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

Ngatanko Iliassa[1*], Ngamo Tinkeu Léonard[2]

[1]*Department of Environmental Sciences, High Institute of the Sahel, University of Maroua, Cameroon*

[2]*Department of Biological Sciences, Faculty of Sciences, University of Ngaoundéré, Cameroon*

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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 LC_5 and the LC_{100} of each essential oil were determinated. These values were used as boundaries of the experimental matrices 2^3 and 2^2 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 ($LC_{100} = 68$ ppm) while *V. heterophylla* essential oil was the least efficient one ($LC_{100} = 669$ ppm). According to the combinations, it was found that the mixture of the three essential oils at their LC_5 level induced 87.5% of mortality whereas the expected mortality was 5 %. The mixture of the three efficient doses (LC_{100}) 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.

* **Corresponding Author:** Ngatanko Iliassa ✉ ngatanko@gmail.com

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 Tschamtke *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 others aromatic plants like *Callistemon citrinus* and *Azardirachta indica* which are efficient against *Chilo auricilius*, (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 Djebbé (14°15,225'E ; 10°41,039N ; 496,93m), *H. spicigera*, collected at Dang (13°33,549'E, 07°25,609N, 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 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 LC_5 and the LC_{100} of the three oils used

Eight concentrations of each essential oil were chosen. They were 31, 37, 43, 50, 56, 62, 68 and 72ppm 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:

$$C = \frac{A \times 0,5}{Vp \times B}.$$

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 considerated 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^n and in this case, the LC_5 and LC_{100} 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 (LC_5 or LC_{100}), was mixed with a defined quantity of another essential (LC_5 or LC_{100}) 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 (LC_5 or LC_{100}), was mixed with a defined quantity a second essential (LC_5 or LC_{100}) and the mixture was combined with a third essential oil (LC_5 or LC_{100}) 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 512^{TM} . Probit analysis were used to calculate the LC_5 and the LC_{100} values of each essential oil according to formula: $LCx = \log_{10} -1(\frac{\frac{x}{10}-b}{a})$.

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.

Factors	LC_5: Bottom level (-)	LC_{100}: Up level (+)
H. spicigera = X_1	31 ppm	182 ppm
O. canum = X_2	31 ppm	68 ppm
V. heterophylla = X_3	182 ppm	669 ppm

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

	Factors		
Combinations	*Xi*	*Xii*	Mortality
1	-	-	Y1
2	-	+	Y2
3	+	-	Y3
4	+	+	Y4

$Y_{Sitophilus} = a_0 + a_iX_i + a_{ii}X_{ii} + a_{i,ii}X_iX_{ii}$

$Y_{Sitophilus}$ = mortality.

X_i, X_{ii} = Plants used.

a_0 is the constant of the model and a_i is the coefficient of the factor X_i.

(-) =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).

Figure 2 shows that the combination of the essential oil of *H. spicigera* at 30 ppm (LC_5) with the essential oil of *V. heterophylla* at 180 ppm (LC_5) caused less than 25% of *S. oryzae* mortality. 100% adult mortality was recorded when 190 ppm (LC_{100}) of *H. spicigera* and 180 ppm (LC_5) of *V. heterophylla* essential oils were combined. The combination of 190 ppm (LC_{100}) of *H. spicigera* with 680 ppm (LC_{100}) of *V. heterophylla* essential oils caused 100% adult mortality. More than 60% of mortality was recorded in the combination of 680 ppm (LC_{100}) of essential oil of *V. heterophylla* with 30 ppm (LC_5) of essential oil of *H. spicigera*.

According to figure 3, the combination of 180 ppm (LC_5) of the essential oil of *V. heterophylla* with 30 ppm (LC_5) of essential oil of *O. canum* caused 40% of mortality. The combination of 180 ppm (LC_5) of essential oil of *V. heterophylla* with 70 ppm (LC_{100}) of essential oil of *O. canum* registered 100% of mortality. The mixture of 680ppm (LC_{100}) of essential oil of *V. heterophylla* with 70ppm (LC_{100}) of essential oil of *O. canum* recorded over 85% of mortality and the combination of 680 ppm (LC_{100}) of essential oil of *V. heterophylla* with 30 ppm (LC_5) 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 2^3**.**

Combinations	Factors and theirs levels			
	X1	*X2*	*X3*	*Mortality*
1	-	-	-	Y1
2	-	+	-	Y2
3	-	-	+	Y3
4	-	+	+	Y4
5	+	-	-	Y5
6	+	+	-	Y6
7	+	-	+	Y7
8	+	+	+	Y8

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:

$\mathbf{Y}_{Sitophilus} = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{1,2}X_1X_2 + a_{1,3}X_1X_3 + a_{2,3}X_2X_3 + a_{1,2,3}X_1X_2X_3$

$\mathbf{Y}_{Sitophilus}$ = mortality.

