander oil at (12.50 ml/ml) (Figure 4).

**Table 1**
**Discrimination quotient (*DQ*)[1] for *Callosobruchus maculatus* exposed to five concentrations of three essential oils for 120 hours.**

| *Concentration (µl/ml)* | *Salvia officinalis* | *Coriandrum sativum* | *Rosmarinus officinalis* |
|---|---|---|---|
| 3.12 | 0.06 | 0.11 | 0.67 |
| 6.25 | 0.31 | 0.43 | 1.00 |
| 12.50 | 0.48 | 1.00 | 1.00 |
| 25.00 | 0.48 | 1.00 | 1.00 |
| 50.00 | 0.70 | 1.00 | 1.00 |

[1]*DQ* [16] values range from –1 indicating that all eggs were oviposited on treated seeds to +1 where all eggs were oviposited on control seeds.

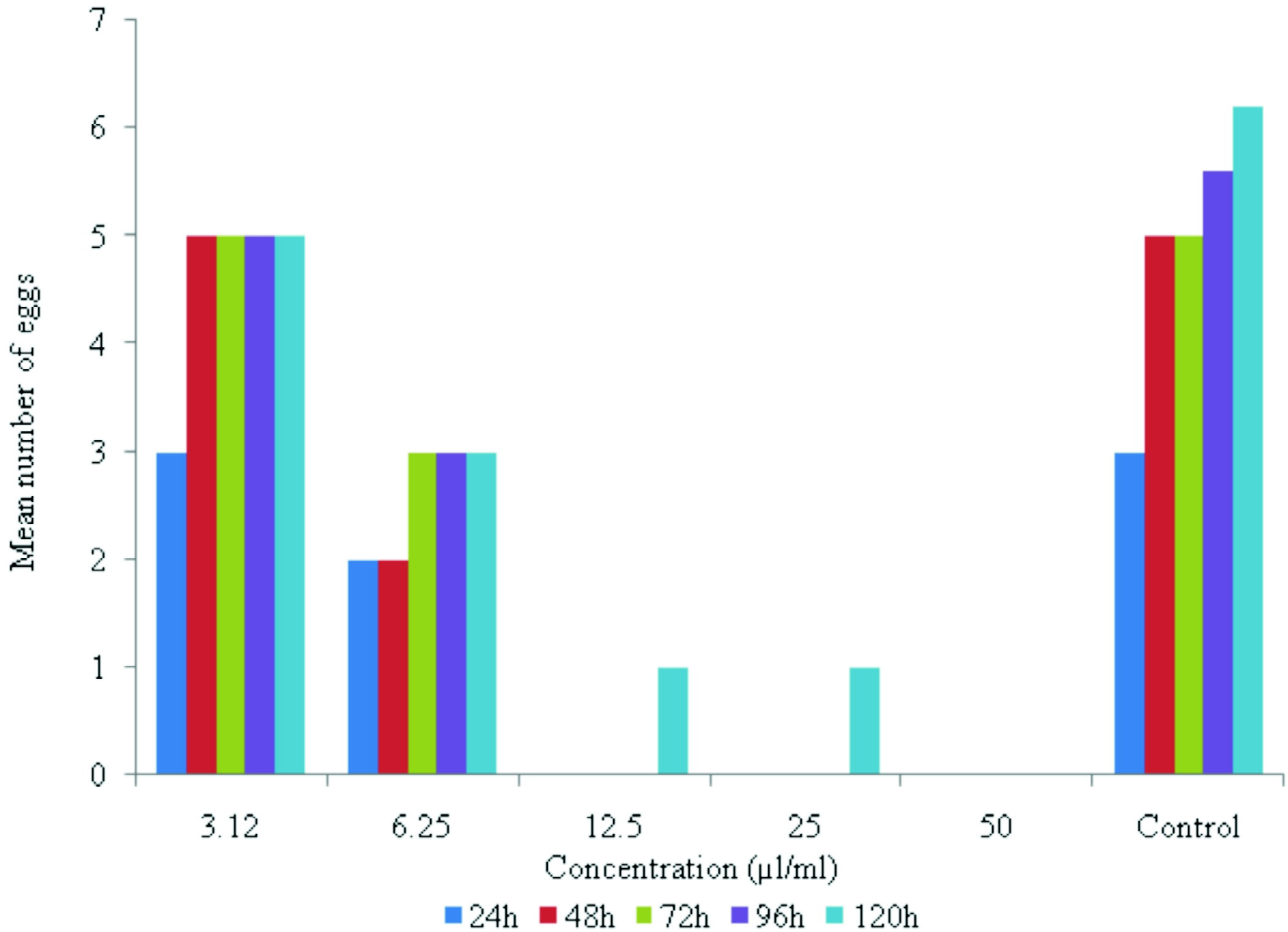


**Figure 1: Mean number of eggs oviposited on *Cajanus cajan* seeds treated with *Coriandrum sativum* oil at different concentrations and five time periods**

