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The Effective Insecticidal Activity of the two Extracts Ethyl Acetate and Hexan of *Trichilia gilgiana* against *Sitophilus zeamaïs*

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Abstract

Post-harvest losses are recognized to be one of the critical constraints upon food security among farmers of poor resource in Africa.The use of botanical pesticides in pest management during storage against insects is often encouraged because synthetic insecticides produce multiple side-effects on human health and environment. Insecticidal activity of the hexane, dichloromethane, ethyl acetate and methanol extracts of bark of *Trichilia gilgiana* was tested on *Sitophilus zeamaïs*, *Tribolium castaneum* and *Rhyzopertha dominica*. The mortality rate was measured variable. The results of analysis showed that the mortality rate has a very highly significant variation following extracts, doses, insects and time considered ($P < 0.001$). Extracts with ethyl acetate and hexane of *Trichilia gilgiana* are effective against *S. zeamais* at the highest doses (1 g/ 10 mL and 0.5 g/10 mL of solvent). These effective extracts were characterized by gas chromatography coupled with mass spectrometry. Molecules such as 2-Oxazalidone; thiocyanic acid; Methanethioamide, N,N-dimethyl; 2-Coumaranone and other were characterizated. These results may consolidate traditional use of *Trichilia gilgiana* in pest management.

Keywords: control, stored grains, indigenous plant, *Trichilia gilgiana*

1. Introduction

Several insects are involved in postharvest and storage, but beetles are also insects that cause not insignificant economic damage in postharvest (Delobel, 1993). Laboratory evaluation showed that when maize was unprotected, damages and losses due to *Sitophilus zeamais* (Motschulsky) reached 20% and 40%, respectively, in four and eight months of storage (Penning de Vries, 2001; Gueye et al., 2012). *S. zeamaïs* can also infest wheat, rice, barley, sorghum, millet, cassava, etc. (Delobel, 1993). One of the consequences of *S. zeamaïs* damage is the development of *Aspergillus* that secretes mycotoxins called aflatoxins and is the most powerful carcinogens known in humans as in animals (Abate et al., 2000; Coombs et al., 1997). *T. castaneum* can infest wheat, corn, barley, sorghum, millet, cassava, yams, peanuts, cotton, castor, cocoa, etc. (Delobel, 1993). *R. dominica* tackles a wide range of stored products, including cereals, wheat, barley, sorghum, rice, etc. (Delobel, 1993; CABI, 2010).

To control *S. zeamaïs*, *T. castaneum* and *R. dominica,* synthetic insecticides remains the primary means for controlling economical damage to crops, but this practice has come under scrutiny as it may pose potential oncogenic risks (Williams et al., 2012). However, *S. zeamaïs*, *T. castaneum* and *R. dominica* have developed resistance to insecticides from different chemical classes including organophosphates, carbamates, synthetic

pyrethroids, and to some newer chemistry insecticides (Regnault-Roger et al., 2008; Ladang et al., 2008; Mahroof et al., 2010).

Bioinsecticidal control of postharvest insects, especially using indigenous plants, has been studied extensively and developed commercially as an alternative to chemical treatments.

Further, bioinsecticidal control is potentially safer and can have reduced impacts on the environment (Markussen. & Kristensen, 2010). Inferences from indigenous traditional practices have uncovered plant chemicals that are useful in pest management, i.e., *Nicotiana tabacum* (Saxena, 1992), *Pistacia lentiscus* (Bachrouch et al., 2010), *Boscia senegalensis* (Gueye et al., 2013), *Azadirachta indica* (Taylor, 2005), *Trichilia martiana* (Saxena et al., 1992), *Drypetes gossweileri* (Aba- Toumnou et al., 2013), *Afrostyrax lepidophyllus* (Aba- Toumnou et al., 2014). In this context, an application of raw or extracts of plants may be a promising alternative for management of insect pests of crops.

