

**POWDERY FORMULATION OF ESSENTIAL OILS FOR THE CONTROL OF RICE
WEEVIL *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE)**

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ABSTRACT

In spite of the effectiveness of the essential oils and flour of leguminous seeds against insect attacks, they are less attractive to producers that continue to use chemicals that have potential adverse effects on consumers and the ecosystems through pollution. The present research was carried out to evaluate insecticidal properties of powdery formulations of essential oils of 3 plants: leaves of *Eucalyptus citriodora* (Myrtaceae), dried fruits of *Xylopia aethiopica* (Dunal) A. Richard (Annonaceae) and the roots of *Echinops giganteus* var. *lelyi* C.D Adams (Asteraceae) with seeds of black bean on *Sitophilus oryzae*. The toxicity by contact-inhalation of essential oils, alone or in combination, in different concentrations is evaluated against young adults of *S. oryzae* with two weeks old after 24 hours. For powdery formulation, the test of contact-ingestion and inhalation is evaluated in different doses each week during 7 weeks. The research carried out reveals that *X. aethiopica* is the most active on *S. oryzae* with the lethal doses 50 and 90 respectively 37.1 and 57 ppm. In combination, *E. giganteus* which alone is not insecticidal, enhance significantly the insecticidal efficiency of the 2 other essential oils. The most insecticidal powdery formulation is that of *E. citriodora* with 100% of mortality two weeks after the application. The present work gives evidence of the insecticidal efficiency of a powdery formulation in the protection of stored cereals from weevil attacks. This method potentially has no adverse effect on consumers and on the environment at the doses used and could be developed and popularized.

Keywords: Cereals; Essentials Oils; Black Bean; Powdery Formulation; *Sitophilus oryzae*.

1. INTRODUCTION

Many pests of stored products are Coleopterans and the most destructive tropical species for cereals belong to the genus *Sitophilus* and *Tribolium* (Tamgno, 2016, Tamgno and Ngamo Tinkeu, 2013; Nukenine et al, 2011, Ngamo Tinkeu and Hance; 2007). Damage due to *Sitophilus* on cereals and others cereal processed products, are mostly impurities and stains which would be able of poisoning consumers (Tamgno and Ngamo Tinkeu, 2013). The control of pests in stored cereal grains, by the application of chemicals which is a common strategy for post-harvest loss avoidance (Ngamo Tinkeu et al, 2016; Tamgno, 2016), had led to the occurrence of many

problems like the pollution of environment, toxicity to human being, emergence of resistant pests strains and many others damages (Idrissi et al, 2010, Regnault-Roger et al, 2008, Glitho et al, 2008). The liberalization of this sector of chemicals has permitted the circulation of the illicit and obsolete synthetic insecticides (Tamgno, 2016, Ngamo Tinkeu et al, 2016). These synthetic pesticides are expensive for the small-scale farmers and pose potential risks due to the lack of technical knowledge on their safe use.

Due to the hazardous effects of chemicals on the environment, human health and useful organisms, the Montreal Protocol recommended their withdrawal from the market in 2010. About 10 years after, few alternative products are proposed in post-harvest grain protection by science to ensure this withdrawal. Recent researches have been undertaken in order to minimize the agro-environmental pollution and health hazards caused by them and to replace them with other methods (Tamgno et Ngamo Tinkeu, 2018, Ngamo Tinkeu et al, 2016, Mebarkia et al, 2012, Folefack et al, 2013, Nguemtchouin Mbouga, 2012, Nukenine et al, 2011, Karimi et al, 2010, Goudoum et al, 2010, Faye, 2010). Hence, there is need to explore new effective biological pests control methods like insecticides based on aromatic plants by essential oils and entomotoxic proteins obtained from legumes which can confer protection to plants against insect pests (Tamgno and Ngamo Tinkeu, 2018, Ngamo Tinkeu et al, 2016, Tamgno, 2016, Nguemtchouin Mbouga, 2012, Goudoum, 2010, Ngamo Tinkeu et al, 2007).

The most efficient crop protection method nowadays must be one which employs a consumer- and environmentally-friendly approach.