**Table 2**
**$LC_{50}$ values for *Callosobruchus maculatus* exposed for 24 hours to three essential oils**

| *Essential oil* | *Probit line* | *$LC_{50}$ ml/ml (95% CI)*[*] | *S.E. of $LC_{50}$* | $\chi^2$ |
|---|---|---|---|---|
| *Salvia officinalis* | Y = 1.31*x* + 2.90 | 39.99 (26.34, 60.73)[a] | 1.24 | 0.21 |
| *Coriandrum sativum* | Y = 0.85*x* + 4.50 | 3.90 (2.01, 7.57)[b] | 1.40 | 0.95 |
| *Rosmarinus* | Y = 0.78*x* + 3.81 | 33.39 (17.36, 64.23)[a] | 1.40 | 1.92 |

[*] Values followed by the same letter along a column are not significantly different (*P* > 0.05) from each other

**Table 3**
**$LT_{50}$ values for *Callosobruchus maculatus* to three essential oils**

| *Essential oil* | *Probit line* | *$LC_{50}$ ml/ml (95% CI)*[*] | *S.E. of $LC_{50}$* | $\chi^2$ |
|---|---|---|---|---|
| *Salvia officinalis* | Y = 1.00*x* + 3.93 | 11.68 (7.18, 19.00)[a] | 1.28 | 0.82 |
| *Coriandrum sativum* | Y = 2.13*x* + 2.62 | 13.19 (10.60, 16.42)[a] | 1.12 | 5.96 |
| *Rosmarinus officinalis* | Y = 1.74*x* + 2.83 | 17.70 (13.22, 23.71)[a] | 1.16 | 1.79 |

[*] Values followed by the same letter along a column are not significantly different (*P* > 0.05) from each other

Coriander oil was also significantly more toxic (P < 0.05) than either sage or rosemary oils with an $LC_{50}$ of 3.90 ml/ml (Table 2), however the time to 50% mortality ($LT_{50}$ = 13.19h) was not significantly different (P > 0.05) from the other two oils (Table 3). The order of decreasing repellent activity of the three oils against *C. maculatus* was coriander > sage > rosemary (Tables 4 and 5). Only coriander oil at the highest concentration exhibited 100% repellent activity after 12h. At lower concentrations and for