Trichilia gilgiana is used in pest management of storage pests at Central African Republic (Lucie et al., 2012a). The objective of this study was to investigate insecticidal effects of extract of bark powder of *T. gilgiana* used by farmers in pest management in Central Africa Republic. Chemical investigate is made to characterize potential chemical compounds.

2.Material and methods

2.1 Plant Material, Method of Extraction and Bioassays

2.1.1 Plant Material

The bark of *T. gilgiana* was collected from Boukoko in Central African Republic in 2012 and authenticated by the researchers of the forestry Ministry of CAR and botanical Professors of University of Bangui (CAR) respectively. *T. gilgiana* bark was dried in the shade and grounder to powder after.

2.1.2 Solvents and Method of Extraction

- n-Hexan (Reag.USP, Ph.Eur.) PA-ACS; Mininum assay (G.C.) : 99,0%; Identity : IR p/t.; Density at 20/20 : 0,659-0,663; Refractive index $n^{20/D}$ 1,375-1,376; Distillation range (>95%dist.) : 67-69°C;

- Dichloromethan stabilized with amylene PA-ACS-ISO; Mininum assay (G.C.) : 99,5%; Identity : IR p/t.; Density at 20/4 : 1,323-1,325;

- Ethyl Acetate PA-ACS-ISO; Mininum assay (G.C.) : 99,5%; Identity : IR p/t.; Density at 20/4 : 0,9000-0,902;

- Mathanol (Reag.USP, Ph.Eur.) PA-ACS-ISO; Mininum assay (G.C.): 99, 8%; Identity: IR p/t.; Density at 20/4: 0,791-0,792.

100 g of powder of *T. gilgiana* has been dissolved in 500 milliliters of Hexan for 5 days in the laboratory. The final hexane extract was recovered after concentration on evaporator at 30°C. The residue of hexane has been dried in air for 2 days and macerated in Dichloromethane. Similarly, the exhausted residue of Dichloromethane has been macerated in ethyl acetate whereas the spent residue in ethyl acetate has been macerated in methanol.

2.1.3 Insects Rearing

Adults of *S. zeamaïs, T. castaneum* and *R. dominica* were respectively collected from corn, millet and sorghum in farms and were reared respectively on 100g of corn , millet and sorghum in laboratory at 27 ± 2 °C

2.1.4 Bioassays

Bioassays tests were conducted at the Regional Centre for Ecotoxicology Studies and Environment Security (CERES-Locustox) in Senegal, West Africa. The aim of bioassays was to identify effective extracts of *T. gilgiana* against *S. zeamaïs*, *T. castaneum* and *R. dominica*. Every extract has six doses (1, 0.5, 0.25, 0.125, 0.62 and 0.03 g/10 mL of solvent) and every dose had been tested with five repetitions.

Theses doses were obtained by cascading dilution (Kéïta et al., 2001). Experimental units were Petrie glass of 90 cm of diameter containing 20 g of grain (maize, millet or sorghum), infested with 25 insects and applied 1 mL of different doses. Insects are put in contact with treaded grains after having evaporated the solvent for 5 min from the hexane and dichloromethane extracts and 25 min from ethyl acetate and methanol extracts. Experimental units were randomly divided into four groups according to the treatments (Abbott, 1925). Insects groups of control were exposed to pure solvent in the same laboratory conditions at 27 °C ± 2 °C and 70% ± 10% r.h.. Dead insects were removed using forceps every 5 d during 35 d. Corrected mortality (CM) is obtained by Abbott formula: CM = [(TM – CM)/(100 – CMt)]100 where, TM = treated mortality, CM = control mortality (Kéïta et al., 2001; Abbott, 1925).

2.1.5 Statistical Analysis

Corrected mortality is submitted to variance analysis, assigned model with four factors (plants, insects, doses and time) (Benelli et al., 2012). Mortality was transformed to $y = 2\arcsin(x/n)$ 0.5 and emerged insects to $y = (x/n)0.5$ (x = mortality rate, n = size of population, $n = 2519$) to normalize the population and stabilize the variance. General linear model in Minitab 14 was used for statistical analysis.

