Saponins-Clay Modified Materials: A New Approach Against Callosobruchus Subinnotatus In Stored Products

J. A. Noudem, M. G. Nguemtchouin Mbouga, K. G. Kaptso, M. Khalfaoui, G. B. Noumi

Abstract: The adsorbent power of montmorillonite clay treated with saponins extracted from kernels of Balanites aegyptiaca was evaluated using the terpenes compounds content in the essential oil of Ocimun gratissimun. The properties of formulations based on O. gratissimum essential oil and montmorillonite clay were modified after treatment of clay with saponins. Sabinene, linalool, terpinen-4-ol, α-gurjunene, neo-allo-ocimene, β-caryophyllene, sabinol and β-pinene terpenic compounds contained in essential oil were chosen to follow their adsorption. The kinetics adsorption of essential oil from a solution onto the adsorbents (treated clay and untreated clay) was explored experimentally. Non-linear form of pseudo-second-order model showed a better fit with good correlation coefficient. Intraparticle diffusion model were also used. Non-linear form of Langmuir isotherm model was applied and the data correlated well and the maximum adsorption capacity was found to be 1.07 mol.g-1 with regards to sabinol. Adsorption capacity of montmorillonite clay treated with saponins is greater than that of the untreated clay. The most adsorbed compounds are the oxygenated terpenic compounds. Treatment of clay with saponins increases the persistence of the insecticidal activity of essential oil adsorbed on montmorillonite clay. The mortality of Callosobruchus subinnotatus decreased from 96% to 70% and from 96% to 13.12% for O. gratissimun essential oil adsorbed on saponins-clay and O. gratissimun adsorbed on untreated clay after 42 days respectively. These results can be used in the production of bioinsecticides.

Index Terms: Saponins-clay, essential oil; adsorption, bioinsecticide, voandzou, Callosobruchus subinnotatus

1 Introduction

In recent years, a significant increase in the yield of food grains and other crops has been required to feed the world's growing population. In Sub Saharan Africa and Cameroon in particular, post-harvest losses of Small-scale farmers are estimated at about 80% of the crops after 6 to 8 months of storage. This is due to the lack of effective means of protection [1]. In the Northern part of Cameroon, the cultivation of cereals occupies a special place because of its high need either by local consumers or industries [2]. However, the cereals from harvests are seriously threatened by insect pests such as Callosobruchus subinnotatus, Sitophilus zemais. To reduce post-harvest loss, farmers/growers have to use high doses of chemical insecticides, which have an immediate effect, but not always affordable, and may have negative effects such as environmental pollution and pesticide residues in food [3]. Moreover, continuous use leads to an increase in pest resistance and enhanced pest resurgence. In this context, efforts are being made worldwide to replace these chemical insecticides with biological alternatives (bioinsecticides), which are less toxic to the environment.

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Aromatic plants are used in folk medicine as antimicrobial agents and their essential oils have been known to have antibacterial and antifungal proprieties [4], [5], [6]. However, the active components of the essential oil are not persistent. Essential oils of Ocimum gratissimum have been tested as grain protectors in Cameroon [6]; Insecticidal activity of clay and Xylopia aethiopica essential oil formulations have already been investigated and shown to increase the persistence of the toxicity and stability of the essential oil [7], [8]. Naturally, crude clays have a relatively low adsorption capacity [9], [10] so they are in most cases modified before use by inorganic molecules such as hydrolyzable cations hydroxides, organics molecules such as hydrocarbon derivatives to 4-diazabicyclo (2, 2, 2) octane or synthetic surfactants such as alkylammonium chlorides. Regarding the adsorption of insecticidal formulations based on O. gratissimum essential oil onto montmorillonite, a treatment with organic cations or metallic polycations significantly improve the adsorption capacity [6]. Moreover the treatment of clay with the synthetic surfactants such as cetyl trimethyl ammonium chloride showed a better affinity of montmorillonite clays onto O. gratissimun essential oil compared to the clays modified with the hydroxide cations [11]. The challenge is now to develop a formulation made of biocompounds that can remain active against insects for a long period. The natural source of surfactants needs to be explored to substitute synthetic surfactants; in order to produce more effective bioinsecticides from modified clay. Balanites aegyptiaca (L.) Del., popularly known as the desert date, is a spiny, evergreen tree commonly grown in the arid regions of Africa, the Middle East, and southern Asia. The extracts of B. aegyptiaca have commonly been used in various traditional folk medicines especially in Africa and southern Asia, and most studies dealing with the diverse biological activities in B. aegyptiaca extracts shown the active compounds to be steroidal saponins [12]. Saponins are a specific class of secondary metabolites. They are generally known as nonvolatile compounds, surfactants which are mainly distributed in the vegetable kingdom [13]. They have the applications in agriculture, where they are used as natural pesticides [12]. Therefore this research aimed at modifying the adsorption capacity of montmorillonite clay with saponins extracted from B. aegyptiaca kernels to increase adsorption of O. gratissimum essential oil as insecticide and also to contribute to the development of pure bioinsecticides.

