

***In-vitro* biocontrol of sugarcane stem borer (*Eldana saccharina* Walker) with essential oils from aromatic plants**

ABSTRACT

Study design and objectives: Sugar cane production is threatened by the devastating action of the larval populations of *Eldana saccharina* Walker. Faced with this situation, the search for sustainable control solutions is a major challenge for sugar producers. This study contributes to the control of the larval populations of the pest through the use of formulations based on essential oils of local aromatic plants.

Methodology: Bioassays on the survival of the larvae were therefore conducted in the laboratory at the Sucrivoire site in Zuénoula. The larvae obtained from the eggs, incubated for 5 days were reared on a nutritional medium and then transferred to empty dishes after one week. Essential oils from the hydrodistillation of three aromatic plants (*Cymbopogon citratus*, *Ocimum gratissimum* and *Zingiber officinale*) were used. Five concentrations of these essential oils (1000, 2000, 4000, 8000 and 10000-ppm) were tested by direct contact on batches of 10 larvae for a total of 30 larvae/concentration. Dead larvae were counted daily and the mortality rate was calculated after 24, 48 and 72 hours of exposure.

Results: As early as 24 hours, larval mortality was significant ($P<0.001$) from 2000-ppm for *Cymbopogon citratus* and *Ocimum gratissimum* essential oils. They caused larval mortality of 63.33 and 80% respectively. *Zingiber officinale* induced a significant mortality of 80% from 4000-ppm. After 72 hours of exposure of the larvae to the essential oils, the mortality rate increased significantly with the concentration ($P<0.001$). The lowest lethal concentrations for 50 and 90% (841.77 and 1905.06 ppm, respectively) were obtained with the essential oil of *Ocimum gratissimum*.

Conclusion: The use of essential oils of *Cymbopogon citratus*, *Ocimum gratissimum* and *Zingiber officinale* can help to control *Eldana saccharina* Walker larval borers for better sugarcane production.

Keywords: Sugar cane; *Eldana saccharina* Walker; Stalk borer; Essential oils, Aromatic plants.

1. INTRODUCTION

Sugar cane is a plant cultivated mainly for the sugar contained in its stalks. Unfortunately, it has been a subject to numerous pest attacks for many years [1]. Of its many insect pests of the crop, *Eldana saccharina* Walker (Lepidoptera; Pyralidae) is the most important [2, 3]. This pest attacks the cane at maturity, resulting in a loss of cane yield [4, 5]. Indeed, the larva feeds on the pulp by digging galleries and its salivary enzymes cause a reddening that favours the proliferation of fungi leading to a weakening of the cane and a drop in its sugar content [6].

From West Africa to South Africa, the major consequences of these pests are reduced sugar production and thus a significant economic impact. This is the case in South Africa and Zimbabwe, where annual crop losses due to this stem borer have been estimated at 5000 tonnes of sugar and about 10 million euros, respectively [7, 4, 5, 6]. Also, the significant increase in cane infestation intensity (13.49%) in the Zuénoula sugar perimeter in Côte d'Ivoire in 2019 contributed to a decrease in sugar production of about 10% compared to the previous season [8]. This demonstrates an upsurge in stem borer populations and calls for better phytosanitary monitoring of sugarcane plots.

Growers generally use synthetic insecticides for the management of this pest. Unfortunately, these products are not always effective because the boring larvae of *Eldana saccharina* Walker are found inside the cane stems. In addition, their harmful effects on the environment and human health show that it is more than necessary to find biological palliatives to control the attacks of this pest [9, 10, 11, 12]. To this end, numerous works undertaken in recent years have highlighted the importance of essential oils in the control of crop pests [13, 14, 15, 16, 17]. These essential oils have been used to control some crop pests due to their remarkable insecticidal properties. This study was conducted to evaluate the insecticidal properties of some essential oils on *Eldana saccharina* Walker, a key sugarcane pest in Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1 Obtaining the larvae

Young *Eldana saccharina* Walker larvae were hatched from eggs and incubated for five days. These larvae were reared in the laboratory on an artificial medium for one week [18]. Prior to testing, they were removed and placed in empty 7 mm diameter plastic boxes. A total of 180 young *Eldana saccharina* Walker larvae were used 10 per dish.

2.2 Production of the essential oils

The essential oils were obtained from the plants of *Ocimum gratissimum*, *Cymbopogon citratus* and *Zingiber officinale*. Fresh leaves of *Ocimum gratissimum* and *Cymbopogon citratus* and rhizomes of *Zingiber officinale* were used to extract essential oils by the hydrodistillation method with a Clevenger type device [19]. The extraction was carried out in the laboratory for 2 hours at a temperature of 25 ± 2 °C and relative humidity of 80%. The obtained essential oils were kept cold (4 °C) in amber-stained bottles and then covered with a layer of aluminium foil to avoid photodegradation [14, 20].