2.2 Chemistry Analysis

2.2.1 Materials and Conditions

Silica Gel, spe 7086-06; colonne HP5MS: 30 mm 0.250 mm sur 0.25 µL; detector (190-400 nm); elution solvent: ethyle acetate; temperature: 325 °C; Gaz: Heluim; volume d'injection: 5 µL; GCMS 5973 Agilent.

2.2.2 Method

Gas chromatography coupled with a mass spectrometer was used in chemical characterization (Gohlke, 1993). 0.5 g of each extract was dissolved in 25 mL of the ethyl acetate or hexane. The mixture was purified with a silica column and then recovered and evaporated with rotavaporator. The concentrate was recovered with 2 mL of ethyl acetate in chromatographic tubes. 5 µL of the extract was vaporized into an injection chamber in the column heading. The oven temperature program was initiated at 50 °C, held for 2 min then raised first at 100 °C, and raised in a second ramp at 3.5 °C/min to 300 °C. Temperature influences the retention time, it allows to further separate the mixture of compounds. At the end of the column, the compounds are identified by an electron impact detector, coupled with a mass spectrometer. The mass spectral library and based Agilent 5973 GCMS data were used to characterize molecules.

3. Results

3.1 Results of Bioassays Tests

The results of analysis of variance (ANOVA) on the insecticidal effect of extracts of *T. gilgiana* against *S. zeamais*, *T. castaneum* and *R. dominica* showed that the mortality rate was a very highly significant variation according to the dose, the insect and the time on the one part and according to the plant, insect and dose on the other part ($P < 0.001$). Moreover it is only the effect of the interaction plant insects dose which was not significant ($P > 0.05$), and implies that the insecticidal effect depends on the nature of the plant, the dose and the insect (Table 1).

Table 1. Variance analysis of studied factors of *Trichilia gilgiana*

Factors/standard	Standard of signification	
	Effect on mortality	Effect on emerged insects
Extracts/4	***	***
Doses/6	***	***
Insects/2	***	***
Times/7	***	0
Extracts x doses/24	0	**
Extract x insects/12	***	***
Extracts x times/28	***	***
Doses x insects/18	***	**
Doses x times/42	***	0
Insects x times/21	***	***
Extracts x doses x insects/72	*	0
Doses x insects x times/126	***	***
Extracts x insects x times/84	**	0

*** Very highly significant effect ($P < 0.001$); ** highly significant ($P < 0.01$); * significant ($P \leq [0.01, 0.05]$); 0: no effect.

significant ($P > 0.05$).

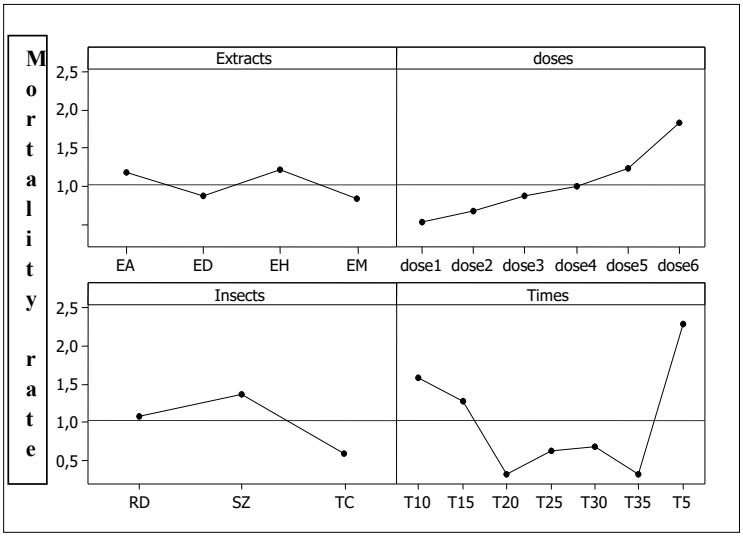

Figure 1. Main effects plot for rate of mortality with many extracts of *Trichilia gilgiana*

EH = hexane extract; ED = dichloromethane extract; EA = acetyl acetate extract; EM = methanol extract; RD = *Rhyzopertha dominica*; SZ = *Sitophilus zeamaïs*; TC = *Tribolium castaneum*; T*x* = *x* days after treatment.