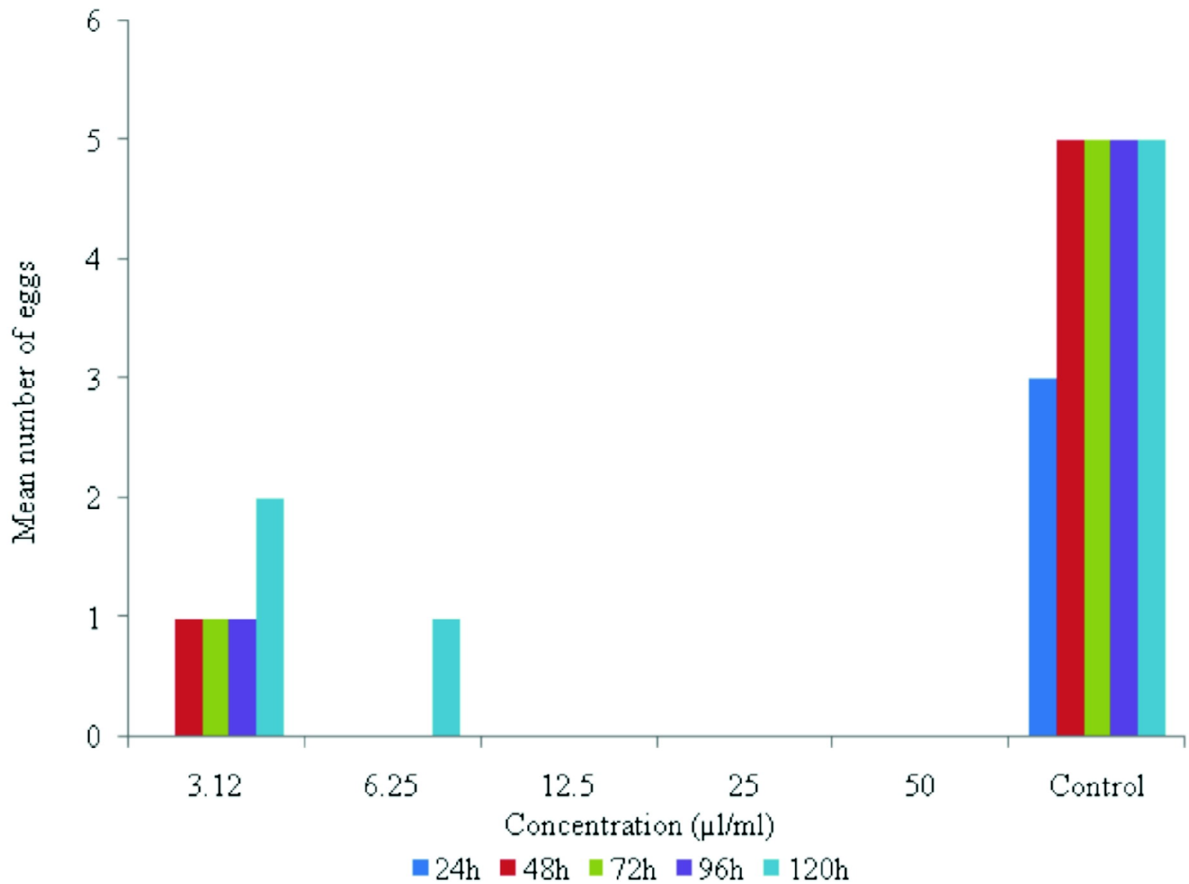


**Figure 2: Mean number of eggs oviposited on *Cajanus cajan* seeds treated with *Rosmarinus officinalis* oil at different concentrations and five time periods**

**Table 4**
**Percent repellency of five concentrations of three essential oils against *Callosobruchus maculatus* at three time periods**

| | | % Repellency (Class)* | | |
|---|---|---|---|---|
| *Essential oil* | *Concentration (µl/ml)* | *12h* | *24h* | *48h* |
| *Salvia officinalis* | 50.0 | 80.0 (IV) | 73.8 (IV) | 60.0 (III) |
| | 25.0 | 73.8 (IV) | 53.0 (III) | 40.0 (II) |
| | 12.5 | 33.0 (II) | 33.3 (II) | 27.0 (II) |
| | 6.25 | 20.0 (I) | 20.0 (I) | 20.0 (I) |
| | 3.12 | 20.0 (I) | 13.0 (I) | 0.0 (I) |
| *Coriandrum sativum* | 50.0 | 100.0 (V) | 80.0 (IV) | 60.0 (III) |
| | 25.0 | 73.3 (IV) | 40.0 (II) | 40.0 (II) |
| | 12.5 | 60.0 (III) | 40.0 (II) | 20.0 (I) |
| | 6.25 | 40.0 (II) | 20.0 (I) | 20.0 (I) |
| | 3.12 | 0.0 (0) | 0.0 (0) | 0.0 (0) |
| *Rosmarinus officinalis* | 50.0 | 40.0 (II) | 40.0 (II) | 26.7 (II) |
| | 25.0 | 40.0 (II) | 40.0 (II) | 20.0 (I) |
| | 12.5 | 20.0 (I) | 20.0 (I) | 20.0 (I) |
| | 6.25 | 20.0 (I) | 20.0 (I) | 0.0 (0) |
| | 3.12 | 0.0 (0) | 0.0 (0) | 0.0 (0) |

* Repellency class [13]: Class 0 – 0-0.1%, Class I – 0.1 – 20%, Class II – 20.1-40%, Class III – 40.1-60%, Class IV – 60.1-80%, Class V – 80.1-100%

longer periods the repellency of the oils was less effective and in some cases became either indifferent or were slightly attractive to *C. maculatus.* All three essential oils demonstrated fumigant activity, nonetheless the fumigant concentration causing 50% mortality ($FC_{50}$) was not significantly different ($P>0.05$) between the three oils tested (Table 6). The 50% Fumigant Time ($FT_{50}$) for coriander oil was significantly longer ($P<0.05$, $FT_{50}$ = 42.99h) compared to either sage ($FT_{50}$ = 14.47h) or rosemary ($FT_{50}$ = 12.14h) (Table 7).