The use of protecting materials which are edible by human and other animals may be a good alternative in pest control. The bio assays on the insecticidal efficacy of crude essential oils of aromatic plants have been explored; but these insecticides are less attractive for producers because of their high volatilization and low persistence (Nguemtchouin Mbouga, 2012, Kouninki et al, 2007, Ngamo Tinkeu et al, 2007). For these reasons, there is therefore a need to formulate them as an easy handling chemical with better persistence. The use of flour of legume seeds that their insecticidal efficacy has been established like support of essential oils can ameliorate their persistence (Tamgno, 2016, Ngamo Tinkeu et al, 2016, Tamgno and Ngamo Tinkeu, 2014, Mebarkia et al, 2012, Fatimé, 2007).

The objective of this study was to determine the sensitivity of the rice weevil *Sitophilus oryzae* adults to the insecticidal properties of crude essential oils of *Eucalyptus citriodora* (Myrtaceae), *Echinos giganteus* (Asteraceae) and *Xylopi aethiopica* Dunal A. Rich (Annonaceae) in formulation with the flour of *Phaseolus vulgaris* by contact –inhalation and contact-ingestion.

2. MATERIALS AND METHODS

2.1. Insect used and its rearing

The millet weevil *Sitophilus oryzae* was used at adult stage. They were obtained from the Unity of Entomological Researches (UER) of the University of Ngaoundéré (Adamaoua-Cameroon) where a permanent rearing is kept in the dark in incubators at 27 ±2.1 °C. The millet weevil belonged to the strain collected in November 2003 in the granary of a peasant at Beka Hosséré

(Ngaoundéré). For the experiments, adults of *S. oryzae* used were two weeks old. Before being tested, these insects were starved for a 24 hours period.

2.2. Origin and preparation of the flour of seeds of *Phaseolus vulgaris*

The seeds of little black bean *Phaseolus vulgaris* coming from Bandjoun in West Region of Cameroon were reduced to flour in the UER. 500 g have been ground using a mill, type WARRING BLENDOR to obtain the flour. Flours obtained were sifted using a sieve, type RETSCH GmbH & Co D-42781, of mesh-size lower than 0.5 mm: the big particles were eliminated. The flour obtained was weighed using a SARTORIUS balance, type 126400 with precision of 0.01g. One part of flours was used directly for biological assays and the rest kept for the preparation with essential oils.

2.3. Aromatic plants collection and hydro distillation

Three parts of three aromatic plants were used for this study: leaves of *Eucalyptus citriodora* (Myrtaceae), dried fruits of *Xylopia aethiopica* (Dunal) A. Richard (Annonaceae) and the roots of *Echinops giganteus* var. *lelyi* C.D Adams (Asteraceae). Leaves of *E. citriodora* were collected in Onaref quarter in Ngaoundéré near the point referenced 14°24.145'E longitude, 07°39.214'N latitude Nord and the altitude 1375 m. Roots of *E. giganteus* were harvested in the guinean savannah of Bini-Dang, Ngaoundéré referenced by 13°32.272'E longitude, 07°24.330'N latitude and 1293 m altitude. The dried fruits of *X. aethiopica* were bought in Mbitom market (Adamaoua-Cameroon). These data were collected with a GPS Garmin Geko 301.

In laboratory before hydro distillation, the leaves were dried at shadow far from sunlight in the laboratory conditions during three days; the roots were washed and cut as fruits in pieces; each part was weighed. The extraction of essential oils of each aromatic plant was carried out in a Clevenger-type apparatus during 4 h. The essential oils obtained were put in the flasks and kept in the refrigerator at 4° C till their use in bioassays.

2.4. Formulation of *Xylopia aethiopica* and *Eucalyptus citriodora* essential oils with flour of *Phaseolus vulgaris* for contact-ingestion toxicity tests

The lethal doses 100 (LD₁₀₀) essential oils of *X. aethiopica* (80 µl) or *E. citriodora* (160 µl) were formulated as powder using various proportions (1 g, 5 g and 10 g) of black bean (BB) flour (*P. vulgaris*) according to a LD₁₀₀ of black bean (BB) flour (10 g) on *S. oryzae* (Tamgno, 2016, Tamgno and Ngamo Tinkeu, 2018). Six treatments were made: BB01X, BB05X and BB10X for *X. aethiopica* and BB01E, BB05E and BB10E for *E. citriodora*. After homogenizing with spatula, two aromatized flours were obtained: one with *X. aethiopica* and the other one with *E. citriodora*, and directly used for bioassays.

2.5. