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Fumigant efficiency of *Plectranthus glandulosus* essential oils against *Sitophilus zeamais* as influenced by site and plant age

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Abstract
Postharvest losses are higher in tropical regions where insects like *Sitophilus zeamais* are more involved in storage damage. *Plectranthus glandulosus*, already used by peasants to protect stored product, and rich in essential oils is well indicated for fumigant test against *S. zeamais*. Essential oil was obtained through hydrodistillation of leaves from plant of two different ages and from three locations. The essential oil yield ranged from 0.19 (Mbang-Foulbe elder plant) to 0.41% (Mbang-Foulbe young plant). The fumigant efficacy was logarithmic with 50% mortality being attained within 4h. This efficacy varied with plant age in Galdi and Mbang-Foulbe. Nguerngaou elder plant, Mbang-Foulbe young plant, and Galdi elder plant were the most efficient with LC_{50} of 0.667, 0.942, and 1.098 µl/l respectively. Therefore, *P. glandulosus* is really fumigant with varying efficacy. However for its usage, it should be harvested instead of cleared in shepherd farms where it grows as a weed.

Keywords: Fumigation, *Sitophilus zeamais*, essential oil, site, plant age.

1. Introduction

Postharvest losses are higher in tropical regions where insects are more involved in storage damage [1]. Eighty to ninety percent of global losses in traditional storage modules are due to insect infestation, with *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) being the most important destructor of stored maize grains [2]. When there is no appropriate protection of stored maize, it could induce up to 30-40% losses although world losses are commonly 4 to 5% [3]. In Zimbabwe, *S. zeamais* is present in 100% of the granaries [1]. In Adamawa region of Cameroon, *Sitophilus* spp. can within six months perforate up to 80.2% of maize traditionally stored [1].

Many synthetic pesticides, including fumigants, have been developed to protect stored crops against insect attacks. Although their usage is effective against pests, some insects have developed resistance to synthetic pesticides [5-8]. Synthetic pesticides have also leaded to many environmental nuisances and health problems [9, 10]. These nuisances are meaningful since farmers have neither appropriate training nor good handling of synthetic insecticides [11, 12], leading to injudicious usage, emphasising the bad consequences. This is why there is a need to develop more efficient, easily affordable, and sustainable pest management products [13] to combat pest problems so as to sustain or increase yields and ensure food security [14]. This situation has lead to the reduction of synthetic pesticides use and the search of safer insecticides [9, 13, 15]. Plant extracts have already shown good result [16-19]. *Plectranthus glandulosus* Hook (Lamiaceae) is effective against the maize and cowpea weevils [20]. However, plant effectiveness varies from one study to the other [20-22], and also varies with spatial location [22, 23, 24], and with plants age [25]. As essential oil composition varies with plant age [26], localities [24] and harvest time [27], even a slight variation in composition can lead to pronounced differences in anti-insect efficacy. In addition, essential oils are well indicated to be applied as fumigant which does not imply contact with insects and food stuff, therefore reducing the problem of residues on food. Nevertheless, little or no works have yet been published as *P. glandulosus* used as fumigant is concerned. The present study is focalized on the protection of crops during storage by using natural protectant. It consists of searching for the appropriate location of plant and the suitable plant age for the better fumigant activity of the leaf essential oils from *P. glandulosus* in order to valorise them by optimising their use against the maize weevil *S. zeamais*.

2. Materials and method

2.1 Test insects

Maize weevil was reared on maize grains under fluctuating laboratory conditions (T = 23.08±2.05 °C, RH = 74.67±14.36%). Adult weevils were obtained from a colony kept since 2005 in the Applied Chemistry laboratory at the University of Ngaoundere. They were reared on maize grains previously frozen at –15°C for two weeks to kill all insects whatever the level of development [16], and kept in laboratory for at least two weeks. Five litre plastic jars were filled up to 1/2 with these maize grains, then 100 unsexed adult weevils were allowed to lay eggs for two weeks [28]. Two to fourteen days old adult weevils were used for the tests [21].