## DISCUSSION

*C. maculatus* is a major pest of several legumes including *V. unguiculata, G. max* and *C. arietinum* [1, 3, 6]. Numerous synthetic insecticides have been utilized to control this pest; however they have all been determined to have negative effects on the environment, ecological habitats and human health and well-being [6, 7, 18]. There has been an upsurge of interest in the use of essential oils in management

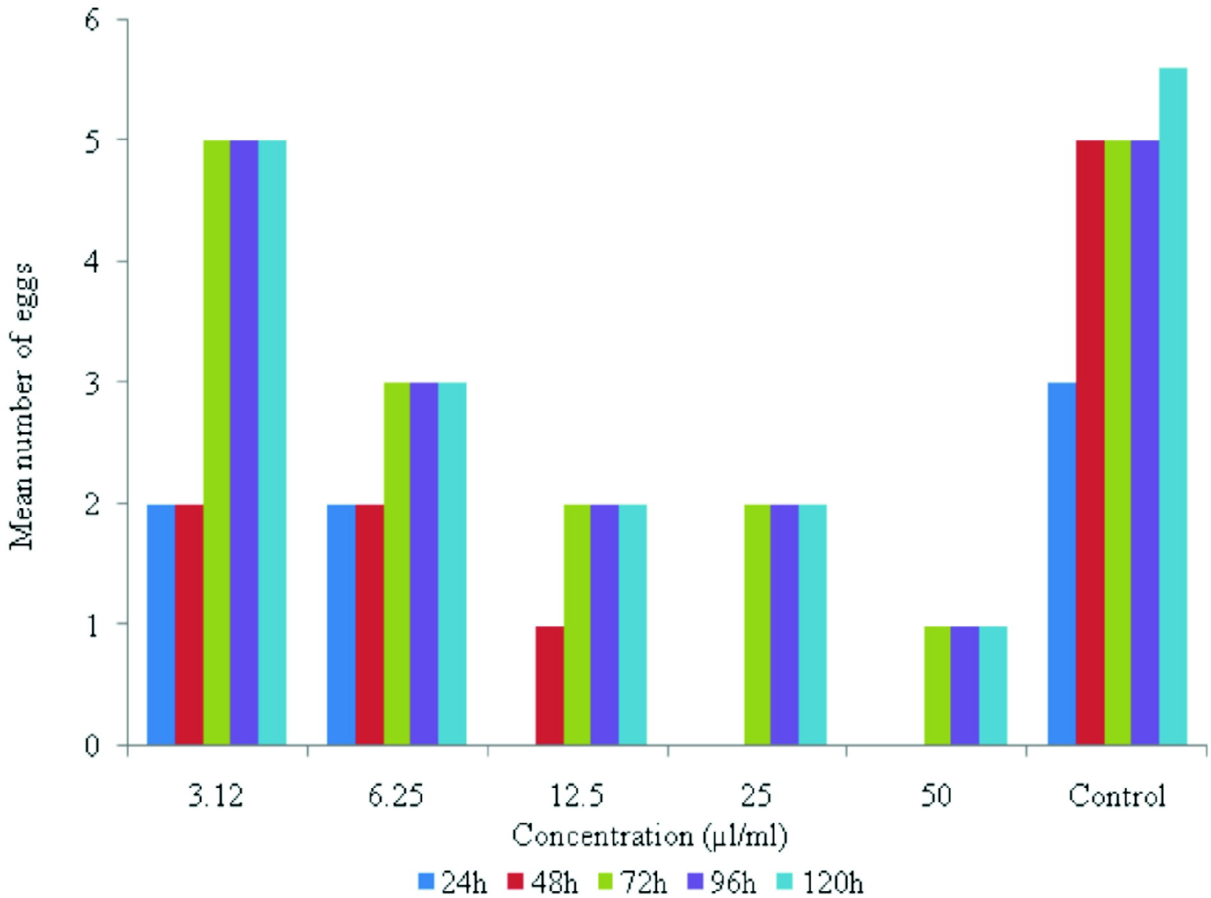


**Figure 3: Mean number of eggs oviposited on *Cajanus cajan* seeds treated with *Salvia officinalis* oil at different concentrations and five time periods**