2 Materials and Methods

2.1 Distillation of essential oil
The O. gratissimum plants used as source of essential oil were collected from Ngaoundere in Adamaoua region (Cameroon) in June 2015. Fresh whole plant samples were introduced in labelled bags and transported to the laboratory. Fresh leaves of the plant were hydro-distilled in a modified Clevenger-type apparatus for 4 h, dried over anhydrous sodium sulphate and stored at 4 °C until analysis and use.

2.2 Essentials oil Analysis
Analysis of ocimun gratissimun were performed on a Shimadzu GC-14B (FID) gaschromatograph (GC), fitted with a fused silica capillary column coated with a stationary phase consisting of 100% methylpolysiloxane (non-polar Rxi-1ms) (30 m × 0.25mm ID, film thickness coating 0.25 μm) The GC operating conditions were as follows: an initial temperature of 40°C (hold 10 min) a ramp at 5°C.min-1 from 40°C to 200°C and 10°C.min-1 from 200°C to 230°C with a final hold at 230°C for 10 min. The detector temperature was 250 °C. Nitrogen was used as carrier gas at a constant flow of 8 mL.min-1. The sample (2 μL) was injected with a split ratio of 1:10. The identification of essential oil components was performed by comparison of their retention index (RI) with reference to a homologous series of n-alkanes (C9–C22) under the same operating conditions and with those of pure authentic samples.

2.3 Extraction of Sqponins
B. aegyptiaca fruits were collected from Maroua in Far-North region (Cameroon), in March 2015. After forwarding these fruits in the laboratory, kernels were obtained after soaking the whole fruit in distilled water and left to stand for 24 h. The pulps of the fruits were removed by simple hand washing and with water from the tap. After this, the nuts were dried in an oven at 50°C for 72 h. The nuts were then decorticated manually to obtain the kernels. The kernels were dried at 40 °C for 24 h and thereafter they were ground. The paste obtained constituted the raw material in the extraction of saponins. Successive extraction of plant material was performed using solvents (non-polar to polar) that were n-hexane, chloroform, dichloromethane acetone and methanol. 850 g of paste were extracted with n-hexane (3 L) for 16h in a soxhlet extractor and the residue was left in the open air for 24 h to evaporate n-hexane. This residue was extracted successively by mechanically shaking with chloroform-dichloromethane (7:8 v/v) and acetone, and the residue was left again in the open air for 24 h. The dried solid residue was then extracted with methanol (1 L) in a Soxhlet extractor for 24 h. The methanol extracted was evaporated, resuspended in 80% aqueous n-butanol and any remaining solid residue removed by filtration with filter paper (Whatman No. 1). The butanol fractions were further partitioned with 1% potassium hydroxide to remove polyphenolic compounds (Hassan et al., 2012). The butanol fractions were then concentrated under reduced pressure, dissolved in methanol and precipitated with diethyl ether. A white-yellowish precipitate of saponin was formed. The precipitate was recovered by decantation, and then air dried at room temperature to yield the dry crude extract.

2.4 Qualitative analysis of saponins
The phytochemical screening was performed on the sample obtained to identify the different families of secondary metabolites. It's an analysis using specific reagents for the characterization of a family of given compound. Although the composition of plants differs according to region and season, the phytochemical screening was done according to Abdallah et al. [14] which showed the presence of saponins, tannins, terpenoids, alkaloids and phenolics compounds in the extracts of B. aegytiaca fruits

2.5 Insect Rearing
The used Voandzeia subterranea weevil C. subinnotatus was of the strain 01Z/LN/01 in vivo collection of insect grain pests of Store Protects Laboratory at the University of Ngaoundere. This strain has been in collection since 2008 in an incubator monitored at local temperature (28±4°C). Adult insects used for tests were one week old.

2.6 Preparation of clay powder
The clay samples used in this study is a montmorillonite clay type that was provided from Maroua locality in the far North region of Cameroon and used in a purified form. The montmorillonite was purified and modified with sodium before it was use. Purification was done by removing stones and other heavy particles were manually removed from the sample, which was then kept dispersed in ultra-pure water for several hours. Fractions less than 50 μm were obtained by using an appropriate sieve. Once the clay fraction was obtained, it was purified using hydrogen peroxide at 50% (V / V) to remove organic materials. Montmorillonite clays were homogenized with a NaCl solution (1 $mol.L^{-1}$), with a solid: liquid ratio of 1/200 (5 g clay/1000mL NaCl). The ion exchange was realized at 25˚C. After each process, the clay was washed with distilled water until no chloride ions were found by the $AgNO_3$ test. To avoid the rapid evaporation of the water, the ion exchanged samples were slowly dried at 70˚C and ground to pass through a 50μm mesh sieve. The obtained sample which is considered as untreated clay was designated by: Mont-Na.