2.2 Plant collection and essential oil extraction

The leaves of *P. glandulosus* were collected from August to September 2012 in three shepherds' farms where this plant grows as weed: Galdi, 50 km south of Ngaoundere (latitude 7.08683N, longitude 13.87959E, and altitude 1259 m); Nguerngaou about 25 km North of Ngaoundere (latitude 7.25426N, longitude 13.35078E, altitude 1151m); and Mbang-Foulbé 45 km North-east of Ngaoundere (latitude 7.53144N, longitude 13.72493E, altitude 989m). Not flowering plants were considered young, whereas those already flowering were considered elder. The harvested leaves were shade dried for two weeks before being ground in a mortar and sieved with a 1 mm mesh to ease essential oil extraction from the leaves. Extraction was carried out by hydrodistillation for 5 h using a modified Clavenger apparatus [26]. The quantity of essential oil extracted from the leaves powder was recorded in an hour bases. After extraction, essential oils were dried over anhydrous sodium sulfate, put in glass tubes, and kept at 4°C in a refrigerator until needed for bioassay [29].

2.3 Fumigant test

Fumigant toxicity test was carried out following the most common method, with modification [15, 17]. After preliminary tests, the concentrations 0, 64, 128, 190, and 255 µl/l was chosen as fumigation method adopted. Talcum was used to wipe the inner part of one litre glass bottle at the half of its height, creating a barrier that prevents the weevils from climbing. Twenty weevils were put at the bottom of the bottle. Then, using a micropipette, the required quantity of essential oil was laid on a 6 cm^2 Whatman filter paper number 1 that was hung in the fumigant chamber, $^1/_3$ up to the cover. Thereafter, the bottle was airtight with its lid, and scotch was used to reinforce the tightness. The concentration was calculated assuming 100% volatilization of the compounds in the exposure vessel [30]. Since *S. zeamais* does not fly vertically, they were not able to touch the piece of filter paper. Therefore, they were just allowed to inhale the essential oil for 24 h after which they were removed and kept in an empty glass Petri dish. Mortality observation was carried out from day 0 post-fumigation to day 7 [31]. Four replications were made. Temperature and relative humidity were recorded throughout the experiment, giving the average of 23.08±2.05°C, and 74.67±14.36% respectively.

2.4 Statistical analysis

Weevil mortality was corrected using Abbott's formula [32] before any statistical analysis involving mortality. Percent mortality of *S. zeamais* were submitted to analysis of variance. Mean separation was carried out using Tukey (HSD), or Student t tests. Probit analysis was performed to estimate lethal concentrations 50% (LC_{50}) [33]. The StatGraphic 15.1.0.2 software was used for the analyses while diagrams were plotted using SigmaPlot 2000 software, Version 11.0.

3. Results and discussion

3.1 Essential oil yield

Within each site, the essential oil yield varies with plant age, and ranged from 0.19 to 0.41% for Mbang-Foulbe elder and young plant respectively. (Figure 1).