**Table 5**
**Repellent effect of five concentrations of three essential oils against *Callosobruchus maculatus* at three time periods**

| | | *Repellent Index (RI)[1] (Mean ± SD)* | | |
|---|---|---|---|---|
| *Essential oil* | *Concentration (µl/ml)* | *12h* | *24h* | *48h* |
| *Salvia officinalis* | 50.0 | 0.27 ± 0.09 (R) | 0.67 ± 0.09 (R) | 0.67 ± 0.09 (R) |
| | 25.0 | 0.27 ± 0.09 (R) | 0.27 ± 0.09 (R) | 0.53 ± 0.19 (R) |
| | 12.5 | 0.87 ± 0.09 (R) | 0.73 ± 0.09 (R) | 0.53 ± 0.09 (R) |
| | 6.25 | 0.87 ± 0.09 (R) | 0.67 ± 0.09 (R) | 1.13 ± 0.09 (A) |
| | 3.12 | 0.73 ± 0.09 (R) | 1.27 ± 0.09 (A) | 1.20 ± 0.07 (A) |
| *Coriandrum sativum* | 50.0 | 0.33 ± 0.09 (R) | 0.20 ± 0.10 (R) | 0.67 ± 0.09 (R) |
| | 25.0 | 0.47 ± 0.09 (R) | 0.80 ± 0.09 (R) | 1.07 ± 0.09 (I) |
| | 12.5 | 0.67 ± 0.09 (R) | 0.80 ± 0.09 (R) | 0.60 ± 0.47 (I) |
| | 6.25 | 0.60 ± 0.09 (R) | 1.07 ± 0.09 (I) | 0.53 ± 0.47 (A) |
| | 3.12 | 1.93 ± 0.09 (A) | 1.43 ± 0.09 (A) | 1.23 ± 0.09 (A) |
| *Rosmarinus officinalis* | 50.0 | 0.87 ± 0.09 (R) | 0.67 ± 0.09 (R) | 0.73 ± 0.09 (R) |
| | 25.0 | 0.73 ± 0.19 (R) | 0.76 ± 0.06 (R) | 1.07 ± 0.09 (I) |
| | 12.5 | 0.80 ± 0.09 (R) | 0.93 ± 0.09 (I) | 1.40 ± 0.07 (A) |
| | 6.25 | 1.07 ± 0.09 (I) | 1.07 ± 0.09 (I) | 1.40 ± 0.07 (A) |
| | 3.12 | 1.13 ± 0.09 (A) | 1.13 ± 0.09 (A) | 1.20 ± 0.16 (A) |

[1] Repellent Index [14]: A – Attractant (*RI* > 1 + *SD*), I – Indifferent (*RI* between 1 – *SD* and 1 + *SD*), R – Repellent (*RI* < 1 – *SD*).