X_1, X_2 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 LC_5 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 (LC_{100}) 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 (LC_5) in the mixture.

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

	Whole essential oils			Combined essential oils			Mortality (%)		
	Hyptis	*Vepris*	*Ocimum*	*Hyp-Vepris*	*Hypt-Oci*	*Oci-Vepris*	observed	expected	**Chi²**
1	-	-	-	+	+	+	87.5	5	***
2	+	-	-	-	+	-	75	36.5	***
3	-	+	-	-	-	+	70	36.5	***
4	+	+	-	+	-	-	87,5	68.3	*
5	-	-	+	+	-	-	80	36.5	***
6	+	-	+	-	-	+	100	68.3	*
7	-	+	+	-	+	-	67.5	68.3	Ns
9	+	+	+	+	+	+	95	100	Ns
	-0.230	-0.023	-0.137	0.001	0,004	-0.001			

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

$Y=97{,}8 - 0{,}23X_1 - 0{,}023X_2 - 0{,}137X_3 + 0{,}001X_1X_2 + 0{,}004X_1X_3 - 0{,}001X_1X_2X_3$

X_1 : *H.spicigera* ; X_2: *V. heterophylla* ; X_3 : *O. canum ;* Y *:* mortality.

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. heterophyla* essential oil, oxygenated sesquiterpenes are the predominant compounds with the rate of more than 42% (Ngassoum *et al.,* 2007).

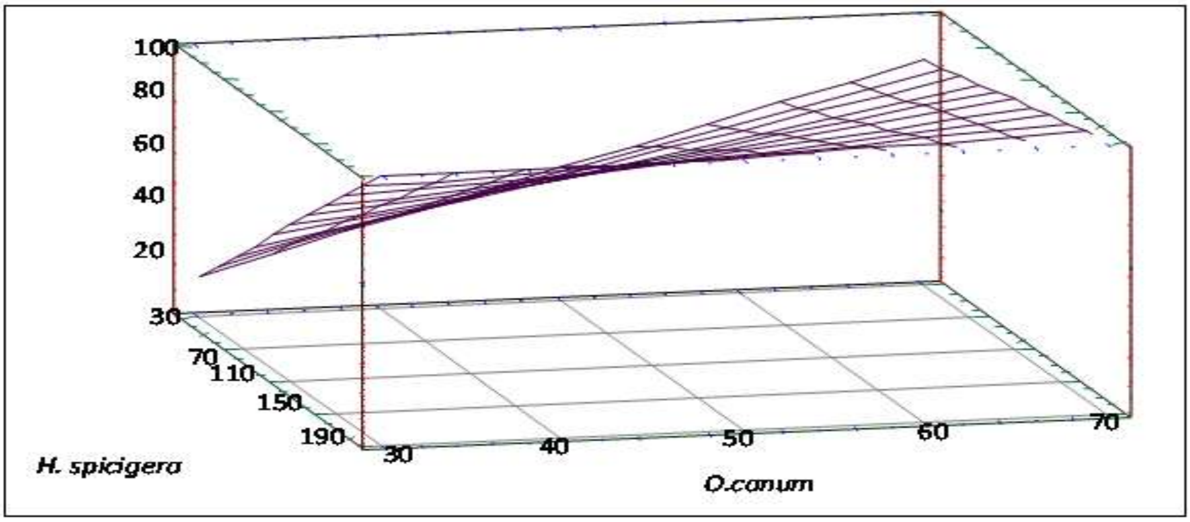

Fig. 1. Variation of adult mortality according to simultaneous variations of quantity of essential oil of *Hyptis spicigera* and *Ocimum canum* in the combination.

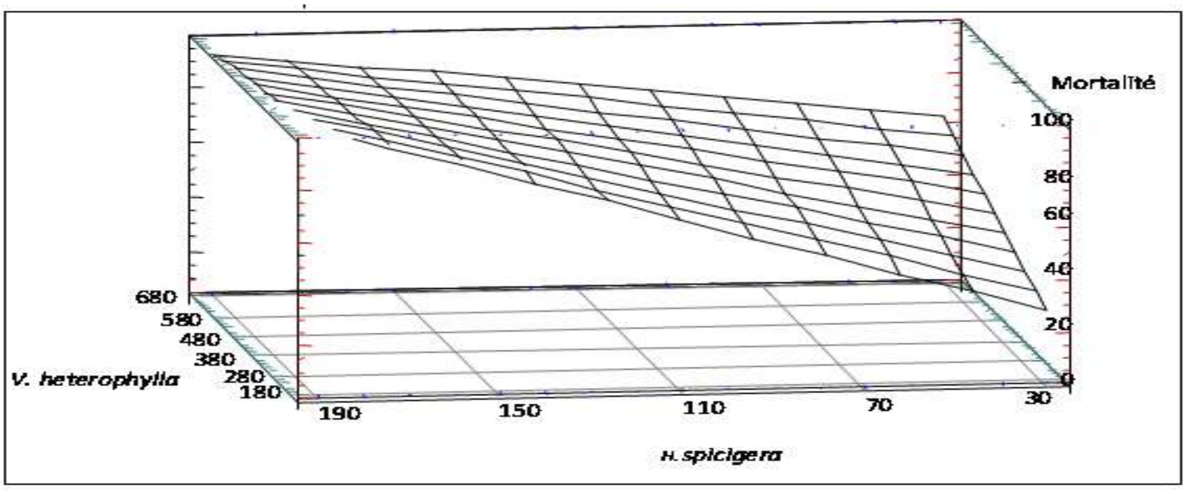

Fig. 2. Variation of insect mortality according to simultaneous variations of quantity of essential oil of *Hyptis spicigera* and *Vepris. heterophylla*in the combination.

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. heterophyla* and that of *O. canum*, the maximum mortality was observed when the volume of *V. heterophyla* 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 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).

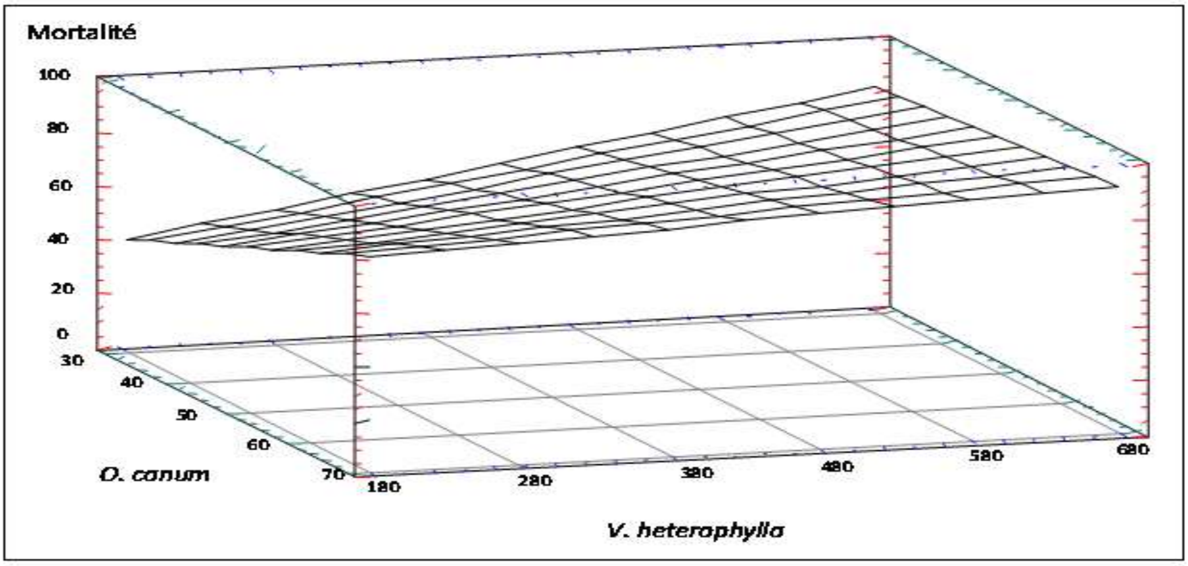

Fig. 3. Variation of adult mortality according to simultaneous variations of quantity of essential oils of *V. heterophylla* and *O. canum* in the combination.

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. heterophyla, 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. hetrophylla* 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.

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