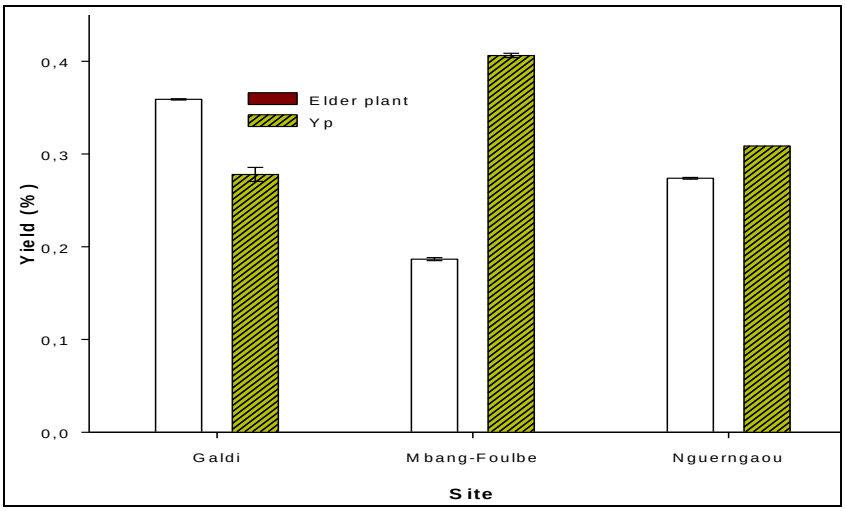

Fig 1: Yield percentage of *Plecthanthus glandulosus* essential oil variation per site, and plant age.

There was a common speeds of extraction, however, no relation was found between this speeds neither with site nor with plant age, since the curve of Ep was always on the one of Young plant except in Mbang-Foulbe (Figure 2). Essential oil yield was determined by plant age, and no relation between yield and site was found. As extraction kinetic is concerned, within the first hour, from 16.67 (Mbang-Foulbe elder plant) to 74.27% (Galdi elder plant) of the total yield of essential oil was already produced. Then from the second to the fourth hour, the rate produced remained almost the same. However, it was for all the sites and plant ages less than the one collected at the end of hydrodistillation.

