# Botanical oils as eco-friendly alternatives for controlling the rice weevil *Sitophilus oryzae*

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## ABSTRACT

The rice weevil Sitophilus oryzae is a major stored grain pest infesting many grains in storage mainly wheat, rice and maize. Toxic effects of certain extracted botanical essential oils (EOs) were evaluated against the adults of Sitophilus oryzae (Linnaeus) (Coleoptera: Curculionidae). Bioassays were carried out by fumigation and guide tables were presented to show the effective range of concentrations of each of the evaluated essential oils and their corresponding mean number of responded insects. The essential oil of fennel (seeds) (Foeniculum vulgare) did not show any toxic effect up to the concentration of 300 µl/370 ml air and Common Sage (Marmaria leaves) (Salvia officinalis) up to 250 µl/370 ml air during the first 48 hrs then they showed a very weak effect. The essential oil of spearmint leaves (Mentha spicata L.) was the utmost toxic and had a lowest  $LC_{50s}$  calculated by 4.43, 3.88 and 3.27 µl/370 ml air after different exposure periods of 24, 48 and 72 hrs, respectively, followed by the essential oil of clove (Syzygium aromaticum) (382.62, 79.95 and 9.23 µl/370 ml air, in respect). In this concern, clove showed its higher toxicity after 72 hrs. The mortality of the exposed adults to EOs increased with the increase of concentrations and time of exposure to each one. The calculated values of toxicity index showed that the essential oil of mint was the most toxic EO (100%) followed by clove (35.43%). Moreover, log (dose)/N.E.D. (response) (Ld-p) regression lines for certain bio assayed botanical essential oils against S. oryzae were also illustrated. Therefore, it could be recommended that the essential oils of spearmint leaves and clove buds could be used to control the rice weevil. S. orvzae.

Keywords: Botanical oils; *Sitophilus oryzae*; Guide tables; LC<sub>50</sub>; Toxicity index; Ld-p lines

## **INTRODUCTION**

Stored grains are subject to loss due to several causes, including physical, sanitary and nutritional degradation, from their maturation to the consumption. Grain loss may be caused by fungi, insect-pests and the inadequate handling from harvest to storage and all these factors can result in important financial losses (Lazzari and Lazzari, 2002). Loss may reach 10% of the total product each year, which translates to 10,000,000 tons of grain lost per year (Smiderle, 2007). Among the stored grain insect-pests, the rice weevil *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), is considered to be the most important insect-pest of

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stored rice causing quantitative and qualitative losses (Sartori et al., 1990). This species is found throughout warm, tropical regions of the world and it may infest grains in the field prior to storage (Pacheco and Paula, 1995). In warmer regions, natural aeration is not sufficient to control infestations, requiring the application of chemical control (Moreira, 1993). However, the indiscriminate application of synthetic products (insecticides) had led to various problems effects, environmental including toxic residual pollution, and development of resistance in insects (Isman, 2006). The use of a mixture of insecticides also favors the development of resistant strains which then makes subsequent pest management difficult (Pereira et al., 1997).

The most effective and fast method to suppress stored grain pests is fumigation. Phosphine is the most used fumigant, but the incorrect use of phosphine has selected resistant pest populations (Calil, 1995). Methyl bromide, a widely used fumigant for insect pest control in stored products is not being used anymore because it has been reported to cause ozone depletion (Lee et al., 2001a). Therefore, there is an urgent need to develop safe, convenient and low-cost alternatives. Considerable efforts have been focused on the use of plant-derived materials including essential oils as bioinsecticides. Essential oils have demonstrated toxic effects against stored-product insects (Rajendran and Sriranjini, 2008). Essential oils and their components are gaining increasing interest because of their relatively safe use and potential for multi-purpose functional use (Feng and Zheng, 2007). These compounds are typically lipophylic, with potential for toxic interference in basic biochemical processes with physiological and behavioral consequences for the insects (Prates and Santos, 2002). Therefore, botanical insecticides may offer an alternative solution for pest control. Botanical essential oils and their constituents have been shown to possess potential for development as new fumigants and they may have advantages over conventional fumigants in terms of low mammalian toxicity and low environmental impact (Franz et al., 2011; Yazdgerdian et al., 2015). Such products of higher plant origin may be exploited as eco-chemical and biorational approach in integrated plant protection programs (Dubey et al., 2010).

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It was acknowledged that various plant oils or extracts can exert toxic activity against some insect species. Essential oil of thyme, *Thymus serpyllum* (rich in thymol and carvacrol) was very effective when treated as fumigants against the bean weevil *Acanthoscelides obtectus* (Bruchidae) (Regnault-Roger *et al.*, 1993). Mint, *Mentha* sp. (Labiatae) produces an essential oil rich in menthone (14–32%) and menthol (30–50%) (Cardoso *et al.*, 2001). Menthol has wide application in the food and pharmaceutical industry; also exhibited insecticidal activity (Agarwal *et al.*, 2001). Fennel extracts was reported to have insecticidal activity against different mites and insects (Mimica-Dukić *et al.*, 2003 and Lee *et al.*, 2006).

The objective of the present investigation was adopted to test the insecticidal properties of the extracted botanical essential oils of spearmint, bitter orange, lemon, the Brazilian pepper, thyme, sweet basil, fennel, clove, eucalyptus and sweet sage(Marmaria) as eco-friendly fumigants to be used for controlling the rice weevil, *S. oryzae*.

## MATERIALS AND METHODS

## **Essential oil tested**

Essential oils (EOs) of different parts of the evaluated botanical materials were isolated by steamdistillation. Table (1) shows the common and family names of the evaluated essential oils, the used parts and their major constituents.