2.7 Preparation of modified clay
To carry out the treatment of montmorillonite-Na clays with saponins, fresh saponins solutions were obtained by solution of an appropriate quantity of saponins in ultra-pure water - methanol (1:2 v/v). The pH of this solution was adjusted to 2.3 by adding HCl solution (0.1$mol.L^{-1}$). Organic solutions prepared at 2.5$g.L^{-1}$ were immediately used for clay treatment. Saponins-clays were synthesized according to the procedure described by Reddy et al. [15] and Nguemtchouin et al. [6] by using a given mass of montmorillonite-Na previously obtained which was dispersed in ultra-pure water in a proportion of 0.5% (W/W). 500mL of saponins solution with the pH 2.3 was added (8$mL.min^{-1}$ using a peristaltic pump) to the previous suspension stirred for 1 h using a magnetic stirrer. The resulting suspension was aged for 24 h at room temperature. After reaction, the clay sample was separated by centrifugation and washed repeatedly with ultra-pure water. The washing and centrifugation steps were repeated until

complete removal of the foam formed due to the saponins. The resulting clay sample was dried at 40°C for 24 h, ground in an agate mortar to a fine powder and identified as Mont-Sa. At the end, fractions of Mont-Sa less than 50 μm were obtained by passing the final modified sample through a sieve of 50 μm mesh.

2.8 Adsorption studies

Adsorption of essential oil by the different samples montmorillonite clays was carried out in batch process. Increasing amounts of clay fractions (0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9; 1.0 g) were dispersed in 10 ml of essential oil solution (1 ml of essential oil in 9 ml of acetone); and equilibrated in an overhead shaker at room temperature (25 °C) for 3 h. In all cases, adsorption equilibrium was reached within 2 h. The particles were allowed to settle and separated by centrifugation. The oil concentration in the supernatant was determined by GC-FID.

2.9 Adsorption isotherms

Adsorption isotherms were determined using the peak area of each component of the essential oils [7]. The adsorbed percentage was calculated as:

$$\%\,Adsorbed = \frac{100\left(A_o - A_x\right)}{A_o} \quad (1)$$

Where A0 is the peak area of each oil component in the initial solution; Ax the peak area of each component in the supernatant. The adsorbed amount per gram is:

$$Q = \frac{\left(C_o - C_e\right)}{m} * V \quad (2)$$

The percentage of each oil component in the initial solution was calculated as:

$$\%\,C = \frac{C_{xi}}{C_{eo}} * 100 \quad (3)$$

Where Cxi is the initial concentration of the each component in the initial solution and Ceo is the concentration of the essential oil solution. The equilibrium concentration was calculated as:

$$C_e = \frac{A_x}{A_o} * \frac{m_{eo}}{V_{eo}} * \%\,C \quad (4)$$

Where V_{eo} the volume of solution (mL):

2.10 Preparation of clay-O gratissimum essential oil formulation

Formulation used in this study was prepared with modified montmorillonite (Mont-Sa) and unmodified one (Mont-Na) by using the following ratio

$$Q = \frac{m_{eo}}{m_{clay}} = 0.1 \quad (5)$$

With m_{EO}: mass of essential oil; mclay: mass of clay. To prepare 10 g of each formulation, 10 g of clay powder (Mont-Na or Mont-Sa) were transferred in a 100 mL flask and the appropriate quantity of O. gratissimum diluted in 10 mL of acetone, was added [6]. After 5 min of manual shaking, the mixture was placed in a water bath set at 30°C for 90 min to complete the evaporation of acetone. The aromatized powders obtained (Mont-Na-O.gratissimum and Mont-Sa-O.gratissimum) were kept in coloured vials tightly closed using aluminium foil.

2.11 Formulation remnant effect on voandzou

To assess the duration of the insecticidal activity of formulations method described by Nguemtchouin et al., [8] was used. Ingestion-contact bioassays were carried out with 10 young adult Callosobruchus subinnotatus per Plexiglas box mixed with 10 g of voandzou and aromatized formulations (Mont-Na-O. gratissimum and Mont-Sa-O. gratissimum) sealed with canvas cloths held in place with rubber bands by noting the C. subinnotatus mortality when beetles were added to voandzou previously treated and conserved for the periods of 1, 7, 14, 21, 28, 35 and 42 days (4 boxes for each). Tests were carried out on C. subinnotatus previously starved for 48 h. Malagrain (Malathion 5%), a synthetic insecticide, was used at 0.01 g per 10 g of voandzou as a positive control (reference) with acetone-treated grain as the blank control. Five days later, mortality was estimated. The mortality rate was corrected for control mortality using Abbott's formula and the results were plotted on log/probability paper.

