Optimization of the insecticidal effect of essential oils of three medicinal plants against rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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**Key words:** Essential oil, *Sitophilus oryzae*, Medicinal plants, Combination, Synergetic effect.

**Abstract**

This study was carried out to find the best combinations amongst essential oils of three medicinal plants, *Vepris heterophylla* (Rutaceae), *Hyptis spicigera* (Lamiaceae) and *Ocimum canum* (Lamiaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae). Eight concentrations of essential oils (from 31 ppm to 669 ppm) were used for contact toxicity with five replications. Mortality was recorded after 24 hours. The LC₅₀ and the LC₁₀₀ of each essential oil were determined. These values were used as boundaries of the experimental matrices 2³ and 2² which were used to determine the maximum synergetic effect of combinations of essential oils. It was found that the essential oil of *O. canum* was the most efficient (LC₁₀₀ = 68 ppm) while *V. heterophylla* essential oil was the least efficient one (LC₁₀₀ = 669 ppm). According to the combinations, it was found that the mixture of the three essential oils at their LC₅₀ level induced 87.5% of mortality whereas the expected mortality was 5%. The mixture of the three efficient doses (LC₁₀₀) induced 95% of mortality although 100% mortality was the awaited. Optimum synergetic effect occurred when essential oil of *H. spicigera* is found in low proportion in the mixture. In order to valorise *V. heterophylla* and to reduce overexploitation of *O. canum* or *H. spicigera*, farmers could used them in combination as potent insecticides to reduce weevil attack of stored rice and sorghum.

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Introduction

Around the world, agriculture is the main source of subsistence for millions of people (Godfray et al., 2010). However, because of harshness of climate at certain sub-Saharan Africa countries, crops are generally harvested once a year and only their proper storage can ensure their availability during the long dry season period (Gnassamo J & Kolyang, 2003; Ngamo 2004). According to Tschanttke et al., (2012), reducing pre- and post-harvest crop losses especially at the smallholder farmer’s level can achieve food security because losses due to insects can reach 20 to 100 percent depending of the type of pests and environmental factors (Ngamo, 2000; Olembo, 2000). To reduce these losses, many tools are commonly used by farmers, amongst them, synthetic chemical products (Nukenine et al., 2002). However, the use of commercial insecticides is limited because of their high costs at the smallholder farmer’s level and their environmental impacts (Hudson and Walker, 1974; Silvie et al., 2014). There is need for cheap and safe alternative methods of controlling pests of stored products.

Use of natural products as ash, bean husk (Deng et al., 2009) or plant extracts with insecticidal value can be one such alternative (Pacheco et al., 1995; De Groot, 1996; Levinson & Levinson, 1998; Huang et al., 1999; Belmain et al., 2001; Boeke, 2002). Previous investigations on powders and extracts of some North Cameroonian aromatic plants have shown real toxic and repellent effect against S. oryzae and S. zeamais (Ngamo et al 2007; Kouninki et al., 2007; Goudoum et al., 2009; Goudoum et al., 2013, Katamssadan et al., 2014). Many studies have confirmed the insecticidal effects of other aromatic plants like Callistemon citrinus and Azadirachta indica which are efficient against Chilo auricularis, (Rashid et al., 2013), Jatropha curcas which is efficient against Callosobruchus maculatus (Boateng et al., 2008), and Alstonia boonei use to protect sorghum and maize against damage of Sesamia calamistis (Oigiangbe et al., 2007).

However, overexploitation of certain plant species because of their effectiveness against insect pests can cause their disappearance. The utilization of these plant species in combination with other species which are less efficient can contribute to reduce the high pressure which prevails over the most efficient ones. According to farmers, simultaneous introduction or combination of different aromatic plants into their granaries during the storage period reduces postharvest losses. It is reported that some small holders are often introduce various different aromatic plants into their granaries just after harvest (Szafranski, 1999). At the north part of Cameroon, amongst the plants used by farmers against stored products ‘pests, V. heterophylla, H. spicigera and O. canum are the most cited and they are combined into the granaries. Investigations of Pol and Smid (1999) and Belaiche et al., (1996) demonstrated synergetic effect between some components of essential oils extracted from aromatic plants. Ngamo et al., (2007) were tested balanced combinations of essential oils of some plants and they were observed a synergetic effect. However, to get the maximum synergetic effect, it is necessary to combine various proportions of oils according to optimization method. Previous studies about combinations of essential oils were conducted to determine the kind of interaction which occurred, while, no study was conducted to quantify volumes of each components of the combination which achieve maximum synergetic effect according the optimization design. The objective of the present study is to find the ideal combination amongst essential oils V. heterophylla, H. spicigera and O. canum in view to propose a method to valorise the less efficient aromatic plants and to reduce the overexploitation of the most efficient ones.

Materials and methods

Plants collection and essential oils extraction

Plant parts were collected in some localities of the North Cameroon. V. heterophylla were collected at Djebbe (14°15,225'E; 10°41,039N; 496,93m), H. spicigera, collected at Dang (13°33,549'E, 07°35,609N, 1100,5m) and O. canum, collected at Touppere (14°24,145'E, 10°39,214N, 375,1m). Leaves of V. heterophylla and inflorescences of H. spicigera...
and *O. canum* were collected, cut in small pieces, dried in the shade and then the dried products were processed for oil extraction. The Clevenger apparatus were used for extraction of essential oils. The duration of the process was about four hours (4h).

**Insect rearing**

The rice weevil, *S. oryzae* used was collected from peasant’s granaries in the locality of Beka Hossere (Adamawa Cameroon) and maintained in vivo collection at the Storeprotect laboratory of the University of Ngaoundéré (Cameroon) since 2003. Jars glasses were kept under controlled conditions in an incubator monitor UNISCOPE SM9082 (Temperature: 30 ± 0.1°C, r. h.: 62 ± 0.1%). Adult insects used for the bioassays were aged between 15 and 20 days.

**Contact toxicity and determination of the LC₅ and the LC₅₀ of the three oils used**

Eight concentrations of each essential oil were chosen. They were 31, 37, 43, 50, 56, 62, 68 and 72 ppm for essential oil of *H. spicigera*, 31, 42, 62, 92, 122, 152, 162 and 182 ppm for essential oil of *O. canum* and 182, 240, 354, 409, 463, 516, 568 and 669 ppm for essential oil of *V. heterophylla*. These concentrations were calculated according to formula:

$$C = \frac{A \times 0.5}{V_p \times B}$$

With C (calculated concentration), A (Volume of essential oil picked up), Vp (Volume of Petri dish) and B (Total volume of the prepared solution). Thus, a defined quantity of considered essential oil was diluted into 0.5 ml of acetone and the solution was picked up with a micropipette (Rainin Magnetic-assist) and wide-spread uniformly on the filter paper (whatman N°1) deposited prior into a 9 mm diameter glass Petri dish. After 4 min, 20 insects about 20 days age were introduced into the Petri dish and then the dish was finally covered and sealed. Control treatment was made with 0.5 ml of acetone without essential oil. Mortality was recorded after a day (24 h). The average mortality is expressed as a corrected mortality according to Abbott's formula (1925). Concerning death, an insect is considered as dead if it fails to react even if it was touched by the fine brush.

**Determination of simultaneous effect of three essential oils on *S. oryzae***

The aim of this test was to assess the effectiveness of a mixture of two or three oils according to fluctuation of their volumes in the mixture. The first step was the determination of simultaneous effect of oils according to $2^2$ matrix (effect of two oils), the second one was the determination of simultaneous effect of oils according to $2^3$ matrix (effect of three oils). Mixtures were done according to standard matrix $2^a$ and in this case, the LC₅ and LC₅₀ values of three essential oils were considered as the inferior and superior boundaries of the matrices or parameters denoted respectively (-) and (+) and the three plants represented the factors as summarized at table 1.

They are four possibilities to combine two oils according to $2^2$ matrix as summarized at table 2 and eight possibilities to combine three oils according to $2^3$ matrix as summarized at table 3. To assess the insecticidal effect of combination of two essential oils, a defined quantity of a given essential oil (LC₅ or LC₅₀), was mixed with a defined quantity of another essential (LC₅ or LC₅₀) as shown at table 2 and the mixture was diluted into 0.5 ml of acetone. The solution was picked up with a micropipette (Rainin Magnetic-assist) and wide-spread uniformly on the filter paper (whatman N°1) deposited prior into a 9 mm diameter glass Petri dish. After 4 min, 20 insects about 20 days age were introduced into the Petri dish and then the dish was finally covered and sealed. Control treatment was made with 0.5 ml of acetone without essential oil. Mortality was recorded after a day (24 h).

To assess the insecticidal effect of combination of the three essential oils, a defined quantity of a given essential oil (LC₅ or LC₅₀), was mixed with a defined quantity a second essential (LC₅ or LC₅₀) and the mixture was combined with a third essential oil (LC₅ or LC₅₀) as shown at table 3, the final mixture of three oils was diluted into 0.5 ml of acetone. The
solution was used for contact toxicity as describe above.