## Sample preparation and essential oil extraction

The buds, leaves or fresh peels were collected and purchased from the local market in Alexandria, Egypt and then they were washed and dried. Firstly, 100g of each sample were air dried at (25°C) and submitted for 4 hours to steam-distillation using the Clevenger typeapparatus. The EOs of all dried samples (100g) were isolated by steam-distillation for 3 h, using a Clevengertype apparatus according to the method recommended by the British Pharmacopoeia (1988). The distillated essential oils (EO) were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 to 5°C until use.

| Table 1. | The evaluated | essential oils | s, the used | plant | parts and | their ma | ajor constituents | ; |
|----------|---------------|----------------|-------------|-------|-----------|----------|-------------------|---|
|          |               |                | 1 0 1       |       |           |          |                   |   |

|   | Essential Oil             |               | - The used | Major constituents  |
|---|---------------------------|---------------|------------|---|
| Scientific Name                                       | Common<br>Name            | Family Name   | part       | of the essential oil  |
| Citrus aurantium L.                                   | Bitter Orange             | Rutaceae      | Peel       | Limonene (89.8% - 94.12)<br>1&2*  |
| Citrus limon L.                                       | Lemon                     | Rutaceae      | Peel       | Limonene $(93.5\%)^3$   |
| Ocimum basilicum L.                                   | Sweet Basil               | Lamiaceae     | Leaves     | Eugenol (75.1%) <sup>4</sup><br>Linalool (95%) <sup>5</sup>                     |
| Schinus molle L.                                      | Brazilian<br>Pepper       | Anacardiaceae | Leaves     | α-Phellandrene (20.6%), β-<br>Phellandrene (10.8%) and α-<br>Pinene $(8.7\%)^6$ |
| Thymus vulgaris L.                                    | Thyme                     | Lamiaceae     | Leaves     | 1,8 cineole (34.69-40.67) and<br>Linalool (8.99-11.75) <sup>7</sup>             |
| Foeniculum vulgare Mill                               | Fennel                    | Apiaceae      | Seeds      | Estragole (34 to 89%) <sup>8</sup>  |
| Syzygium aromaticum<br>(Linn.) (Merrill. &<br>Perry.) | Clove                     | Myrtaceae     | Buds       | Eugenol (71.56 %) and<br>eugenol acetate<br>(8.99 %) <sup>9</sup>               |
| <i>Cinnamomum camphora</i><br>Nees & Eberm            | Eucalyptus                | Myrtaceae     | Leaves     | Camphor (68.03%) <sup>10</sup>  |
| Salvia officinalis L.                                 | Common Sage<br>(Marmaria) | Lamiaceae     | Leaves     | α-Thujone (40.90 %) and<br>camphor<br>(26.12 %) <sup>11</sup>                   |
| Mentha spicata L.                                     | Spearmint                 | Lamiaceae     | Leaves     | Carvone (40.8% ± 1.23%)<br>and limonene (20.8% ±<br>1.12%) <sup>12</sup>        |

\*1(Camara *et al.*, 2015), 2(Bendaha *et al.*, 2016), 3(Verzera *et al.*, 2004), 4(Joshi, 2013), 5(Dambolena *et al.*, 2010), 6(Abrha and Unnithan, 2014), 7(Cases *et al.*, 2009), 8(He and Huang, 2011), 9(Nassar *et al.*, 2007), 10(Frizzo *et al.*, 2000), 11(Porte *et al.*, 2013) and 12 (Snoussi *et al.*, 2015).

#### The tested insect

A susceptible laboratory strain of the rice weevil, *Sitophilus oryzae*, was obtained from a stock culture maintained at the laboratories of Plant Protection Dept., Faculty of Agric. (Saba Basha), Alex. Univ., Egypt. The adults of 2-3 weeks old were used for bioassay tests.

## Bioassay

A Whatman (#1) filter paper was treated with different concentrations of pure essential oil (a range of  $1-300 \mu l/370 \text{ ml air}$ ). A piece of filter paper (2x2 cm for the concentrations of 1- 100 and 4x4 cm for the other ones of  $150-300 \mu l/370 \text{ ml air}$ ) was fixed in the center of the inner surface of a plastic lid of a 500ml glass jar. Each replicate (glass jar) implied 20 weevils. Mortality was assessed after 24, 48 and 72hrs while the glass jar was still closed. There were three replicates of 20 *S. oryzae* for each concentration and the untreated check (control). Mortality of treatments was adjusted according to Abbott (1925) formula if a proportion of insect control died during the experiment.

## Data analysis

Probit analysis was used to calculate  $LC_{50}$  (concentration causing 50% mortality) compared with the control ,  $Lc_{90}$  values and their fiducial limits (confidence intervals) for each evaluated essential oil that give a reasonable relation between dose and mortality (response) that could be used easily for probit analysis (Finney, 1971). Toxicity Index (%) (based on  $LC_{50}$  after 72 hrs) was calculated according to Sun (1950). Toxicity Index= Lc50 of the most toxic oil /Lc50 of the other compared oil. Meanwhile, the Ld-p lines of these essential oils were plotted.

## **RESULTS AND DISCUSSION**

Mortality of S. oryzae varied from 10 to 100% after 24, 48 and 72 hrs exposure to each of the bioassayed botanical essential oil (EOs) evaluated by the fumigation technique which was taken into consideration after testing a wide range of these EOs. The obtained results elucidated that the efficiency of the oils was directly related to concentration and response. The guide Tables: 2, 3 and 4 represent the detected effective range of the evaluated EOs concentrations, the mean number of dead insects (S. oryzae adults) and mortality percentages after the different adopted periods of exposure [24 (Table 2), 48 (Table 3) and 72 hrs (Table4), respectively).