Figure 1 shows the evolution of treatment based extracts, dose, time and insects. Ethyl acetate and hexan extracts of *T. gilgiana* are effective against *S. zeamais* and *R. dominica* at high doses (1 g/10 mL and 0.5 g/10 mL of solvent). The mortality curve over time shows that at T5, T10 and T15, the mortality rate is high. It implies that the efficacy of the extracts with ethyl acetate and hexan is limited in time.

Table 2. Chemical characterization of hexane and ethyle acetate extracts of *Trichilia gilgiana*

TR (min)	*Chemical compounds*
	Hexan extract of Trichilia gilgiana
1.201	Methylene Chloride
1.750	Methanethioamide, N, N-dimethyl; 2,3-Dihydrothiophene 1,1-dioxide
1.842	2-Oxazalidone; Thiocyanic acid, ethyl ester; 2-[2-[2(-Methoxyethoxy) ethoxyl et hoxyl] ethyl acetate
1.916	Butane, 1-ethoxy-
2.076	d-Glycer1-gluco-heptose; Octanoic acid, hexyl ester; 6-Desoxy-1-gulitol
2.379	Octane ; Heptane, 2,4-dimethyl-
6.768	Hexadecane; Decane, 2, 3,6-trimethyl-; Silane; trichlorooctadecyl-
7.054	Decane, 2, 3,7-trimethyl-; Dodecane, 1-iodo-; Hexadecane, 2,6, 10,14-tetramethyl-
7.488	Tetradecane
8.123	Heneicosane ; Heptadecane ; Hexadecane
8.306	Butylated Hydroxytoluene
8.426	Dodecane ; Tridecane
8.500	Dodecane ; 2-Bromo dodecane
8.569	Hexadecane ; Tridecane ; Heneicosane
8.740	2-Bromotetradecane ; Heptadecane ; Hexadecane, 2, 6,10, 14-tetramethyl-
8.849	Hexadecane
9.694	Heptadecane
9.832	Eicosane, 2-methyl- ; Heptacosane ; Heneicosane
10.077	2,6-Diisopropylnaphthalene ; 1-Acetyl-4, 6,8-trimethylazulene
10.134	Naphthalene, 2-butyl-3-hexyl-1, 2, 3,4-tetrahydro- ; Naphthalene, 6-butyl-7-hexyl-1, 2, 3,4-tetrahydro-
	6-Propyl-7H-benz [de] anthracen-7-on