Data analysis
Data of mortalities were expressed as a corrected mortality according to Abbott’s formula (1925). Values were submitted to ANOVA procedure of Statview 512TM . Probit analysis were used to calculate the LC$_5$ and the LC$_{100}$ values of each essential oil according to formula:

\[
LCx = \log_{10} - 1 \left( \frac{x}{a} \right).
\]

Test of $t$- Students was used to compare the effect oils and that of their combination at 95% ($P= 0.05$).

Results
The LC$_5$ and LC$_{100}$ of the three essential oils used
The LC$_5$ values of essential oils of H. Spicigera, O. canum and V. heterophylla, were respectively 31, 31 and 182 ppm. In the same order, the LC$_{100}$ values of 128, 182 and 669 ppm were obtained for essential oils of O. canum, H. spicigera and V. heterophylla respectively.

Table 1. Summary statement of factors and parameters used in experimental matrices.

<table>
<thead>
<tr>
<th>Factors</th>
<th>LC$_5$: Bottom level (–)</th>
<th>LC$_{100}$: Up level (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. spicigera = $X_1$</td>
<td>31 ppm</td>
<td>182 ppm</td>
</tr>
<tr>
<td>O. canum = $X_2$</td>
<td>31 ppm</td>
<td>68 ppm</td>
</tr>
<tr>
<td>V. heterophylla = $X_3$</td>
<td>182 ppm</td>
<td>669 ppm</td>
</tr>
</tbody>
</table>

Table 2. Different possibilities of combination of essential oils according to experimental matrix $2^2$.

<table>
<thead>
<tr>
<th>Combinations</th>
<th>$X_1$</th>
<th>$X_2$</th>
<th>$X_3$</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Y1</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Y2</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Y3</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Y4</td>
</tr>
</tbody>
</table>

$Y_{Sitophilus} = a_0 + a_1X_1 + a_2X_2 + a_1a_2X_1X_2$

$Y_{Sitophilus} = $ mortality.

Xi, Xii = Plants used.
a$_0$ is the constant of the model and a$_i$ is the coefficient of the factor Xi.

(-) = LC$_5$

(+) = LC$_{100}$.

Simultaneous effect of the three essential oils combined two by two
It appears that combination of 30 ppm (LC$_5$) of essential oil of H. spicigera with 30 ppm (LC$_5$) of essential oil of O. canum caused 25% of mortality of the experimental population of targeted insects. The mixture of 190 ppm (LC$_{100}$) of essential oil of H. spicigera with 30 ppm (LC$_5$) of essential oil of O. canum and the combination of 190 ppm (LC$_{100}$) of essential oil of H. spicigera with 70 ppm (LC$_{100}$) of essential oil of O. canum gave similar result (100% of mortality). The mixture of 70 ppm (LC$_{100}$) of essential oil of O. canum with 30 ppm (LC$_5$) of the essential oil of H. spicigera induced over 75% adult mortality (Fig.1).

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According to figure 3, the combination of 180 ppm (LC5) of the essential oil of *V. heterophylla* with 30 ppm (LC5) of essential oil of *O. canum* caused 40% of mortality. The combination of 180 ppm (LC5) of essential oil of *V. heterophylla* with 70 ppm (LC100) of essential oil of *O. canum* registered 100% of mortality. The mixture of 680 ppm (LC100) of essential oil of *V. heterophylla* with 70 ppm (LC100) of essential oil of *O. canum* recorded over 85% of mortality and the combination of 680 ppm (LC100) of essential oil of *V. heterophylla* with 30 ppm (LC5) of essential oil of *O. canum* caused over 80% of mortality.

**Table 3.** Different possibilities of combination of essential oils of *H. spicigera*, *O. canum* and *V. heterophylla* according to experimental matrix 2³.

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Factors and theirs levels</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>X</em>₁</td>
<td><em>X</em>₂</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The experimental response expressed in term of rate of mortality after 24 h was modelling as a first order intrinsic polynomial materializes by following equation:

\[
Y_{Sitophilus} = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{1,2}X_1X_2 + a_{1,3}X_1X_3 + a_{2,3}X_2X_3 + a_{1,2,3}X_1X_2X_3
\]

\[Y_{Sitophilus} \] = mortality.

\[X_1, X_2 \text{ et } X_3\] = reduced variables (plants used).

\[a_0\] is the constant of the model and \(a_i\) is the coefficient of the factor \(X_i\).