Such guide tables would be of great importance for the research workers since they will facilitate the selection of the concentrations that can be tested against the target insect. Generally, it could be noticed that two essential oils showed little bioactivity (sweet basil and thyme), six oils caused significant adult mortality and two had none (fennel and common sage). Thyme was found to have a weakened delayed effect and was only efficient and more toxic after 72 hrs of exposure but it was still the least effective oil.

The toxic effects of the essential oils are depending on both of the mode of action and the target pest (Liu *et al.*, 2006), besides, the species of the botanical material and its freshness.

Six EOs were found to be active as fumigants against S. oryzae exhibiting higher mortality at the initial evaluated concentrations and at the end of the exposure periods (after 72 hrs) as shown in Table 4. These oils showed a concentration - mortality relationship and therefore, LC<sub>50</sub> values can be calculated and Ld-p lines can also be drawn. The essential oil of fennel (seeds) did not show any toxic effect up to the concentration of 300 µl/370 ml air and Common Sage (Marmaria leaves) up to 250 µl/370 ml air). Nevertheless, extracts of fennel were found to be toxic against *Culex pipiens* larvae, and terpineol and 1,8-cineole were the most effective components against Anopheles dirus and Aedes aegypti, as shown by Kim and Ahn (2001), Traboulsi et al. (2005) and Lee et al. (2006) who suggested that fennel could be used as an alternative of synthetic insecticides.

Meanwhile, the essential oil of *O. basilicum* exhibited weak fumigant toxicity against *S. oryzae* adults. The essential oil of *O. basilicum* might be required in higher concentrations than those of the other tested EOs to kill stored- grain insects. The essential oil of spearmint leaves (*Mentha spicata* L.) was found to have the greatest toxicity and lower LC<sub>50s</sub> (LC<sub>50</sub> =12.32, 9.77 and 7.79  $\mu$ l/370 ml air) after different periods of exposure (24, 48 and 72 hrs, respectively), followed by the essential oil of clove (382.62, 79.95 and 9.23  $\mu$ l/370 ml air, in respect) (Table 5). It could be also noticed that clove showed its higher toxicity after 72 hrs. The essential oil of bitter orange peel was more toxic than that that of lemon against *S. oryzae* (LC<sub>50</sub> values = 39.76 and 115.76  $\mu$ l/370 ml air, respectively).

The mortality of exposed adults to EOs increased with the increase of concentration and time of exposure.

Therefore, based on the deduced  $LC_{50}$  values of the tested oils, extracted essential oils of spearmint (*Mentha spicata* L.) and clove buds were the most active fumigants against the rice weevil *Sitophilus oryzae*, presenting safer alternatives to conventional insecticides.

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| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $   | concentrations (µl/ | 370 ml air)  |          |           |               |        |
|---|---------------------|--------------|----------|-----------|---------------|--------|
| Spearmint     0.0     1.0*     13.0     15.0     19.0     .   | 60.0 70.0           | 80.0 90.0    | 100.0    | 150.0 2   | 00.0 250.0    | 300.0  |
| (Learve)     (0.0)     (0.5)**     (55.0)     (75.0)     (95.0)     2.0     3.0     ****     5       Clove     0.0     2.0     2.0     2.0     3.0     ****     -   | 3                   |              |          | 3         | 3             | 8      |
| Clove     0.0     2.0     2.0     3.0     ****       Budd:     (0.0)     (10.0)     (15.0)     ****     ****       Excalyptus     (10.0)     (10.0)     (15.0)     ****     ****       Excalyptus     ***     ***     ***     ****     ****       (Lazvei)     ***     *     *     *     *     *       Sweet Bazil     (Lazvei)     *     *     *     *     *     *       Thyme     *     *     *     *     *     *     *     *     *       Thyme     *     *     *     *     *     *     *     *     *       Thyme     *   | •                   | ,            |          |           |               | ,      |
| (Bud:)   7   (0.0)   (10.0)   (15.0)   7     Eucalyptus   7   7   7   7   7     (Leaves)   Sweet Basil   7   7   7   7     Sweet Basil   6   7   7   7   7     Sweet Basil   8   6   7   7   7     Sweet Basil   8   7   7   7   7     Sweet Basil   8   7   7   7   7     Sweet Basil   8   7   7   7   7     Raykan   7   7   7   7   7   7     Sweet Basil   0.0   0.0   0.0   0.0   7   7   7     Thyne   7   7   7   7   7   7   7   7     Thyne   7   7   7   7   7   7   7   7     Thyne   7   7   7   7   7   7   7   7   7     Femal   6   7   7   7   7<  |                     |              | 6.0      |           | 7.0 8.0       | 11.0   |
| Eucalyptic   Learves)   Fucalyptic     (Learves)   Sweet Basil     Sweet Basil   Sweet Basil     (Rayhan   Earves)     Inyme   Earves)     Learves)   Earves)     Thyme   0.0     Thyme   0.0     (Learves)   0.0     Female   0.0     (Jearves)   0.0     Female   0.0     Seedic)   0.0     (Jearves)   0.0     Common   Sage     (Mamania)   (Learves)     Common   2.0     Sage   2.0     Prepper   2.0     Pepper   2.0     Pepper   0.00     Solo   0.00     Pepper   0.00  |                     | ,<br>,       | (30.0)   |           | 35.0) (40.0)  | (55.0) |
| (Larve)   |                     |              | 0        | 6         | 3 6           | 9      |
| Sweet Basil     Rayhan     Novet Basil       Leaves)     Leaves)     1       Thyme     0.0     0.0     0.0       Thyme     0.0     0.0     0.0     0.0       Femel     0.0     0.0     0.0     0.0     0.0       Femel     0.0     0.0     0.0     0.0     0.0     0.0       Seeds)     -     (0.0)     (0.0)     (0.0)     0.0   |                     |              | (0.0)    | (10.0) () | (30.0) (30.0) | (30.0) |
| (Rayhan   |                     |              | 1        | 1         | 1             | 2      |
| Learnery   Learnery     Thyme   C.   C   O.0   O.0   O.0   O.0   Common   C <thc< th="">   C   C</thc<>   |                     | ,            | (2:0)    | (5.0)     | 5.0) (5.0)    | (10.0) |
| Turgane   (Larves)   0.0   0.0   0.0   0.0   0.0     Femal   0.0   0.0   0.0   0.0   0.0   0.0   0.0   0.0     Femal   0.0 <td></td> <td></td> <td>c</td> <td>-</td> <td>,<br/>,</td> <td>P</td>  |                     |              | c        | -         | ,<br>,        | P      |
| Femel     0.0     0.0     0.0     0.0       (Seeds)     (0.0)     (0.0)     (0.0)     (0.0)     (0.0)       Lenon (Peel)     (Peel)     (Peel)     (Peel)     (Peel)     (Peel)     (Peel)       Common     Sage     (Peel)     (Peel) | •                   | •            | (0.0)    | (2:0) ()  | 10.01 (10.01  | (20.0) |
| Femal     0.0     0.0     0.0     0.0       (Seeds)     .     (0.0)     (0.0)     (0.0)     .       Lenon (Peel)     .     .     (0.0)     (0.0)     .     .       Lenon (Peel)     .     .     .     .     .     .     .       Common     .     .     .     .     .     .     .     .       Common     .   |                     |              | 10.00    | 1         | farmer farmer | 12:22  |
| (Seeds) (0.0) (0.0) (0.0) (0.0)   Lemon (Peel) - - - -   Common Sage - - - -   Common Sage - - - - -   Common Sage - - - - -   Common Common - - - - -   Common Sage - - - - -   Camea Sage - - - - -   Orange - - - - - -   Brazilian - - - - - -  |                     |              | 0.0      | 0.0       | 0.0 0.0       | 0.0    |
| Lemon (Peel)  |                     |              | (0.0)    | (0.0)     | (0.0) (0.0)   | (0.0)  |
| Lemon (Peel)  |                     |              | 01       | 0.01      | 150           | 14.0   |
| Common<br>Sage<br>(Marmania)<br>(Leaves)<br>(Leaves)<br>(Peel)<br>(Peel)<br>Brazilian<br>Pepper   | ,<br>,              | ,            | (2:0)    | (0 0)     | 55.0) (75.0)  | (000)  |
| Sage     Sage       (Mamnania)     (Mamnania)       (Leaves)     2.0     4.0       Orange     (10.0)     (20.0)       Brazilian     2.0     5.0     11.0     15.0       pepper  |                     |              |          |           |               |        |
| (Marmania)<br>(Leaves)<br>Orange<br>(Peel)<br>Brazilian<br>Pepper<br>Pepper   |                     |              | 0.0      | 0.0       | 0.0 0.0       | 1.0    |
| (Leaves)<br>Orange 2.0 4.0<br>(Peel) (20.0) (20.0)<br>Brazilian 2.0 5.0 11.0 15.0<br>pepper 75.0  |                     |              | (0.0)    | (0.0)     | 0.0) (0.0)    | (2:0)  |
| Orange 2.0 4.0<br>(Peel) (10.0) (20.0) 2.0 5.0 11.0 15.0<br>Pepper 75.0   |                     |              |          |           |               |        |
| (Peel) (10.0) (20.0) 20.0) Brazilian 2.0 5.0 11.0 15.0 Pepper 1.0 15.0  |                     |              | 11.0     | 11.0      | 12.0 18.0     | 19.0   |
| Brazilian 2.0 5.0 11.0 15.0 pepper  | •                   |              | (55.0)   | (55.0) (( | (0.09) (0.03  | (0.56) |
| bepper  | 50 110              | 15.0 12.0    | 0.00     |           |               |        |
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| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | 10                   |       |          |        |         |        | Su     | ggested o | concentra | ations (µl | 370 ml a | ir)    |         |        |        |        |        |
|--|----------------------|-------|----------|--------|---------|--------|--------|-----------|-----------|------------|----------|--------|---------|--------|--------|--------|--------|
| Spannint     0.0     2.3*     14.0     17.0     200     14.0     17.0     200     14.0     17.0       Clove     Clove     Clove     Clove     14.0     17.0     2000     11.0     14.0     17.0       Clove     Clove     Clove     70.0     (35.0)     (100)     35.0     (100)     12.0     (25.0)     (25.0)     (25.0)     (25.0)     (25.0)     (25.0)     (20.0)   | OII                  | 1.0   | 2.5      | 5.0    | 7.5     | 10.0   | 20.0   | 50.0      | 60.0      | 70.0       | 80.0     | 90.0   | 100.0   | 150.0  | 200.0  | 250.0  | 300.0  |