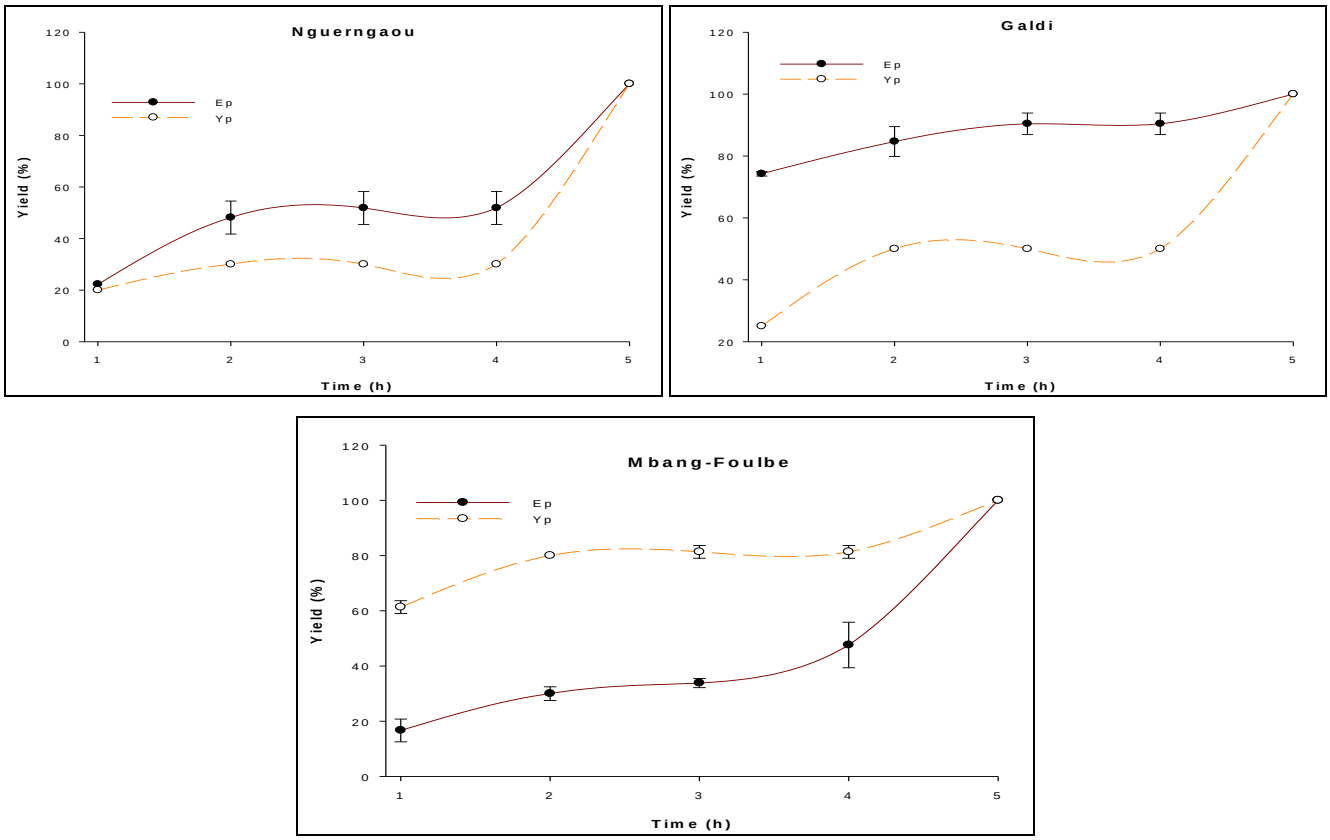

Fig 2: Rate of *Plectranthus glandulosus* essential oils produced per time for each site.

3.2. Fumigation efficacy

All the essential oils possess fumigant activity against *S. zeamais*, and the mortality increased significantly with rising time. Amongst the factors considered, concentration was more determinant of fumigant efficacy all over the days. In the overall concentration and plant age, fumigant efficacy of the essential oils from Nguerngaou was higher than the one from Galdi and Mbang-Foulbe all over the seven periods (Figure 3).

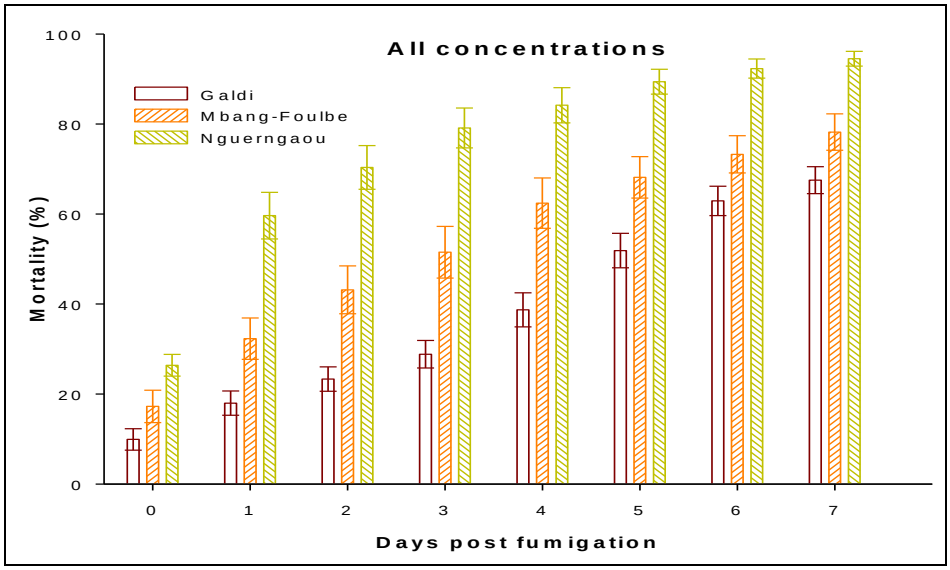

Fig 3: Overall percentage mortality (mean+S.E., n=32) of *Sitophilus zeamais* per site within 7 days after being fumigated for 24 h with essential oil of *Plectranthus glandulosus*.

Generally, the fumigant efficacy was logarithmic with the mortality rate of 50% being attained within the fourth day for all the essential oils at least at the concentration 128µl/l. Three essential oils, Nguerngaou elder plant, Nguerngaou young plant and Mbang-Foulbe young plant, at 64 µl/l air caused more than 50% mortality to *S. zeamais* within the first day. Nevertheless, at the concentration 16µl/l air, Galdi young plant and Mbang-Foulbe elder plant effectively remained less than 50% even within day 7 (Figure 4).