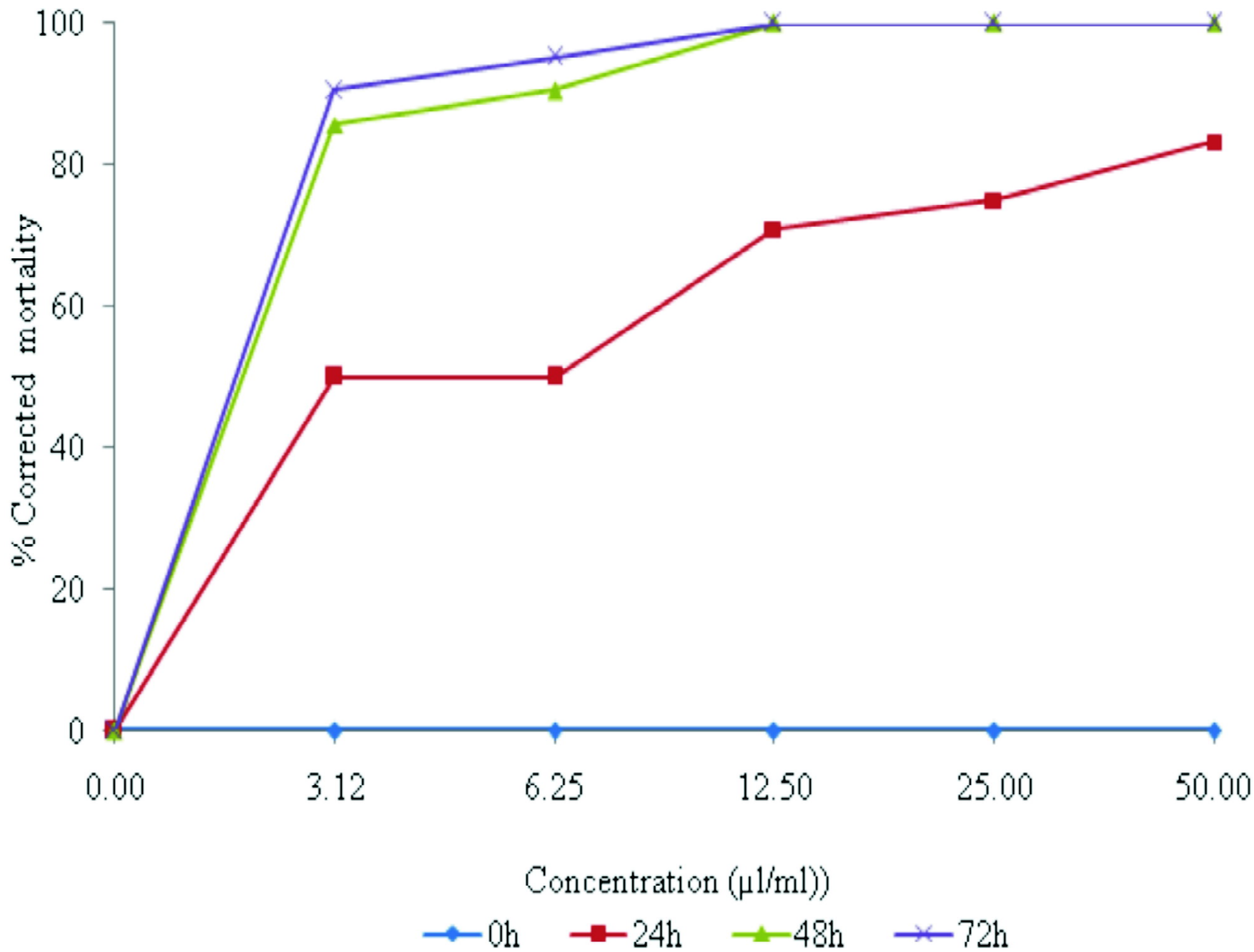


**Figure 4: Percent corrected mortality of *Callosobruchus maculatus* adults exposed to five concentrations of coriander oil for 72 hours.**

**Table 6**
**Fumigant mortality ($FC_{50}$) values for *Callosobruchus maculatus* exposed for 24 hours to three essential oils**

| *Essential oil* | *Probit line* | *$FC_{50}$ µl/L air (95%CI)** | *S.E. of $LC_{50}$* | *$\chi^2$* |
|---|---|---|---|---|
| *Salvia officinalis* | $Y = 0.64x + 3.89$ | 1.80 (0.86, 3.75)[a] | 0.05 | 0.07 |
| *Coriandrum sativum* | $Y = 0.94x + 2.97$ | 4.74 (2.72, 8.28)[a] | 0.04 | 3.22 |
| *Rosmarinus officinalis* | $Y = 1.29x + 2.18$ | 5.12 (3.04, 7.95)[a] | 0.04 | 5.26 |

* Values followed by the same letter along a column are not significantly different ($P > 0.05$) from each other.

**Table 7**
**Fumigant time ($FT_{50}$) for *Callosobruchus maculatus* exposed to three essential oils**

| *Essential oil* | *Probit line* | *$FC_{50}$ µl/L air (95%CI)** | *S.E. of $LC_{50}$* | *$\chi^2$* |
|---|---|---|---|---|
| *Salvia officinalis* | $Y = 2.55x + 2.04$ | 14.47(12.03, 17.42)[a] | 1.56 | 1.10 |
| *Coriandrum sativum* | $Y = 1.37x + 2.76$ | 42.99 (30.17, 61.25)[b] | 1.20 | 1.58 |
| *Rosmarinus officinalis* | $Y = 2.61x + 2.17$ | 12.14 (10.13, 14.54)[a] | 1.10 | 5.30 |

* Values followed by the same letter along a column are not significantly different ($P > 0.05$) from each other.

of *C. maculatus* as well as other pests associated with stored products. Essential oils are said to be effective against *C. maculatus* because of the presence of mono- and sesquiterpenes with monoterpenes being more effective [11]. Monoterpenes reportedly found in the three essential oils tested in this study include high levels of α and α-thujone, camphor, camphene, 1,8-cineole and α-pinene in sage [19], α-pinene, β-cymene, 1,8 cineole, camphor, linalool and γ-terpinene in rosemary [20] and linalool,

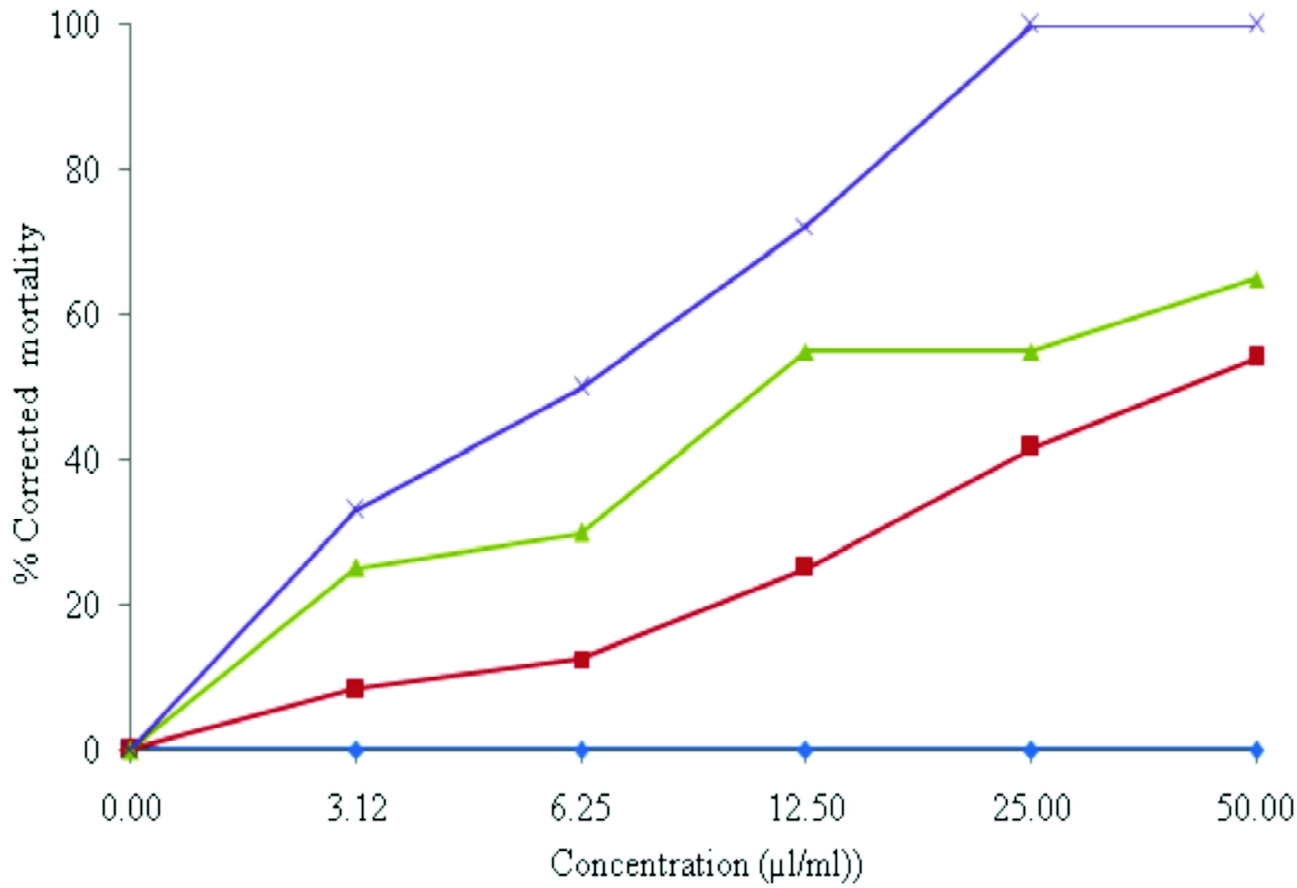


**Figure 5: Percent corrected mortality of *Callosobruchus maculatus* adults exposed to five concentrations of sage oil for 72 hours.**

α-pinene, geranyl acetate and γ-terpinene in coriander [21]. Both sage and coriander essential oils reduced the number of eggs oviposited especially at higher concentrations. This is similar to the results obtained by others [21, 22] where oviposition by *C. maculatus* was significantly reduced with increasing concentrations of 1,8 cineole and α-pinene which are present in sage and coriander. Toxicity was also significantly higher ($P < 0.05$) in coriander essential oil ($LC_{50} = 3.90$µl/ml) compared to either sage or rosemary.

These results were comparable to that obtained by others [11, 21, 22]. Coriander at the highest concentration (50ml/ml) was the only oil which was received a Class V (strongest) repellent rating among the oils tested (Table 4), however as expected, its repellent abilities decreased after 24h and 48h to Classes IV and III respectively. There was a similar trend observed for the other oils and at different concentrations [23]. Fumigant mortality is in part as a result of toxicity of the compound(s) and vapour pressure. While the 50% fumigant concentration ($FC_{50}$) for the three oils tested against *C. maculatus* were not significantly different ($P >$ 0.05) from each other (Table 6), both sage and rosemary had significantly lower ($P < 0.05$) 50% Fumigant Times ($FT_{50}$) compared to coriander (Table 7). Both essential oils also contained high levels of 1,8 cineole, a-pinene and a-pinene all of which are reported to have high vapour pressures [22].