2. 12. Theoretical background: isotherm models

Under ideal saturated conditions the solid liquid ratio should not influence the amount of organic or inorganic molecules adsorbed per unit of adsorbent. However some interested studies have shown that both organic and inorganic contaminant adsorption is dependent on solid-liquid ratio to some degree and the competition system of mixture [16]. In order to optimize the design of adsorption system and to optimize the use of capsules in various formulations, it is important to establish the most appropriate correlation for equilibrium curves. In this respect, the equilibrium experimental data of the adsorption of each component in the crude essential oil on clay were studied using Freundlich and Langmuir models. The Freundlich model is an empirical equation employed to describe heterogeneous system; characterized by the heterogeneity factor nF describes reversible adsorption and is not restricted to the formation of the nonlayer [17]. The Freundlich model, usually applied to the adsorption on heterogeneous solid surfaces is defined by equation:- Where kF is the Freundlich constant related to the adsorption capacity ($mg.g^{-1}$) and 1/n is related to the adsorption intensity and C the concentration of the medium. The logarithm linear form of the Freundlich equation is:

$$\log q = \log k_F + \frac{1}{n}\log C \quad (6)$$

The Langmuir equation model assumes that the solid adsorbent has a limited adsorption capacity (qm), all the active sites are identical, they are only a complex of solute-molecule (monolayer adsorption) and that there is no interaction between the adsorbed molecules. This model supposes unique adsorption energy and no transmigration of sorbate along the surface plane.The following linear form relation can represent the Langmuir isotherm equation:

$$\frac{C_e}{q} = \frac{1}{ab} + \frac{1}{a} C_e \quad (7)$$

Where q ($cg.g^{-1}$) is the amount of terpenic compound adsorbed onto the clay at the equilibrium concentration, Ce. a indicated an effective adsorption of components by studies adsorbents (the theoretical monolayer capacity in $dm^3.g^{-1}$), b is the Langmuir equilibrium constant related to the affinity of bending sites ($cg.g^{-1}$) and Ce the equilibrium solution concentration ($cg.L^{-1}$). The values of a and b can be evaluated from the intercept and the slope of the linear plot of experimental data of Ce/q versus Ce or 1/q= f(Ce). One of the essential characteristic of the Langmuir equation could be expressed by the dimensionless constant called equilibrium parameter RL, that was calculated by using the equation.

$$R_L = \frac{1}{1 + bC_0} \quad (8)$$

Where b is the Langmuir constant related to the energy of adsorption (L/mg) and C0 is the initial adsorbate concentration (cg/L) and these values were derived from Langmuir isotherm. The character of the adsorption isotherm was related to the value of RL. The adsorption is unfavorable (RL > 1), linear (RL = 1), favorable (0 < RL < 1), or irreversible (RL = 0).

3 Results and discussion

3.1. O. gratissimun analysis

The identification of the compounds was made through a comparison of retention time and kovats indices with results obtained by GC-FID. Chromatographic analysis showed that O. gratissimun essential oil contain more than 50 components. Thirty compounds, representing about 98.32% of the essential oils have been identified; their retention indices and percentage composition, listed in order of elution are given in Table 1. Percentages yield and the identified volatile constituents from O. gratissimum show a composition rich in order of decreasing amounts: Sabinol (35.13%), terpinene-4-ol (15.53%), α-gurjunene (10.57%), sabinene (7.65%), β-caryophyllene (6.76%), linalool (4.61) and β-pinene (2.59). Five chemotypes of O. gratissimum are currently identified and are represented by the type eugenol, thymol, citral, ethyl cinnamate, and linalool (Cortez et al., 1998). Essential oils can contain more than 50% of any one of these chemotypes but, the results found in this study shows that this essential oil contains a chemotype sabinol which is different from the results found by [18]. In contrast, Ogendo et al. [3] showed that the chemical composition of O. gratissimum obtained from Kakamega forest in western Kenya is a methyl eugenol/ocimene chemotype with 64.28% and 10.40% methyl eugenol and b-(Z)-ocimene, respectively. Documented information from Asian, African and Latin American countries has shown the existence of significant intra-species variations in chemical composition with up to nine different chemotypes for O. gratissimum. Its chemical composition varies with season, time and stage of harvest and geographical origins [19], [20].

Table 1. *O. gratissimum essential oil composition.*

Components		Formula	Percentage (%)	KI
1	α-pinene	$C_{10}H_{16}$	0.25	931
2	sabinene	$C_{10}H_{16}$	7.65	974
3	β-pinene	$C_{10}H_{16}$	2.59	980
4	α-phellandrene	$C_{10}H_{16}$	0.27	1004
5	γ-terpinene	$C_{10}H_{16}$	1.24	1060
6	linalool	$C_{10}H_{14}O$	4.61	1095
7	1,3,8-p-menthratriene	$C_{10}H_{14}$	0.25	1106
8	trans-thujone	$C_{10}H_{16}O$	0.30	1112
9	neo-allo-ocimene	$C_{10}H_{16}O$	3.22	1124
10	Sabinol	$C_{10}H_{16}O$	35.13	1139
11	citronellal	$C_{10}H_{18}O$	0.46	1148
12	terpinen-4-ol	$C_{10}H_{16}O$	15.53	1173
13	p-cymen-8-ol	$C_{10}H_{16}O$	0.56	1180
14	myrtenol	$C_{10}H_{16}O$	0.25	1192
15	verbenone	$C_{10}H_{14}O$	1.16	1200
16	trans-carveol	$C_{10}H_{16}O$	0.28	1204
17	carveol	$C_{10}H_{16}O$	0.23	1222
18	geranial	$C_{10}H_{16}O$	0.25	1271
19	2-undecanone	$C_{11}H_{22}O$	1.28	1287
20	bicycloelemene	$C_{15}H_{24}$	0.25	1343
21	α-gurjunene	$C_{15}H_{24}$	10.57	1416
22	β-caryophyllene	$C_{15}H_{24}$	6.76	1418
23	α-humulene	$C_{15}H_{24}$	0.27	1458
24	Germacrene D	$C_{15}H_{24}$	0.66	1485
25	trans-mumurola-4(14)-diene	$C_{15}H_{24}$	0.25	1498
26	α-cadinene	$C_{15}H_{24}$	1.78	1538
27	epoxide of humulene II	$C_{15}H_{24O}$	0.29	1594
28	zingiberene	$C_{15}H_{24}$	1.24	1601
29	1,10-di-epi-cubenol	$C_{15}H_{26}O$	0.35	1610
30	cubenol	$C_{15}H_{26}O$	0.39	1635
31	Unidentified components		1.68	

KI: Kovats Index as determined on an Rxi-1ms column using the homologous series of n-hydrocarbons. In bold: Main compounds.

3.2. Extraction of Saponins

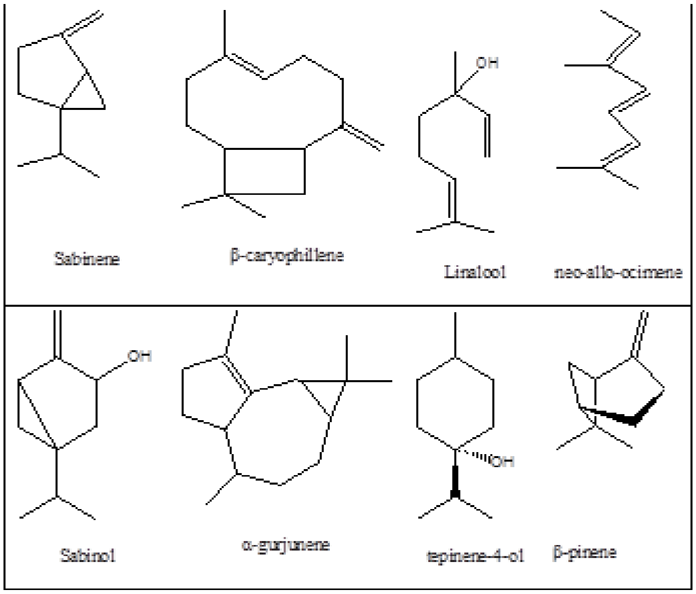
The yield of total saponins of B. aegyptiaca kernels extracted using 100% methanol was about 8.81%. Phytochemical screening performed on the crude saponins is presented in Table 2. It appears from this table that the extract obtained contains only saponins. Saponins known as surfactants are mainly distributed in the vegetable kingdom and exist in plants in biologically active form.

Table 2: *Phytochemical sceening for n-butanol extract of B. aegyptiaca kernels.*

Active principle	Occurrence
Saponins	+
Flavonoids	-
Polyphenols and phenols compounds	-
Tannins	-
Triterpenes	-
Steroids	-
Terpenoids	-

+ = Present; - = absent

Table 3: *Major components in Ocimum gratissimun essential oil with adsorption isotherms*



3.3. Adsorption isotherms

The adsorption isotherms of teterpenics compounds current in an essential oil of O. gratissimum by untreated and treated clays were made. For adsorption studies, 8 major compounds of O gratissimun essential oil were selected (Table 3): Adsorption isotherms of O gratissimun components are shown in Figures 1 and 2. The isotherms are of L-type according to the classification of Giles et al. [21]. Adsorption decreased in the order sabinol> terpinen-4-ol> linalool > α-gurjunène> sabinene> neo-allo-ocimene> β-pinene> β-caryophyllene for untreated montmorillonite clay (Mont-Na); and in the order sabinol> terpinen-4-ol> linalool > sabinene >α-gurjunène > neo-allo-ocimene> β-pinene> β-caryophyllene for montmorillonite clay treated with saponins (Mont-Sa). Adsorption curves clearly show that saponins modifications strongly improve the clay mineral affinity for the terpenic compounds particularly for the oxygenated mono terpenic compounds (Fig. 2). The amount adsorbed by the treated montmorillonite is more than twice that of the untreated montmorillonite. This adsorption amount of terpenic components between Mont-Na and Mont-Sa could be as a result of many factors. The selectivity was affected by the abundance of each component in the crude essential oil; Sabinol, terpinene-4-ol, α-gurjunene, sabinene which are the most adsorbed were the most abundant compounds (35.13%, 15.53%, 10.57% and 7.65% respectively), while β-pinene (2.59%) and β-caryophyllene (6.76) were the less adsorbed in all case. The selectivity of adsorption was affected by the polarity of terpenic components; sabinol, terpinen-4-ol and linalool were adsorbed in larger amounts than some monoterpene hydrocarbons; particularly for Mont-Sa. The maximum of adsorption of 0.0065 cg/g (sabinol) and 0.0021cg/g (β-caryophyllene) measured for the Mont-Sa increased to 0.0145 and 0.0037cg/g for Mont-Na as shown on Figure.1and 2. The high adsorption of oxygenated terpenes compounds could be explained by the fact that hydrogen bonds and van-der-walls forces attraction that exist between the terpene compounds, the heteroatoms contained in glycosidic functions of the sugars and the structure of the saponins. The two common isotherm models have been tested in the present study: Langmuir and Freundlich models. Applicability of the isotherm equations was compared in table 4 base on the correlation coefficient. Jaynes and Boyd [22] proposed that the adsorption mechanism fits the Langmuir or Freundlich models when the correlation coefficient value is greater than 0.89. According to this study, all the correlation coefficients obtained were greater than 0.89 for Freundlich and Langmuir model with Mont-Sa. Regarding Mont-Na all the correlation coefficient values were greater than 0.89 for Freundlich model except sabinol which presented a correlation coefficient of 0.87 and for Langmuir model, except sabinène (0.82) and neo-allo-ocimen (0.84). Those coefficients obtained indicated that the adsorption isotherm of terpenic compounds of essential oil on Mont-Na and Mont-Sa can be applicable to both models: Langmuir and Freundlich. Nevertheless Freundlich model seems to be more coherent than that of Langmuir model for the untreated montmorillonite clay (Mont-Na). The applicability of these two adsorption models to the investigated systems implies that both: monolayer adsorption and heterogeneous surface conditions could occur simultaneously during the adsorption of terpenic compounds on Mont-Na and Mont-Sa [23].

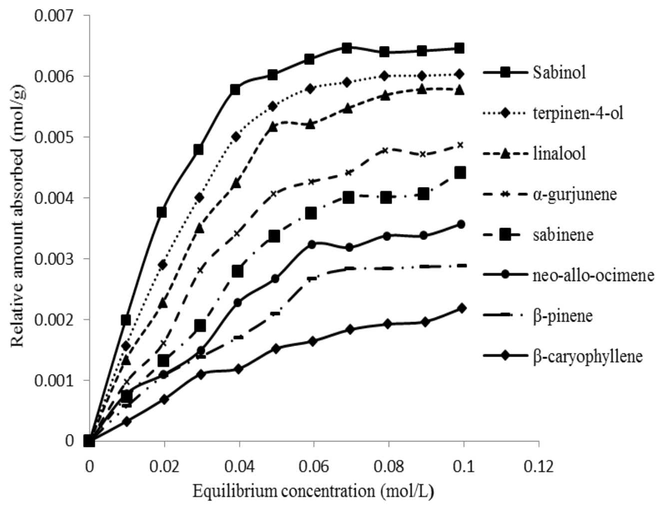


Figure 1: *Adsorption isotherms of the essential oil of Ocimum gratissimum by untreated montmorillonite (Mont-Na).*

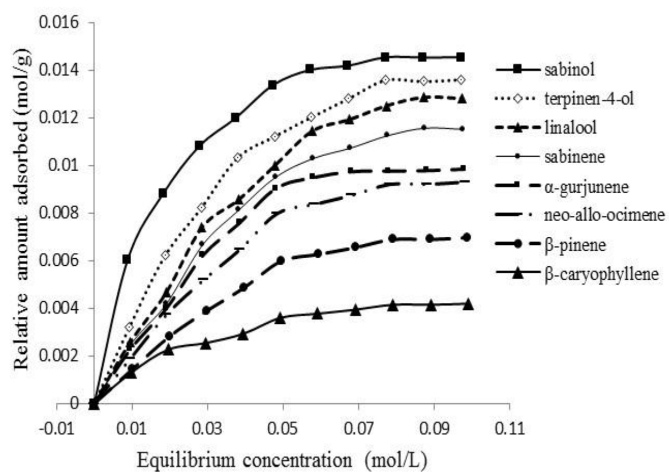


Figure 2: *Adsorption isotherms of the essential oil of Ocimum gratissimum by montmorillonite treated with saponins (Mont-Sa).*

The isotherms yield constants whose values express the affinity of terpenic compounds for the surface of adsorbent. For practical reasons, the adsorption capacity of an adsorbent should be as high as possible. Appling the Langmuir isotherm model, it was observed that the (a) value from the Langmuir isotherm was 2 times larger for Mont-Sa than for Mont-Na (Table 4). Furthermore, the values of RL calculated are between 0 and 1 indicating a so-called favorable adsorption [24]. The higher values of KF (applying the Freundlich model) indicated more sorption, so the results show that Mont-Sa offered a maximum adsorption capacity compared to Mont-Na adsorbent. In addition, KF values obtained are not significant for Mont-Na, it corresponds to few active sites in those adsorbents. While the KF of Mont-Sa are significant compared to Mont-Na and indicate an increase of more active sites in surface after modification with saponins molecules. Moreover, the other Freundlich constant: “n” an important parameter which is the function of the adsorption strength can also give information about the type of adsorption. It firstly indicated favorable adsorption of terpenic compounds by Mont-Na and Mont-Sa with values obtained: 1.24 to 2.70. The values of n >1 corresponds to a physical adsorption mechanism which is beneficial for the present process. It can be concluded that, the use of saponins treatment used is a good method to enhance the adsorption capacity of montmorillonite clay.

Table 5. *Freundlich and Langmuir Parameters*

Adsorbent	compound	Langmuir				Freundlich		
		a	b	RL	r^2	KF	n	r^2
Mont-Na	sabinol	0.37±0.08	0.02±0.00	0.41	0.97	0.02±00	2.10±0.13	0.87
	terpinen-4-ol	0.25±0.05	0.03±0.00	0.45	0.94	0.03±00	1.75±0.11	0.91
	linalool	0.18±0.01	0.05±0.01	0.42	0.95	0.03±00	1.55±0.05	0.94
	α-gurjunene	0.12±0.00	0.07±0.01	0.43	0.9	0.02±00	1.39±0.14	0.95
	sabinene	0.08±0.01	0.11±0.03	0.42	0.82	0.07±00	1.24±0.19	0.97
	neo-allo-ocimene	0.07±0.00	0.09±0.02	0.46	0.84	0.02±00	1.38±0.22	0.96
	β-pinene	0.06±0.01	0.08±0.01	0.47	0.9	0.01±00	1.37±0.13	0.97
	βcaryophyllen	0.04±0.00	0.11±0.00	0.44	0.9	005±00	1.26±0.16	0.97
Mont-Sa	sabinol	1.07±0.12	0.01±0.00	0.42	0.99	0.03±00	2.70±0.15	0.94
	terpinen-4-ol	0.47±0.06	0.04±0.00	0.41	0.98	0.06±00	1.65±0.11	0.94
	linalool	0.34±0.03	0.06±0.02	0.38	0.94	0.07±00	1.42±0.08	0.96
	α-gurjunene	0.32±0.06	0.06±0.01	0.4	0.94	0.06±00	1.46±0.12	0.95
	sabinene	0.33±0.00	0.04±0.01	0.46	0.93	0.05±00	1.56±0.19	0.92
	neo-allo-ocimene	0.26±0.04	0.05±0.02	0.47	0.94	0.05±00	1.45±0.11	0.95
	β-pinene	0.19±0.05	0.06±0.01	0.48	0.93	0.04±00	1.44±0.14	0.95
	β-caryophyllene	0.17±0.02	0.03±0.00	0.49	0.98	0.01±00	1.98±0.12	0.96

a: indicated effective adsorption (the theoretical monolayer capacity) (dm^3 cg^{-1}).
b: Langmuir equilibrium constant related to the affinity of bending sites ($cg\ g^{-1}$).
K_F: is the Freundlich constant related to the adsorption capacity ($cg\ g^{-1}$).
n is related to the adsorption intensity.

3.4. Formulations remnant effect

Each formulation previously mixed with grain at day 1 and infested 7, 14, 21, 28, 35 and 42 days later, was found to be active on C. subinnotatus for a longer period than the same formulation mixed with voandzou and tested immediately. As shown in Figure 3, for both essential oil formulations and Malagrain, insecticidal activity progressively decreased in the order: synthetic insecticide formulation (Malagrain)> Mont-Sa-EO> Mont-Na-EO (p<0.05).). It is also observed in this figure that the formulation and the time significantly influence the mortality rate of the insects brought into contact with the various formulations. The decrease in the mortality rate of C. subinnotatus over time reflects a decrease in the insecticidal activity of the powder formulations previously sprinkled on voandzou. At the end of the 42th day, Mont-Na-EO induced less than 15% of C. subinnotatus mortality, while Mont-Sa-EO still induced 70% of mortality. These remnant bioassay results indicated that insecticidal effect of O. gratissimum essential oil could persisted for about 60 days after its adsorption on Mont-Na. These remnant bioassay results indicated that insecticidal effect of O. gratissimum essential oil could persisted for about 60 days after its adsorption on Mont-Na. More ever, the high mortality and the persistence could be one of the effects of saponin which has shown a toxicity against beans weevil Acanthscelides obtectus [25]. Treatments of clay by saponin which are natural surfactants improve the insecticidal activity of O. gratissimum. These results are comparable to those of Nguemchouin et al. [6], which found that the Mont-Na-EO formulation completely lost its insecticidal activity after 45 days and the formulations obtained with synthetic surfactant-modified clays after 80 days. The poor retention of activity by the Mont-Na-EO formulation was a consequence of less terpenic components being adsorbed initially, and a higher release rate of these components, compared to Mont-Sa-EO. This rapid loss of insecticidal activity can also be explained by the fact that the terpenic compounds have an almost zero interaction with the Mont-Na, which would facilitate the volatilization of these compounds. Since many terpenic compounds are oxygenated, this has a very low affinity with Mont-Na except that the latter are responsible for the effectiveness of the insecticidal activity on insects such as C. Subinnotatus. Concerning the Mont-Sa-EO formulation, the latter formulation was benefited firstly by the capacity for hydrogen bonds to form between the clay adsorbent and oxygenated terpenic compounds, which are the majors compounds in O. gratissimum, and secondly the interactions Van-der-waals to be formed between the terpenic compounds and the heteroatoms contained in glycosidic functions of the sugars contained in the structure of the saponins. In addition to the advantages mentioned above, the effectiveness of the Mont-Sa-EO formulation can also be explained by the fact that the quantity of terpenic compounds adsorbed is greater relative to the amount adsorbed by Mont-Na thanks to the structures of the new Molecules present in Mont-Sa. The insecticidal activity of crude saponins is not to be overlooked in the Mont-Sa-EO formulation since it has been demonstrated in the literature that saponins possess insecticidal properties. This was verified and Mont-Sa was found to have a mortality rate higher than that of Mont-Na. It can therefore be argued that there is an association of the insecticidal activities of Mont-Sa and the terpenic compounds.

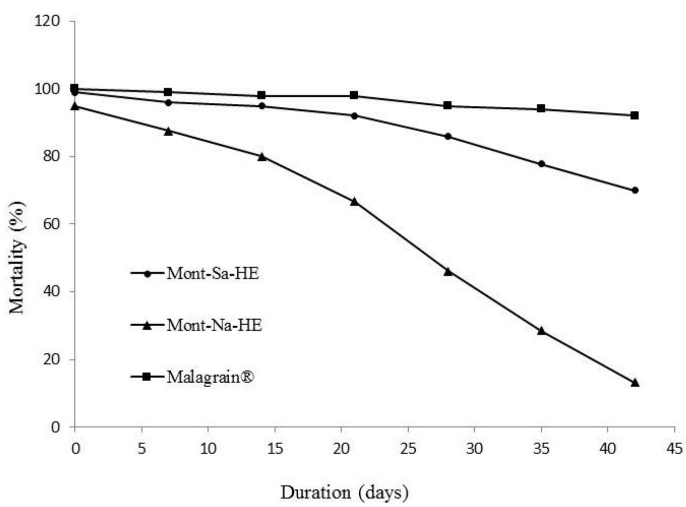


Figure 3: *Insecticidal effect of formulations previously mixed with voandzou.*

Table 5. *ST_{50} and ST_{90} values (days) of formulations based on O. gratissimum essential oil adsorbed on Mont-Na or Mont-Sa, and Malagrain® against C. subinnotatus.*

Formulations	ST_{50}^a	ST_{90}^a	Slope ± SE^b	r^2
Mont-Na-EO	18.62	6.28	-1.32±0.22	0.67
Mont-Sa-EO	432.31	9.30	-0.76±0.13	0.87
Malagrain®	1563.86	24.85	-0.71±0.09	0.75

[a]: Number of days at which formulation could induce 50% or 90% mortality.
[b]: Slope of mortality-day regression line.

According to Jaya et al. [26], the biological activity in some plants may be due to synergistic effects of different active principles leading to different mode of action during their pesticide action, which in the present study could be the synergy effect of terpenics components and saponins. For all formulations tested, the previous storage or holding days (legal days) at which each formulation could induce 90% or 50% mortality of C. subinnotatus (ST90 or ST50) (i.e. corresponding to a loss of insecticidal potency of about 10% and 50% of mortality) was also determined by Probit analysis using a logarithmic transformation of days (Table 5). It was found that formulations with grains conserved in boxes lost 50% of their insecticidal potency after about 18.62 days, 432.31 days and 1563.86 days for formulations with unmodified clay, modified clay and finally Malagrain. These results are similar to those of Nguemchouin et al. [6] which found that the ST50 of the Mont-Na formulation was 519.25 days and that of the synthetic surfactant treated clay was 508.87 days.

4 CONCLUSION

The present study is a contribution in research of environmentally safe ways of pest management through use of a formulation based on modified clay with natural surfactants (saponins) and essential oil. The treatment of the clay using the saponins molecules exhibited the highest adsorption capacity of clay treated than untreated clay. The adsorption of the terpenic compounds by clay treated with saponins molecules was affected by the polarity of terpenic compound; sabinol linalool and terpinen-4ol were adsorbed in large amounts than other monoterpene hydrocarbons. The Freundlich and Langmuir isotherms were found to be applicable for the adsorption equilibrium data of terpenic compounds on treated and untreated clays. Treatment of clay with saponins is a good method to enhance the remnant effect of bioinsecticide obtained by adsorption of essential oil on clays. The use of bioinsecticides based clays treated with saponins and essential oil could become very important elements of integrated pest management strategies, potentially because they are very common, easy to use and environmentally friendly.

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