2.3 Preparation and application of the essential oils

The solutions used were prepared by diluting a quantity of each essential oil in distilled water with an emulsifier, Tween 20, at 0.5% of the final volume. Five concentrations: 1000, 2000, 4000, 8000 and 10000-ppm, were prepared for each product. Finally, a control consisting of distilled water with Tween 20 added was used. Each treatment consisted of three boxes containing 10 larvae each for 30 larvae tested per concentration.

The tests were carried out using the direct contact method between the product and the larva, described by [14]. Thus, a 40 µl drop of the solution was placed on the larva using a syringe. The bioassays were performed under laboratory conditions at a temperature of 25 ± 2 °C and relative humidity of 80%. All tests were repeated 3 times under the same conditions of temperature, relative humidity and methodology.

2.4 Evaluation of the effect of essential oils on *Eldana saccharina* Walker larvae

The treated larvae were observed for 72 hours. During these observations, dead larvae were counted every 24 hours. As young *Eldana saccharina* Walker larvae are highly mobile, mortality was observed when they do not react to any contact and remain immobile. Observed mortality was calculated and corrected according to [21] formula in case of mortality observed in the control batch:

$$M_c = M_e - M_t / 100 - M_t \quad (i)$$

M_c = Adjusted mortality in percent

M_e = Mortality in the test sample

M_t = Mortality in the untreated control sample

2.5 Determination of lethal concentrations

The concentrations that caused the death of 50 and 90% of the larvae (LC50 and LC90) were determined 72 hours after their exposure to each solution. These LC50 and LC90 lethal concentrations were determined using the ED50plus version 1.0 software in EXCEL.

2.6 Data analysis

The statistical analysis of the data was performed with XLSTAT 2016 in EXCEL. An analysis of variance was used to show statistical differences at the 5% threshold and the mean mortalities were classified using the Newman and Keuls test.

3. RESULTS

3.1 Effect of *Zingiber officinale* essential oil

The mortality rates obtained after exposure of the larvae to different concentrations of *Zingiber officinale* essential oil are presented in Table 1. After 24 hours of exposure of the larvae to increasing concentrations, the mortality rates varied from 30 to 100% and total mortality was observed from 8000 ppm. After 48 and 72 hours of exposure, mortality rates were above 50% for all concentrations tested.

They ranged from 53.33 to 100% after 48 hours and from 63.33 to 100% after 72 hours of exposure. Mortality increased over the observation time and for all concentrations of the essential oil. The analysis of variance performed at the 5% tolerance level showed a statistical difference ($P < 0.0001$) between the concentrations for their effects on the larvae.

3.2 Effect of *Cymbopogon citratus* essential oil

Table 2 shows the larval mortality rates obtained after exposure to different concentrations of the essential oil of *Cymbopogon citratus*. At 24 hours after testing, larval mortality rates ranged from 20 to 100%. After 48 hours of exposure, mortality rates were 43.33 and 76.67% for 1000 and 2000 ppm while total mortality was observed from the 4000 ppm concentration. After 72 hours of exposure, the mortality rates were 56.67 and 86.67% for 1000 and 2000 ppm, respectively. Increasing concentrations of the essential oil induced high mortalities with increasing exposure time. The results of analysis of variance at a 5% threshold showed a significant difference between the concentrations studied. The minimum concentration of 4000 ppm that caused total larval mortality during the 72 hours of exposure.

Table 1. Influence of *Zingiber officinale* essential oil on average mortality of *Eldana saccharina* Walker larvae

Concentration (ppm)	Average mortality rate (%)		
	24 hours	48 hours	72 hours
10000	100.00 c ± 0.0	100.00 d ± 0.0	100.00 c ± 0.0
8000	100.00 c ± 0.0	100.00 d ± 0.0	100.00 c ± 0.0
4000	80.00 c ± 20.0	80.00 c ± 20.0	83.33 c ± 20.8
2000	46.67 b ± 11.5	73.33 c ± 5.8	86.67 c ± 5.8
1000	30.00 b ± 17.3	53.33 b ± 11.5	63.33 b ± 5.8
Control	0.00 a ± 0.0	0.00 a ± 0.0	0.00 a ± 0.0
Significance (P)		0.00 ***	

Means followed by the same letter do not differ statistically at the 5% level (Newman and Keuls test); ***: Highly significant at 0.01%.