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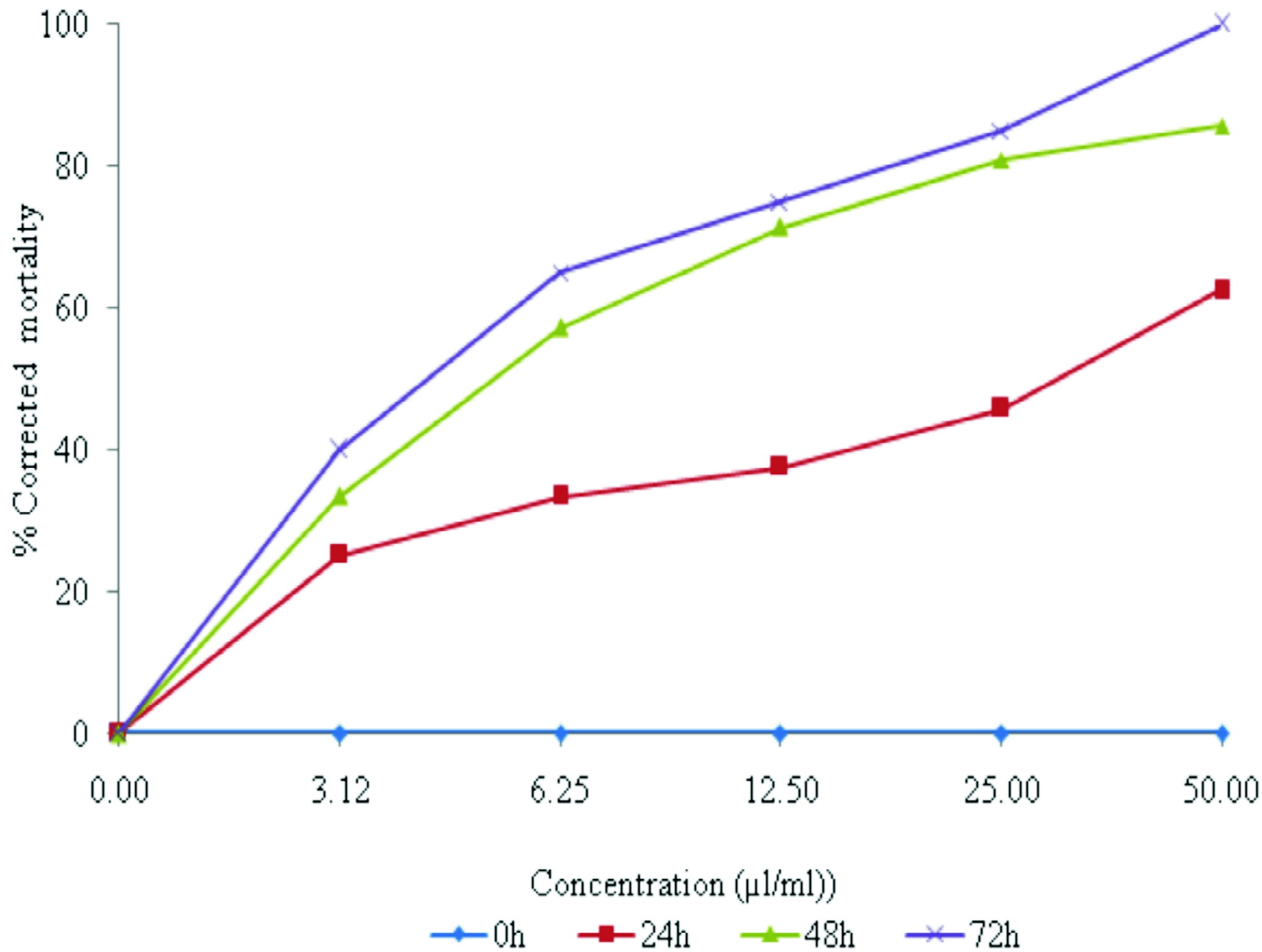


**Figure 6: Percent corrected mortality of *Callosobruchus maculatus* adults exposed to five concentrations of rosemary oil for 72 hours.**


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