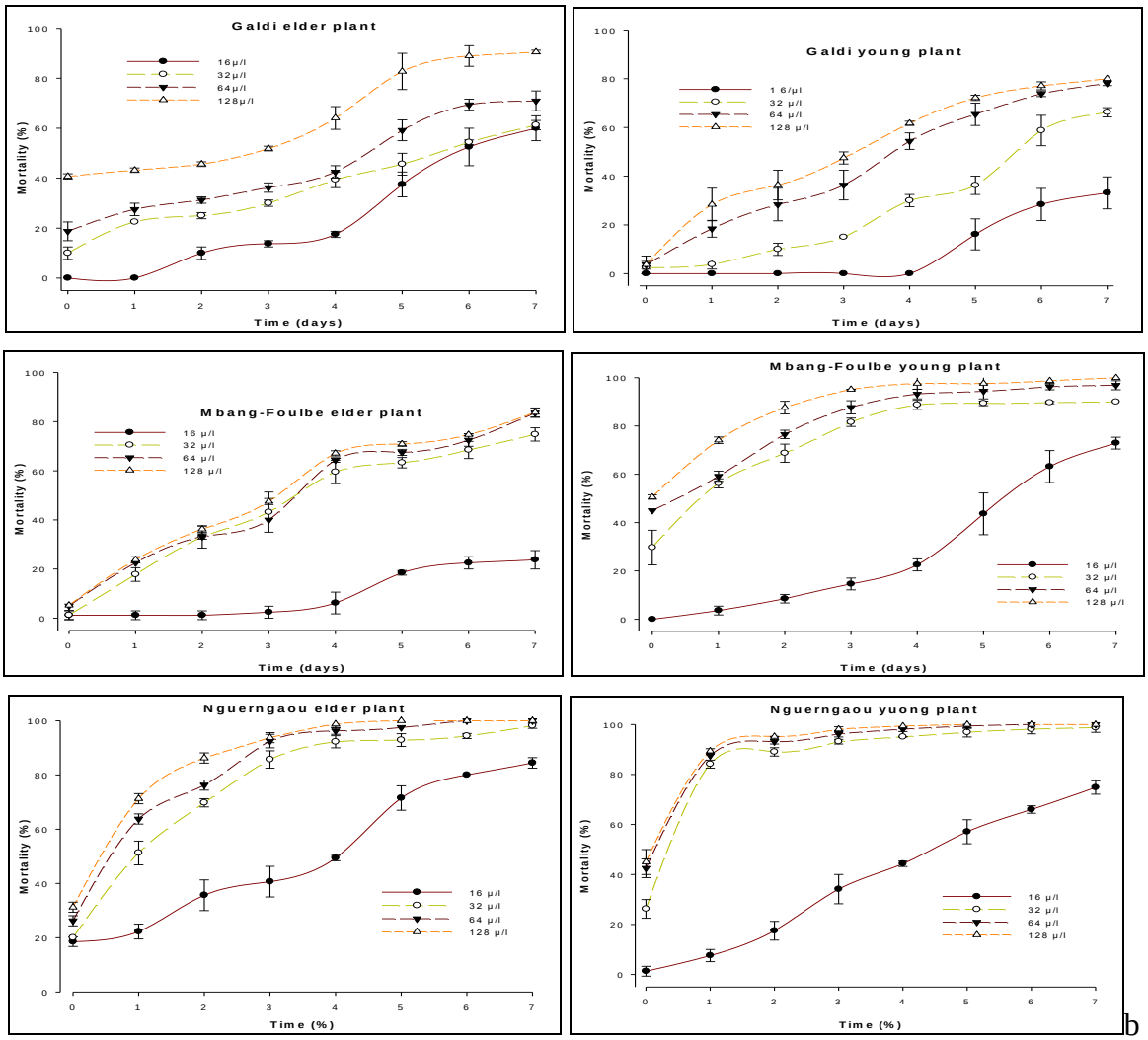

Fig 4: Fumigant evolution of *Plectranthus glandulosus'* essential oils.