|  | Spearmint            | 0.0   | 2.3*     | 14.0   | 17.0    | 20.0   |        |           |           |            |          |        |         |        |        |        |        |
| Clove     4.0     4.0     6.0 </td <td>(Leaves)</td> <td>(0.0)</td> <td>(10.0)**</td> <td>(0.01)</td> <td>(\$5.0)</td> <td>(100)</td> <td></td> <td>e</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>c</td> <td>6</td>   | (Leaves)             | (0.0) | (10.0)** | (0.01) | (\$5.0) | (100)  |        | e         |           |            |          |        |         |        |        | c      | 6      |
|  | Clove                |       |          | 4.0    |         | 4.0    | 6.0    | 6.0       |           |            |          |        | 10.0    |        | 11.0   | 14.0   | 17.0   |
|  | (Buds)               | ,     |          | (20.0) | •       | (20.0) | (30.0) | (30.0)    | i,        | ,          | ,        | X      | (50.0)  |        | (55.0) | (0.0)  | (85.0) |
|  | Eucalyptus           |       |          |        |         |        |        |           |           |            |          |        | 5.0     | 5.0    | 7.0    | 10.0   | 12.0   |
| Sweet Baril     Sweet Baril     10     10     10     30     40     40     40       Rayian     1  | (Leaves)             |       |          | •      | •       |        |        | 5         |           | i.         |          | •      | (25.0)  | (25.0) | (35.0) | 50.0)  | (60.0) |
| (Jayikan   (Jayikan <th< td=""><td>Sweet Basil</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>01</td><td>01</td><td>0.0</td><td>0.0</td><td>0.1</td></th<> | Sweet Basil          |       |          |        |         |        |        |           |           |            |          |        | 01      | 01     | 0.0    | 0.0    | 0.1    |
| Leaves)   Leaves)   (3.0)  | (Rayhan              | •     | e        | 5      | •       | i      | c      | 5         | •         | P          | ï        | £      | 0.1     | 1.1    | 0.0    | 0.4    | 1000   |
| Thyme     3.0     2.0     5.0     8.0     100       (Leaves)     0.0     0.  | Leaves)              |       |          |        |         |        |        |           |           |            |          |        | (n·c)   | (n·c)  | (n.cr) | (n.u)  | (0.02) |
| (Leaves)   (15 0)   (10 0)   (25 0)   40 0)   (50 0)     Famel (Seeds)   0.0   | Thyme                |       |          |        |         |        |        |           |           |            |          |        | 3.0     | 2.0    | 5.0    | 8.0    | 10.0   |
| Famel (Seeds)     0.0     <  | (Leaves)             | •     |          | •      | •       |        | •      | •         | •         |            |          | •      | (15.0)  | (10.0) | (25.0) | 40.0)  | (50.0) |
| remnet (Seeds)     (0.0)   |                      |       |          | 0.0    |         | 0.0    | 0.0    | 0.0       |           |            |          |        | 0.0     | 0.0    | 0.0    | 0.0    | 0.0    |
| Lemon (Peel)   3.0   14.0   14.0   15.0   19.0     Common Sage   (15.0)   (70.0)     | rennet (Seeds)       |       | ,        | (0.0)  |         | (0.0)  | (0.0)  | (0.0)     |           |            | ł.       | ÷      | (0.0)   | (0.0)  | (0.0)  | (0.0)  | (0.0)  |
| Lemon (rest)   15.0   (70.0)   70.0)(   (75.0)   95.0)     Common Sage   0.0   0.0   0.0   0.0   0.0   0.0   20     (Marmaria)   0.0   0.0   0.0   0.0   0.0   0.0   0.0   20     (Marmaria)   1.   1.0   1.0   1.0   0.0   0.0   0.0   20     (Marmaria)   1.   1.0   1.0   1.0   1.0   0.0   0.0   20     (Leaves)   3.0   6.0   115.0   30.0)   30.0   14.0   17.3   19.0   19.6     Brazilian   5.0   9.0   15.0   17.0   18.7   20.0   19.6   19.6   10.6     Pepper   1.2.0   (75.0)   (75.0)   (85.0)   93.0   (100.0)   19.0   19.6   19.6   19.6   10.6   |                      |       |          |        |         |        |        |           |           |            |          |        | 3.0     | 14.0   | 14.0   | 15.0   | 19.0   |
| Common Sage     0.0     0.0     0.0     0.0     0.0     0.0     20       (Mammaia)     (10.0)     (10.0)     (0.0)     (0.0)     (0.0)     (0.0)     (10.0)       (Leaves)     3.0     6.0     11.0     17.0     17.3     19.0     19.6       Orange (Peel)     10.0     (15.0)     (30.0)     30.0     15.0     17.0     17.3     19.0     19.6       Brazilian     5.0     9.0     15.0     17.0     17.0     17.3     19.0     19.6       Pepper     .  | Lemon (Feel)         |       |          |        | •       |        |        | ł         | •         | ï          | ,        |        | (15.0)  | (70.0) | 70.0)( | (75.0) | (95.0) |
| (Mammana)   (0.0)   (10.0)     Orange (Peel)   .   .   .   .   .   .   .   .   19.6   19.6   19.6   19.6   19.6   19.6   .   .   .   .   .   .   19.6   19.6   .   .   .   .   .   .   .   19.6   19.6   .   .   .   .   .   .   19.6   19.6   . <td>Common Sage</td> <td></td> <td>0.0</td> <td>0.0</td> <td>0.0</td> <td>0.0</td> <td>2.0</td>   | Common Sage          |       |          |        |         |        |        |           |           |            |          |        | 0.0     | 0.0    | 0.0    | 0.0    | 2.0    |
| Orange (Peel)     3.0     6.0     14.0     17.0     17.3     19.0     19.6       Drange (Peel)     (15.0)     (30.0)     (30.0)     (30.0)     (90.0)     (95.0)     (98.0)       Brazilian     5.0     9.0     15.0     17.0     17.0     17.0     (90.0)     (95.0)     (98.0)       Pupper     5.0     9.0     15.0     17.0     18.7     20.0     (90.0)     (95.0)     (98.0)       I Laves     5.0     9.0     15.0     17.0     18.7     20.0     (90.0)     (95.0)     (98.0)       I Laves     20.0     93.0)     (100.0)     93.0)     (100.0)     17.0  | (Mamana)<br>(Leaves) | •     | 5        | s      |         | r.     | C      | 6         | ·         | C          | C        | C      | (0.0)   | (0.0)  | (0.0)  | (0.0)  | (10.0) |
| Orange (Peel) (70.0) (90.0) (95.0) (98.0)<br>Brazilian 5.0 9.0 15.0 17.0 18.7 20.0 (90.0) (95.0) (98.0)<br>pepper (Leaves) (100.0) (93.0) (100.0)  |                      |       |          |        |         |        | 3.0    | 6.0       |           |            |          |        | 14.0    | 17.0   | 17.3   | 19.0   | 19.6   |
| Brazilian<br>pepper 5.0 9.0 15.0 17.0 18.7 20.0<br>(Leaves) (25.0) (45.0) (75.0) (93.0) (100.0)  | Orange (Feel)        | •     |          | 5      | •       | C      | (15.0) | (30.0)    | ł         | i.         | ı.       | £1     | (0.0)   | (0.06) | (0.06) | (95.0) | (98.0) |
| pepper (Leaves) (25.0) (45.0) (75.0) (85.0) (93.0) (100.0)   | Brazilian            |       |          |        |         |        |        | 0 5       | 00        | 15.0       | 17.0     | 18.7   | 0.00    |        |        |        |        |
| (Leaves)   | pepper               | •     |          | •      | •       | ÷      | ×      | 0220      | (45.0)    | 02200      | (82 0)   | (03.0) | (100 0) | •      | •      | ł      | •      |
|  | (Leaves)             |       |          |        |         |        |        | 10.000    | 12-21     | laint      | 10       | (area) | (n-n-1) |        |        |        |        |