Table 2. Influence of *Cymbopogon citratus* essential oil on average mortality of *Eldana saccharina* Walker larvae

Concentration (ppm)	Average mortality rate (%)		
	24 hours	48 hours	72 hours
10000	100.00 b ± 0.0	100.00 d ± 0.0	100.00 c ± 0.0
8000	100.00 b ± 0.0	100.00 d ± 0.0	100.00 c ± 0.0
4000	93.33 b ± 11.5	100.00 d ± 0.0	100.00 c ± 0.0
2000	63.33 b ± 28.9	76.67 c ± 5.8	86.67 c ± 11.5
1000	20.00 a ± 34.6	43.33 b ± 15.3	56.67 b ± 15.3
Control	0.00 a ± 0.0	0.00 a ± 0.0	0.00 a ± 0.0
Significance (P)		0.00 ***	

Means followed by the same letter do not differ statistically at the 5% level (Newman and Keuls test);
 ***: Highly significant at 0.01%.

3.3 Effect of *Ocimum gratissimum* essential oil

Table 3 shows the mortality rates after treatment of the larvae with various concentrations of the essential oil. Larval mortality rates ranged from 33.33 to 100% after 24 hours of exposure and total mortality was obtained from the 4000 ppm concentration. After 72 hours, larval mortality rates changed to 56.67 and 93.33% for the 1000 and 2000 ppm concentrations, respectively. The analysis of variance of the mortality rates showed that the concentrations tested are significantly different because the p probability was well below the critical threshold of 5%. Thus, at the minimum concentration of 4000 ppm of the essential oil achieved the total mortality of larvae.

3.4 Comparative larvicidal effect of the three essential oils

The mortality rates obtained after 72 hours of exposure of the larvae to the different concentrations of the essential oils are recorded in Table 4. At the 1000 ppm concentration, the three essential oils studied induced larval mortality of more than 50%. The rates ranged from 56.67 to 63.33% for *Cymbopogon citratus*, *Ocimum gratissimum* and *Zingiber officinale* essential oils, respectively. The essential oils of *Cymbopogon citratus* and *Ocimum gratissimum* caused 100% mortality with the concentration of 4000 ppm onwards, however, *Zingiber officinale* caused total mortality at the minimum concentration of 8000 ppm. The results obtained show a remarkable larvicidal effect of the three essential oils on the young larvae of *Eldana saccharina* Walker. The analysis of variance of the mortality rates showed a highly significant difference ($P < 0.0001$) between the concentrations of all essential oils tested on the larvae. Thus, the classification of the means by the Newman and Keuls test, with a 95% confidence interval, showed that the optimal concentration causing the death of all larvae was 4000 ppm for the essential oils of *Cymbopogon citratus* and *Ocimum gratissimum*, and 8000 ppm for that of *Zingiber officinale*.

Table 3. Influence of *Ocimum gratissimum* essential oil on average mortality of *Eldana saccharina* Walker larvae

Concentration (ppm)	Average mortality rate (%)		
	24 hours	48 hours	72 hours
10000	100.00 d ± 0.0	100.00 c ± 0.0	100.00 c ± 0.0
8000	100.00 d ± 0.0	100.00 c ± 0.0	100.00 c ± 0.0
4000	100.00 d ± 0.0	100.00 c ± 0.0	100.00 c ± 0.0
2000	80.00 c ± 20.00	93.33 c ± 11.5	93.33 c ± 11.5
1000	33.33 b ± 11.5	53.33 b ± 41.6	56.67 b ± 37.9
Control	0.00 a ± 0.0	0.00 a ± 0.0	0.00 a ± 0.0
Significance (P)	0.00 ***		

Means followed by the same letter do not differ statistically at the 5% level (Newman and Keuls test);
 ***: Highly significant at 0.01%.

Table 4. Influence of *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum* essential oils on average mortality of *Eldana saccharina* Walker larvae

Concentration (ppm)	Average mortality rate (%) after 72 hours		
	<i>Zingiber officinale</i>	<i>Cymbopogon citratus</i>	<i>Ocimum gratissimum</i>
10000	100.00 c	100.00 c	100.00 c
8000	100.00 c	100.00 c	100.00 c
4000	83.33 bc	100.00 c	100.00 c
2000	86.67 bc	86.67 bc	93.33 bc
1000	63.33 bc	56.67 b	56.67 b
Control	0.00 a	0.00 a	0.00 a
Average	72.22 ± 5.4	73.89 ± 4.5	75.00 ± 8.2
Significance (P)		0.00 ***	

Means followed by the same letter do not differ statistically at the 5% level (Newman and Keuls test);

***: Highly significant at 0.01% in the rows.

3.5 Determination of lethal concentrations (LC50 and LC90)

The LC50 and LC90 of the essential oils used in this study are presented in Table 5. After 24 hours of exposure, the LC50 and LC90 were low for the essential oil of *Ocimum gratissimum* compared to the other two essential oils. They were 1302.33 and 3255.81 ppm, respectively. But after 48 hours of exposure, the essential oil of *Zingiber officinale* gave the lowest LC50 but *Ocimum gratissimum* had the lowest LC90. After 72 hours of exposure of the larvae to the essential oils, *Ocimum gratissimum* recorded the lowest LC50 and LC90. These were 841.77 and 1905.06 ppm, respectively. During the 72 hours of observation, the essential oil of *Ocimum gratissimum* was the most effective at low concentrations.