Nguerngaou elder plant, Mbang-Foulbe young plant, and Galdi elder plant were the most efficient with LC_{50} of 0.667, 0.942, and 1.098 µl/L respectively (Table 1). When we divided the LC_{50} by the essential oil yield, the situation remains the same, however LC_{50} increased highlighting the small yield rate of our essential oils.

Table 1: *Plectranthus Glandulosus* LC_{50} per essential oil.

Product	Slope	R^2	LC_{50} (95% fiducial limits)	□2 (p)	LC_{50}/Yield
Galdi					
Young plant	1.433	0.547	1.371 (1.230-1.471)	46.485 (0.000)	4.931
Elder plant	1.039	0.875	1.098 (0.767-1.270)	41.200 (0.000)	3.059
Mbang-Foulbe					
Young plant	2.282	0.644	0.942 (0.837-1.019)	10.915 (0.693)	2.318
Elder plant	1.882	0.450	1.390 (1.215-1.507)	112.600 (0.000)	7.446
Nguerngaou					
Young plant	2.846	0.371	0.938 (0.762-1.039)	24.423 (0.041)	3.039
Elder plant	0.788	0.047	0,667 (0.005-.901)	209.088 (0.000)	2.435

Fumigant efficacy of *P. glandulosus* varied with plant age in Mbang-Foulbe ($2.54252 \leq t \leq 5.37345$; $0.0000 \leq p \leq 0.0164$). However, in Galdi, the difference was statistically significant just at days 0 and 1 ($-3.7092 \leq t \leq -2.0823$; $0.0008 \leq p \leq 0.0459$) (Table 2). Whereas for Nguerngaou, no variation was found all over the experiment ($|t| <$ "2.04227", t_{table}). Surprisingly, the variation was not the same in all the sites. Within the first day, essential oil from the elder plant of Galdi was more insecticidal ($t = -2.0823$; $p=0.0459$) whereas for essential oil from Mbang-Foulbe, young plants led to the highest weevil mortality whatever the day (Table 2).

Table 2: Comparison of fumigant efficacy against *Sitophilus zeamais* (mean+S.E., n=16), of essential oils of *Plectranthus glandulosus*, by plant age.

Plant	Day	Galdi		Mbang-Foulbe		Nguerngaou	
		Mean□SE	t student (p)	Mean□SE	t student (p)	Mean□SE	t student (p)
Young	0	2.46±0.77	T= -3.7092 (0.0008)	31.32±5.20	t = 5.3735 (0.0000)	28.77±4.62	t = 0.9877 (0.3312)
Elder		17.37±3.95		3.18±0.64		24.00±1.40	
Young	1	12.65±3.18	t = -2.0823 (0.0459)	48.30±6.90	t = 4.3822 (0.0001)	67.15±8.91	t = 1.4781 (0.1498)
Elder		23.30±4.01		16.30±2.37		52.13±4.90	
Young	2	18.69±4.01	t = -1.7755 (0.0860)	60.36±7.95	t = 3.9092 (0.0005)	73.71±8.42	t = 0.6890 (0.4961)
Elder		27.97±3.35		25.93±3.78		66.97±4.99	
Young	3	24.72±4.87	t = -1.3684 (0.1814)	69.75±8.34	t = 3.7945 (0.0007)	80.41±6.96	t = 0.2508 (0.8037)
Elder		32.97±3.55		33.28±4.78		78.15±5.73	
Young	4	36.53±6.28	t = -0.5669 (0.5750)	75.53±7.97	t = 2.5425 (0.0164)	84.21±5.97	t = 0.0085 (0.9933)
Elder		40.87±4.38		49.30±6.55		84.14±5.24	
Young	5	47.49±5.97	t = -1.1544 (0.2575)	81.25±5.84	t = 3.2634 (0.0027)	88.34±4.73	t = -0.3778 (0.7082)
Elder		56.26±4.69		55.04±5.52		90.46±3.02	
Young	6	59.53±5.13	t = -1.0438 (0.3049)	86.98±3.79	t = 4.0589 (0.0003)	91.03±3.75	t = -0.5954 (0.5561)
Elder		66.31±4.00		59.56±5.59		93.59±2.12	
Young	7	64.38±4.96	t = -1.0510 (0.3016)	89.95±2.74	t = 3.3374 (0.0023)	93.39±2.83	t = -0.6819 (0.5005)
Elder		70.68±3.36		66.46±6.48		95.64±1.71	