mean number of dead insects (out of 20 S ortzae adults) after a period of 48 hrs exposure to different and - and the second Table 3. A guide for the effective range of

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|                   |            |          |          |              |         | Sug    | gested c | oncentra | ntions (µ | 1370 ml | air)   |         |        |         |         |         |
|-------------------|------------|----------|----------|--------------|---------|--------|----------|----------|-----------|---------|--------|---------|--------|---------|---------|---------|
| 0II               | 1.0        | 3.5      | 5.0      | 7.5          | 10.0    | 20.0   | 50.0     | 60.09    | 70.0      | 80.0    | 90.06  | 100.0   | 150.0  | 200.0   | 250.0   | 300.0   |
| Spearmint         | 0.0        | 4.0*     | 16.0     | 19.3         | 20.0    | 3      |          |          |           |         | ŝ      |         |        | Ş       |         |         |
| (Leaves)          | (0.0)      | (20.0)** | (80.0)   | (0.96)       | (100.0) | •      |          | •        | •         |         | •      |         | ¢      | •       |         |         |
| Clove             |            |          | 8.0      |              | 10.0    | 12.0   | 17.0     |          |           |         |        | 18.0    |        | 18.0    | 19.0    | 19.3    |
| (Buds)            |            |          | (40.0)   |              | (50.0)  | (0.09) | (85.0)   | ţ        |           |         |        | (0.06)  |        | (0.06)  | (95.0)  | (0.96)  |
| Eucalyptus        |            |          |          |              |         |        |          |          |           |         |        | 8.0     | 10.0   | 12.0    | 15.0    | 16.0    |
| (Leaves)          | c          |          |          |              |         | ·      | C        |          | •         |         |        | (40.0)  | (20.0) | (00.09) | (75.0)  | (80.0)  |
| Sweet Basil       |            |          |          |              |         |        |          |          |           |         |        | 01      | 0.2    |         | 0.9     | 00      |
| (Rayhan           | ,          | ,        | ,        | ,            | •       | •      | ,        | •        | •         | ,       | •      | 0.4     | 0.0    | 0.4     | 0.0     | 0.0     |
| Leaves)           |            |          |          |              |         |        |          |          |           |         |        | (0.02)  | (0.07) | (0.02)  | (0.05)  | (40.0)  |
| Thyme             |            |          |          |              |         |        |          |          |           |         |        | 4.0     | 5.0    | 7.0     | 14.0    | 16.0    |
| (Leaves)          | •          |          |          |              |         | •      |          | ,        | •         | ·       |        | (20.0)  | (25.0) | (35.0)  | (0.0)   | (80.0)  |
|                   |            |          | 0.0      |              | 0.0     | 0.3    | 0.3      |          |           |         |        | 1.0     | 1.0    | 1.0     | 1.0     | 2.0     |
| Lennel (Seeds)    |            | ,        | (0.0)    |              | (0.0)   | (1.6)  | (1.6)    | ł        |           | 8       |        | (2:0)   | (5.0)  | (2:0)   | (5.0)   | (10.0)  |
|                   |            |          |          |              |         |        |          |          |           |         |        | 7.0     | 16.0   | 16.0    | 17.0    | 20.0    |
| Lemon (Peel)      | c          | •        | c        | e:           | •       | ·      | c        |          | ŀ         | e.      |        | (35.0)  | (80.0) | (80.0)  | (85.0)  | (100.0) |
| Common Sage       |            |          |          |              |         |        |          |          |           |         |        | 00      | 00     | 00      | 00      | 00      |
| (Marmaria)        | ,          |          | ,        |              | •       | •      |          |          | •         | ,       | •      | 000     | 0.00   | 000     | 000     | 1000    |
| (Leaves)          |            |          |          |              |         |        |          |          |           |         |        | (0.0)   | (0.0)  | 10-01   | (0-0)   | (0.01)  |
| 1-0               |            |          |          |              |         | 6.0    | 9.0      |          |           |         |        | 17.0    | 19.3   | 20.0    | 20.0    | 20.0    |
| Orange (reel)     |            |          |          |              | •       | (30.0) | (45.0)   |          | •         |         |        | (85.0)  | (0.96) | (100.0) | (100.0) | (100.0) |
| Brazilian         |            |          |          |              |         |        | 7.0      | 13.0     | 16.0      | 18.0    | 19.0   | 20.0    |        |         |         |         |
| pepper (Leaves)   | •          |          |          |              | •       | •      | (35.0)   | (65.0)   | (80.0)    | (0.06)  | (98.0) | (100.0) |        | -       |         |         |
| "Mean No. of dead | individual | uls      | ** Morta | dity percent | age     | ONwee  | t tested |          |           |         |        |         |        |         |         |         |