Table 5. Influence of lethal concentrations (LC50 and LC90) of *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum* essential oils on average mortality of *Eldana saccharina* Walker larvae after 72 hours of exposure

Exposure time	Lethal concentration	<i>Cymbopogon citratus</i> (ppm)	<i>Zingiber officinale</i> (ppm)	<i>Ocimum gratissimum</i> (ppm)
24 hours	50%	1948.45	2299.7	1302.33
	90%	3680.41	6394.66	3255.81
48 hours	50%	1026.67	775.42	916.67
	90%	3266.67	7029.66	1916.67
72 hours	50%	919.63	952.12	841.77
	90%	3052.55	6787.07	1905.06

4. DISCUSSION

The essential oils extracted from the aromatic plants used in this study had a remarkable larvicidal effect on *Eldana saccharina* Walker. Indeed, the mortality rates were significant as the concentrations

increased. Also, the larval mortality evolved in an increasing way from 24 to 72 hours after exposure of the larvae to the different essential oils. This increase in mortality rates as a function of concentration and observation time shows the toxicity of these essential oils for *Eldana saccharina* Walker larvae. These results confirm those obtained by various authors in their studies on crop pests [13, 14, 15, 16, 22]. These authors showed the efficacy of essential oils of *Ocimum canum* Sims, *Ocimum sanctum* L., *Ocimum gratissimum*, *Cymbopogon citratus* (DC.) Stapf, *Cymbopogon nardus* L. and *Citrus* sp. on *Aphis gossypii* adults, *Dysdercus voelkeri* larvae and adults, *Pectinophora gossypiella* adults and *Cylas puncticollis* Boheman adults.

This insecticidal property of natural substances such as essential oils is believed to be due to their chemical constituents. Indeed, several authors have highlighted the chemical composition of essential oils of aromatic plants such as *Cymbopogon citratus*, *Ocimum gratissimum* and *Zingiber officinale* [23, 14, 17, 15, 16] and the insecticidal efficacy of these essential oils could be explained by their high levels of oxygenated monoterpenes such as α -Citral and Neral for the essential oil of *Cymbopogon citratus* and Thymol for the essential oil of *Ocimum gratissimum*. Also, [17], showed that the insecticidal effect of *Ocimum canum* essential oil on *Sitophilus zeamais* adults was due to various monoterpenic compounds including 1,8 Cineole. As for [20], they demonstrated the biological anti-cancer and antibacterial activity of *Zingiber officinale* essential oil against pathogenic bacteria. The inhibitory effect on the growth of these bacteria would be due to the majority of chemical compounds such as 1,8 Cineole and Camphene, which are respectively oxygenated and hydrocarbon monoterpenes. For [23] the efficacy of *Zingiber officinale* essential oil would be due to its high content of Camphene that is a hydrocarbon monoterpene. This important biological action of monoterpenes contained in essential oils has been demonstrated in various studies on crop pest control in Cameroon, India, Burkina Faso and Côte d'Ivoire [24, 25, 26, 15, 16]. This biological action would be due not only to nature but also to the chemical structure of the monoterpene compounds contained in essential oils [27, 28]. However, the biological action of essential oils of aromatic plants would be due to the existing synergy between the main and secondary chemical constituents and not to the activity of the main chemical components alone [29,30, 31].

The essential oil of *Ocimum gratissimum* was found to be the most toxic at low concentrations to *Eldana saccharina* Walker larvae after 72 hours of observation. The effectiveness of this essential oil could be explained by the fact that it is mainly composed of oxygenated monoterpenes such as Thymol. This oxygenated monoterpene acts directly on the cuticle of insects and mites, particularly those with soft bodies [32, 33]. These results confirm the remarkable insecticidal efficacy of oxygenated terpenoid compounds as reported by various authors [34, 35, 36]. The high mortality rates obtained in this study show that the tested essential oils are effective in the control of crop pests such as *Eldana saccharina* Walker. These results confirm those obtained in various studies conducted on pathogenic microorganisms and other crop pests [37, 38, 14, 17].

5. CONCLUSION

The essential oils of *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum* used in this study caused significant larval mortality rates during the 72 hours of exposure. At low concentrations, the *Ocimum gratissimum* essential oil formulation was the most effective of the essential oils tested. These local aromatic plant essential oils are a promising solution for the control of *Eldana saccharina* Walker larval populations in sugarcane. A study of their use under conditions of natural infestation in sugarcane plots would confirm their effectiveness against this pest.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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