t_{tab} (α=0.05, df= 30) = 2.04227

3.3 Discussion

The 0.19-0.46% essential oil yield obtained by hydrodistillation was close to 0.46% [21] and 0.4% [34] obtained previously. This closeness can be explain by the fact that we all dried leaves before hydrodistillation. However, the range we obtained can be simply due to the separation of plants by age. Since essential oil composition varies with the yield [26, 27], it can also varies with plant age. The small yield can be counteract by the fact that *P. glandulosus* grows as a weed in sheeper farms and around some animal paddocks in the Adamawa region of Cameroon. The variation in hydrodistillation kinetic can support the fact that light and heavy molecules rates are not the same in all our samples. Even if essential oil components are usually no longer than 300 Daltons [35], light components are extracted first, and heavier ones require longer periods of hydrodistillation [36]. Therefore, young plant of Galdi and elder plants of Mbang-Foulbe may have heavier molecules.

The high incidence of the factor concentration in bio tests, we noticed in this work, has since been stated [37] and is common in all insecticidal tests [13, 17, 19, 28]. Evolution with concentration and time is common with tests using essential oils [13, 21, 38], and was also found with volatile compounds like α-Phellandrene, β-myrcene and β-ocimene [39]. The fumigant efficacy is therefore due to some of the essential oils components.

The LC_{50} values obtained are really small as compared to other plants against the same insect. 36.89 mg/l was obtained with *Evodia Rutaecarpa* Hort essential oil [40]. They were even less than those of some essential oil components like pulegone (11.8 µl/l), R-carvone (17.5 µl/l), S-carvone (28.1 µl/l) and E-Z-ocimenone (42.3 µl/l) [17]. This difference can directly be linked to the fact that they gave food to weevils after fumigation [39], or observed mortality directly after fumigation [17], while we calculated LC within 7 days. However, by using a solvent and allowing it to evaporate for 15 s [39], or 2 min [17], there can also be volatilisation of some light essential oil components. Moreover, the absence of cage for weevils in our test may have also reduced adsorption phenomenon of essential oil molecules on to cage material.

Our samples, especially Nguerngaou elder plant, Mbang-Foulbe young plant, and Galdi elder plant may have more insecticidal and volatile compound. This is reinforced by the fact that 100% volatility has just been an assumption [30]. Assumption confirmed by the deep essential oil smell still coming from the pieces of Whatman paper number 1 even weeks after fumigant test.

However, LC_{50}, values as small as 0-1.7 ml/l and 0.6-1.4 ml/l was already found against *S. granarius* for rosemary and common sage respectively [18]. These authors observed an increase of fumigant activity with temperature and a decrease with RH. But, we worked at temperature and RH of 23.08±2.05°C, and 74.67±14.36% respectively. Meaning that an increase of temperature and a decrease in relative humidity can lead to a decrease in our LC_{50} because essential oil volatility increases with temperature.

P. glandulosus's essential oil has been found, in tests with maize, effective in repelling and killing *S. zeamais*, in also reducing its F_1 progeny [21]. We found that this essential oil is also highly effective in fumigant test against *S. zeamais*, with effectiveness varying with plant age and site.

4. Conclusion

Young plant from Mbang-Foulbe gave the highest essential oil yield. However, Nguerngaou was the best site with overall mortality over 50% attained within the first day post fumigation, and mortality always higher than the one of other sites. Essential from elder plant of Nguerngaou was the most fumigant. Since the yield were low (<0.5%), and *P. glandulosus'* essential oils found really fumigant, it is advisable to harvest instead of clearing it in our farms, or to produce it.

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