**Simultaneous effect of the three essential oils on rice weevil**

ANOVA analyses show that the three essential oils used have significant effect (P>0.05) on the experimental response. Meanwhile, they have shown negative index which means that they may have negative influence one to another. According to the table 4, the combination of the three LC5 of essential oils induced 87.5% adult mortality, while the expected one was 5%. The mixture of the three essential oils at their higher dose levels (LC100) caused 95% adult mortality whereas 100% mortality expected. The maximal percentage of adult mortality was reached when essential oil of *V. Heterophylla* was at its lowest rate (LC5) in the mixture.

**Table 4.** Observed and expected mortality of different combinations of the three essential oils.

<table>
<thead>
<tr>
<th>Whole essential oils</th>
<th>Combined essential oils</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hypsis</em></td>
<td><em>Vepris</em></td>
<td><em>Ocimum</em></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

According to this table above, the equation of the model is:

\[Y=97.8 - 0.23X₁ - 0.023X₂ - 0.137X₃ + 0.001X₁X₂ + 0.004X₁X₃ - 0.001X₂X₃\]

\[X₁ : H.spicigera ; X₂ : V. heterophylla ; X₃ : O. canum ; Y : mortality.\]

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Discussion

There is a substantial difference concerning the efficacy of the three essential oils used on *S. oryzae*. Indeed, essential oil of *O. canum* has presented the lowest LC$_{100}$ value which means that this plant is the most efficient and the least efficient one is *V. heterophylla*. This difference may be due to variation of the composition of their essential oil. According to previous studies (Ngassoum *et al.*, 2002), monoterpenes are the majors compounds found in essential oils of *O. canum* and *H. spicigera*, while in *V. heterophylla* essential oil, oxygenated sesquiterpenes are the predominant compounds with the rate of more than 42% (Ngassoum *et al.*, 2007).

![Fig. 1. Variation of adult mortality according to simultaneous variations of quantity of essential oil of *Hyptis spicigera* and *Ocimum canum* in the combination.](image)

Results of combination of essential oils of *H. spicigera* and *O. canum* express the synergetic effect between these two essential oils. This result confirms that the peasant practice which consists to combine these plants during the harvest period to fill their granaries must be widely vulgarized. Concerning the combination of essential oils of *V. heterophylla* and *Iliassa* and Léonard that of *O. canum*, the maximum mortality was observed when the volume of *V. heterophylla* is low in the combination. According to the efficacy of these oils used alone, *O. canum* has appeared to be the most effective. To reduce the overexploitation of *O. canum* because of its efficiency, the result of this study showed that peasants can combine it with *V. heterophylla*. Consider the following graph for a visual representation of these results.

![Fig. 2. Variation of insect mortality according to simultaneous variations of quantity of essential oil of *Hyptis spicigera* and *Vepris heterophylla* in the combination.](image)
heterophylla and their effectiveness remains the same. Previous studies mentioned that combining a little volume of thymol to carvacrol, citral and eugenol have created a synergic effect. The combination of carvacrol with nisine induces synergic effect (Pol and Smid 1999). Citronellol, menthol and terpineol have shown positive interaction when they are combined, particularly menthol increased the efficiency of the two others compounds (Belaiche et al., 1996).

According to simultaneous combination of the three essential oils, mixing essential oil of H. spicigera with two others has a benefit effect. It is also disclose from the result that the mixture O. canum and V. heterophylla at their maximum contents was not advantageous but H. spicigera has a benefit interaction with V. heterophylla and O. canum. Finally, the combination of essential oils of V. heterophylla, O. canum and H. spicigera as insecticide against S. oryzae has presented a benefit effect regarding their efficacy. More ever, this practice of mixing O. canum and V. heterophylla can be handle in reasonable exploitation of these plants within the same area to ensure their sustainability.

Conclusion
The present study examined possibilities to combine essential oils of three aromatic plants in order to optimize their reasoned utilization against rice weevils. It's showed that essential oil of O. canum which was the most effective can be used in few quantity in combination with H. spicigera and V. heterophylla to get higher efficacy on S. oryzae. This result indicates also some potentially applications at the farmers level to better manage the utilization of O. canum in combination with other aromatic plants as component of integrated storage of rice and sorghum against rice weevils.

Acknowledgements
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References


Goudoum A, Ngamo LST, Ngassoum MB, Mbofung CM. 2013. Persistence of active compounds of essential oils of Clausena anisata (Rutaceae) and Plectranthus glandulosus (Labiateae) used as insecticides on maize grains and flour. African Journal or Food, Agriculture, Nutrition and Development 1, 7325-7338.