10.249	Heptadecane; Octadecane, 1-iodo-; Eicosane, 10-methyl-
10.489	Tetratriacontane ; Pentadecane, 2, 6 ,10-trimethyl- ; Pentacosane
12.186	Octacosane ; Pentacosane ; Tetratriacontane
12.289	Octadecane; Silane, [1,4-phenylenebis (oxy)] bis [trimethyl-Heptadecane
12.724	Pentacosane ; Docosane ; Octadecane, 2-methyl-
13.055	Hexadecanoic acid, ethyl ester
15.678	Octacosane ; Hentriacontane ; Heneicosane
	Ethyle acetat extract of Trichilia gilgiana
1.213	Methylene Chloride
1.739	Methanethioamide, N, N-dimethyl; 2,3-Dihydrothiophene 1,1-dioxide
1.807	Formic acid hydrazide; Ethyne, chloro-; Thiirane
2.007	Hydrazine, 1,2-dimethyl-; Acetic acid; Thiirane
3.625	Dimethyl sulfone ; 1,4-Cyclohexadiene, 1-methyl-
5.962	Cyclotrisiloxane, hexamethyl- ; 1,2-Benzisothiazol-3-amine
6.265	Methysulfinyl; Disulfide, methyl; Methanamine, N-methoxy
6.528	2-Coumaranone; Benzeneacetic acid, 2-hydroxy-, methyl ester
6.734	3,3-Diisopropoxy-1, 1, 1, 5,5, 5- hexamethyltrisiloxane ; 7-Chloro-4-methoxy-3-methylquinoline ; Propiophenone, 2-(trimethylsiloxy)-
7.043	2-Methoxy-4-vinylphenol ; Ethanone, 1-(3-methoxyphenyl)-
7.128	3,27-Dioxa-2,28-disilanonacosane, 2, 2,4, 28,28-pentamethyl-; Silane, ethenyldimethoxymethyl-3-Dimethylsilyloxy-6-ethyloctane
7.248	Dimethyl sulfoxide ; 5-Methoxybenzofurazan, 1-oxide
7.363	3-5-Dihydrotoluene ; 1,4-Benzenediol, 2-methyl-
7.494	Tetradecane ; Heptadecane, 8-methyl- ; Tetradecane
7.523	2H-1-Benzopyran-2-one, 3, 4-dihyro; 2(3H)-Benzofuranone, 3-methyl-
7.580	Benzaldehyde, 3-hydroxy-4-methoxy- ; Vanillin
7.688	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis); Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans ; Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis
7.814	Naphthalene,1, 2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methyllene-1-(1-methylethyl)-(1.alpha., 4a.alpha.,8a.alpha.)-; Cyclohexene, 6-ethenyl-6-methyl-1-(1-methyllethyl)-3- (1-methylethylidene)-, (S)-
	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methyllethyl)-, [S-(E, E)]
7.991	Silane, methoxytripropyl-; Silane, ethoxytrimethyl-; Octadecanoic acid, 3-hydroxy-, methyl ester
8.123	Hexadecane ; Pentadecane
8.254	Phenol, 2,4-bis (1,1-dimethylethyl) ; Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters
8.369	2-Propanone, 1-cyclopentyl-3-ethoxy-; 5-Amino-1-ethylpyrazole; Methanone, dicyclohexyl-
8.414	5, 5, 8a-Trimethyle-3, 4,6,7,8,8a-hexahydro-2H-chromene; 1,3-Benzenediol, 5-pentyl-
8.666	6-Methoxycoumaran-7-ol-3-one; Ethanone ; 1-(3,4-dimethoxyphenyl)-; Ethanone ; 1-(2,5-dimethoxyphenyl)-
8.711	Fumaric acid, ethyl 2- fluorophenyl ester; 4-Amino-2,6-dihydroxypyrimidine; Isopropylphoshonic acid, fluoroanhydride, 4-methylcyclohexyl ester
8.877	6H-Purin-6-one, 2-amino-1,7-dihydro-; Thiazolo [5,4-d] pyrimidine, 5-methyl-; Benzaldehyde, 3-methoxy-, oxime
8.957	Phenol, 3, 4,5-trimethoxy; 1-1'-Biphenyl, 2-methoxy-

3.2 Results of Chemistry Analysis

Chemical compounds were characterized using data base of Agilent database (5973) in the range of 50 m/z to 550 m/z (Figure 2). Hexane extract of *T. gilgiana* showed the presence of large peaks, additioned to other minor peaks. The signals for the main peak about 1.750 min (Figure 2) match the library mass spectra to 2-Oxazalidone and thiocyanic acid.

GC-MS profile of the Chemical compounds contained in ethyl acetat extract showed the presence of large peaks including other minor peaks.The signal for the peak about 1.739 min match to the library mass spectra to Methanethioamide, N,N-dimethyl (Figure 3).

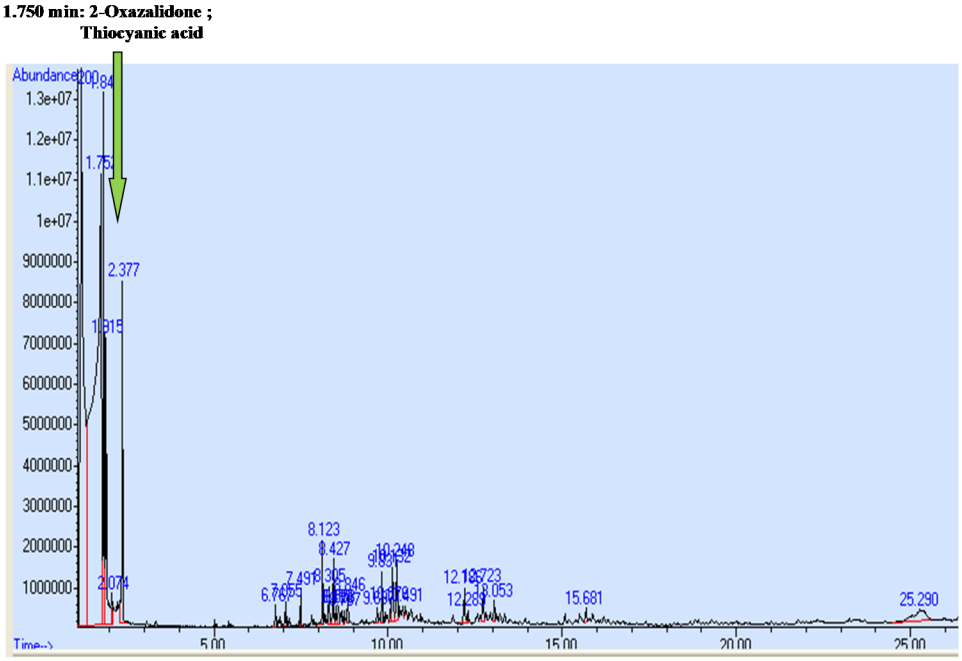

Figure 2. Chromatographic profile GC-MS of hexan extract of *Trichilia gilgiana*

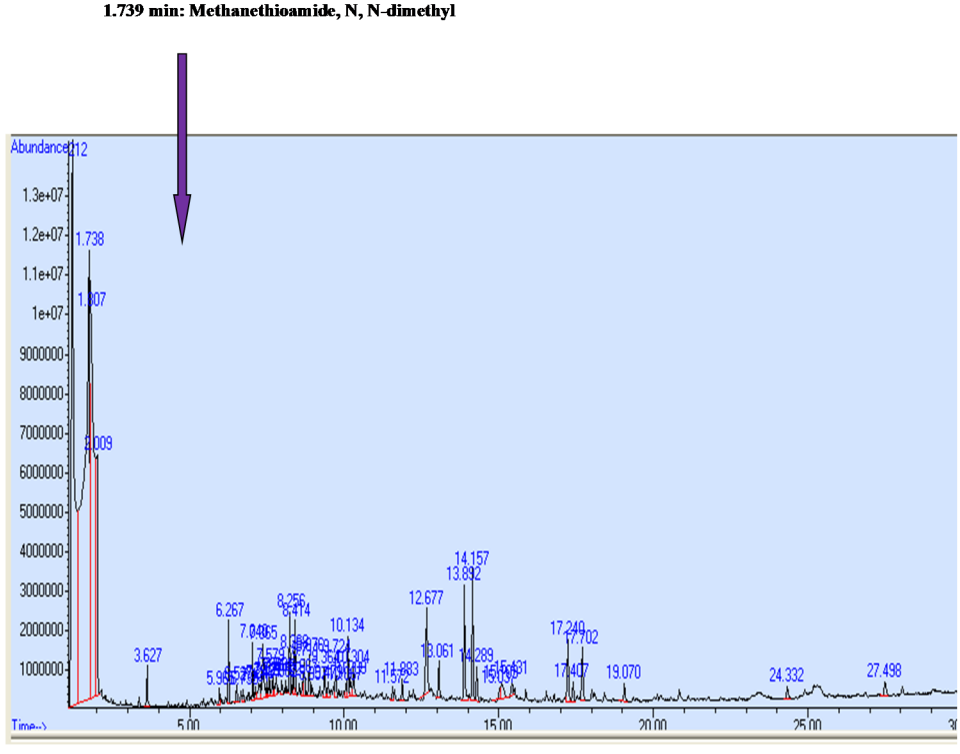

Figure 3. Chromatographic profile GC-MS of ethyl acetate extract of *Trichilia gilgiana*