Table 4. A guide for the effective range of concentrations and mean number of dead insects (out of 20 S. oryzoe adults) after a period of 72hrs exposure to different tested botanical essential oils

Table 5. Response of the rice weevil S. oryzae to different botanical essential oils (LC50 and LC20 values and their corresponding FL [Fiducial Limits] through

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| fumigant toxicity bit             | oassay at different exposure times                                     |                         |                            |                              |                           |
|-----------------------------------|--|-------------------------|----------------------------|------------------------------|---------------------------|
|                                   |  |                         | $LC_{50}$ (µl/370 ml air)) | LCps (µl/370 ml air)         | Toxicity Index            |
|                                   | Regression of N.E.D. response(y) o                                     | n log                   |                            |                              | (%)                       |
| Oil                               | dose (x)   | **d                     | (95% Fid                   | lucial Limits)               | (based on                 |
|                                   | (Maximum likelihood estimate   | (                       | Tow                        | er-Upper)                    | LC <sub>50</sub> after 72 |
|                                   |  |                         |                            |                              | hrs)                      |
|                                   | $y_1^* = -4.49 + 2.24x$  | 0.52                    | 100.68 (78.26-129.38)      | 544.59 (321.46-929.34)       |                           |
| Orange                            | y2=-4,42+2.44x   | 0.94                    | 64.17 (48.80-84.18)        | 302.15 (201.43-455.99)       |                           |
|                                   | y <sub>3</sub> =-4.47+2.79x  | 0.81                    | 39.76 (29.60-53.23)        | 154.18 (107.16-223.23)       | 8.22                      |
|                                   | $y_1 = -8.40 + 3.73 x$   | 0.48                    | 179.37 (152.20-211.38)     | 495.51 (312.87-786.25)       |                           |
| Lemon                             | y <sub>2</sub> =-9.60+4.43x  | 0.41                    | 146.68 (124.77-172.38)     | 344.77 (254.52-467.51)       |                           |
|                                   | y <sub>3</sub> =-8.66+4.11x  | 0.67                    | 115.76 (92.77-144.35)      | 285.34 (211.86-384.76)       | 2.82                      |
|                                   | y1=-2.39+3.70 x  | 0.47                    | 4.43 (3.62-5.40)           | 12.32 (8.61-18.04)           |                           |
| Spearmint                         | y2=-2.41+4.10x   | 0.49                    | 3.88 (3.18-4.70)           | 9.77 (7.20-13.53)            |                           |
|                                   | y3=-2.24+4.36x   | 0.64                    | 3.27 (2.67-3.97)           | 7.79 (5.86-10.58)            | 100                       |
|                                   | y1=-2.45+0.95x   | 16.0                    | 382.62 (160.55-945.63)     | 20596.29 (1298.12-379011.41) |                           |
| Clove                             | y <sub>2</sub> =-1.73+0.91x  | 0.54                    | 79.65 (46.88-136.29)       | 5088.88 (922.89-31888.16)    |                           |
|                                   | y3=-1.10+1.14x   | 0.98                    | 9.23 (4.75-17.23)          | 275.581 (109.76 -649.87)     | 35.43                     |
|                                   | y1=-7.36+2.79x   | 0.82                    | 434.73 (210.15-901.97)     | 1689.23 (200.09-14392.20)    |                           |
| Eucalyptus                        | y <sub>2</sub> =-4.98+2.06x  | 0.82                    | 259.67 (180.67-373.87)     | 1627.43 (314.22-8534.03)     |                           |
|                                   | y <sub>3</sub> =-5.03+2.34x  | 0.95                    | 139.02 (102.10 -189.04)    | 698.98 (279.67-1757.98)      | 2.35                      |
|                                   | y <sub>1</sub> =-20.20+11.03x  | 0.89                    | 67.93 (64.01-72.10)        | 95.78 (86.06-106.61)         |                           |
| Brazilian pepper                  | y2=-15.77+8.85x  | 0.86                    | 60.47 (55.82-65.51)        | 92.75 (81.41-105.68)         |                           |
|                                   | y <sub>3</sub> =-14.68+8.43x   | 0.97                    | 55.18 (49.93-60.97)        | 86.47 (75.54 - 99.00)        | 5.93                      |
| Thyme                             | $y_3 = -8.62 + 3.74x$  | 0.52                    | 201.60 (171.26-237.33)     | 554.98 (335.76-919.14)       | 1.62                      |
| * y <sub>1</sub> =N.E.D. after 2- | 4 hrs, y <sub>2</sub> = after 48 hrs and y <sub>3</sub> =after 72 hrs. | **P= Probability corres | ponding to chi-square.     |                              |                           |

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Fig. 1. Log (dose)/N.E.D. (response) (Ld-p) regression lines for *certain* bioassayed botanical essential oils for 72 hrs against *Sitophilus oryzae* 

Moreover, the calculated values of the toxicity index proved that EO of spearmint was the most effective tested compound (100%), followed by clove (35.43%), orange (8.22%) and Brazilian pepper EOs (5.93%) (Table 5).

In this concept, these results could be attributed to the compounds presented in the essential oils of spearmint, clove, orange and Brazilian pepper, whereas the toxicity of spearmint is due to its biologically active major compounds that have been found in peppermint (menthol, isomenthone, limonene and 1,8cineole) (Snoussi et al., 2015). The Brazilian pepper leaves was found to contain up to 5% essential oil including biologically active triterpenes and sesquiterpenes. The essential oil present in the leaves, bark, and fruit of the Brazilian pepper tree is a rich source of chemicals (over constituents identified 50 thus far, including biologically active triterpenes and sesquiterpenes) (deNascimento et al., 2012).

Figure (1) is showing the log (dose)/N.E.D. (Ld-p) regression lines for certain (response) bioassayed botanical essential oils for 72 hrs against Sitophilus oryzae. The presented results are in agreement with those of Derbalah and Ahmed (2011) who showed that the oil and powder of M. viridis were effective against S. oryzae with the respect to adults mortality. Relevant results have been described for Mentha species essential oils in controlling diverse pests of stored products. Benavad et al. (2012) evaluated the chemical composition and insecticidal effect of essential oils of M. suaveolens and M. pulegium against S. oryzae and R. dominica. The essential oils were very toxic for the two Coleopteran species within the first 24 hours, with 100% mortalities when concentrations of 50 µl and 12 µl/ Petri dish of 9 cm diameter were used, respectively.

The toxic effects of essential oils involve many factors, among which are the entry points of toxins (inhaled, ingested or absorbed) and which may have contact, fumigation and phagoinhibitory effects (Regnault-Roger, 1997). The variation in toxicity was found to depend upon the type of the evaluated essential oils. The essential oils, especially those oils of spearmint and clove can be used as effective control agent for stored grain pests by fumigation. However botanical compounds have some limitations such as low bioavailability, high volatility and photodegradation that restrict their use in several occasions (Madhusudhanamurthya et al., 2013).

A number of investigations (Ho *et al.*, 1997; Huang *et al.*, 1998) have demonstrated contact, fumigant and antifeedant effects of a range of essential oil

constituents (cinnamaldehyde,  $\alpha$ -pinene, anethole), as well as extracts of cloves (*Syzygium aromaticum*) and star anise (*Illicium verum*) against the red flour beetle (*Tribolium castaneum*) and the maize weevil (*Sitophilus zeamais*). Eugenol, the major constituent of oil of cloves and holy basil, *Ocimum suave*, was shown to be effective against **S. zeamais** and **T. castaneum** and another two additional coleopterans, *S. granarius* and *Prostephanus truncatus* (Obeng-Ofori and Reichmuth, 1997). Essential oils and their combinations were also useful as fumigants for the protection of stored rice against the rice weevil (Lee *et al.*, 2001b).

Essential oils from different plant species possessed ovicidal, larvicidal and repellent effects against various insect species and are regarded as environmentally compatible pesticides (Isman, 2000; Cetin *et al.*, 2004). This study has explored the potential for development of certain essential oils (spearmint, clove, orange and Brazilian pepper) especially from conifers to be effective, economically and environmentally friendly commercial insecticides for controlling the rice weevil *Sitophilus oryzae*.

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## الملخص العريي

الزبوت النباتبة كبدائل آمنة ببئباً لمكافحة سوسة الأرز عبدالفتاح سيد عبدالكريم سعد ، السيد حسن محمد تايب، حورية لطفي مطر او

وأظهرت النتائج أن الزيت المستخرج من النعناع البلدي ۵۰% من الأفراد المعرضة (LC<sub>50s</sub>) (۳,۸۸، ۳,۸۸، ٣.٢٧ ميكرولتر بعد ٢٤، ٤٨، ٧٢ ساعة على التوالي). التركيز وزمن التعرض. وقد تأكدت السمية العالية للنعناع بحساب دليل السمية(١٠٠%) تلاه في ذلك القرنفل الإرتداد التى توضح العلاقة بين لوغاريتم التركيزات المستخدمة والإستجابة لحشرة سوسة الأرز. وعلى هذا يمكن التوصية بإستخدام الزيوت الناتجة من أوراق النعناع وبراعم القرنفل لإستخدامها لمكافحة سوسة الأرز .

تعتبر سوسة الأرز من الحشرات الهامة التي تصيب الحبوب المخزونة وبالذات القمح والأرز والذرة. وقد تم كان له سمية عالية وأعطى أقل قيم تركيزات قاتلة لنسبة تقييم بعض الزيوت النباتية الأساسية المستخلصة من أوراق كل من النعناع، الريحان، الزعتر، الفلفل البرازيلي، الكافور، المرمرية وبذور كل من الشمر وبراعم القرنقل لبينما أظهر زيت القرنفل سمية عالية بعد ٧٢ ساعة. وقد وقشر البرتقال والليمون كمدخنات ضد هذه الحشرة. وقد تم وُجد أن نسبة الموت في الحشرات تزداد بزيادة كل من إجراء التقييم الحيوي لهذه الزيوت كمدخنات وتم عرض جداول استرشادية تبين المدى الفعال من هذه الزيوت الذي يمكن إستخدامه وتأثيراته على الإستجابة ضد الحشرة ممثلة (٣٥,٤٣). علاوة على ذلك تم توضيح خطوط السمية أو في تعداد ونسبة الحشرات الميتة. وقد وُجد أن الزيت المستخرج من بذور الشمر لم يظهر اي تأثير حتى ٣٠٠ ميكرولتر من الزيت وكذلك زيت المرمرية حتى ٢٥٠ لهما تأثير طفيف وضعيف (بعد ٧٢ ساعة).