4. Discussion

Insecticidal activity of four extracts of *T. gilgiana* was tested on *S. zeamais*, *T. castaneum* and *R. dominica*. Hexan and ethyl acetate extracts of *T. gilgiana* are effective against *S. zeamais*. Previously, the authors have proved insecticidal activity of bark of *T. gilgiana* against *S. zeamais* and *T. castaneum* (Lucie et al., 2012b). Authors reported that powder of bark of *T. gilgiana* presented respectively on *S. zeamais* and *T. castaneum* the LC50 of 5.60 g/100 g and 5.86 g/100 g of corn or millet. Bioassays of hexan and ethyle acetate confirm preliminary insecticidal activity of *T. gilgiana.*

In general plants belonging to species families recognized for their pesticides action are widely used by farmers. The family of Meliaceae including 14 genuses (Lemmens, 2008) which one is *Trichilia* was largely counted. This family offers opportunities to isolate new compounds of insecticide property (Wheeler, 2000). The genus *Trichilia* mainly on the fruits of *Trichilia martiana* showed that this species contains two limonoids and a furan having an unsaturated chain c16 to c22 (Wheeler, 2000), similar to the structure of insecticidal compounds of avocado. Other studies (Satasook, 1992; Ewete et al., 1996b) on Asian Meliaceae have isolated the rocaglamide, a benzofuran having a comparable activity to that of azadirachtin, a natural insecticide.

Three families of secondary metabolites (alkaloids, tannins and terpenes) are often used by plants to defend themselves against external aggression (Regnault-Roger, 2008; Berenbaum, 1983; Feeny, 1976; Hagerman & Butler, 1989).

Hexane extract reveals some molecules such as Thiocyanic acid, Methanethioamide, N, N-dimethyl, etc.

Thiocyanic acid and Methanethioamide, N, N-dimethyl are part of glucosinolates and are known for their involvement in the defense system of the plant against external aggression (Bodnaryk, 1991; Müller et al., 2001; Gueye, 2011).

The remaining molecule then quickly converts an Thiocyanic acid are the active substances that serve as defense for the plant (Smolinska, 2003; Gueye, 2011). Insecticidal activity of methyl isothiocyanate in *Boscia senegalensis* is proved on *Caryedon serratus*, *S. zeamais*, *Prostephanus truncatus*, *Callosobruchus maculates* and *T. castaneum* by enymatical degradation of glucocapparin in presence of water (Gueye, 2011). These results revealed the presence of chemical compounds belong to the group of glucosinolates. It is likely that the glucosinolates can play a key role in the insecticidal activity against *S. zeamais* in this study.

5. Conclusions

The objective of this study was to investigate insecticidal effects of extract of bark powder of *T. gilgiana* used by farmers in pest management in Central Africa Republic.The results of analysis showed that the mortality rate has a very highly significant variation following extracts, doses, insects and time considered ($P < 0.001$). Extracts with ethyl acetate and hexane of *Trichilia gilgiana* are effective against *S. zeamais* at the highest doses (1 g/ 10 mL and 0.5 g/10 mL of solvent). These effective extracts (ethyl acetate and hexane) were characterized by gas chromatography coupled with mass spectrometry. Molecules such as 2-Oxazalidone; thiocyanic acid; Methanethioamide, N,N-dimethyl; 2-Coumaranone and other were characterizated in effective extracts (ethyl acetate and hexane). These results may consolidate traditional use of *Trichilia gilgiana* in pest management.

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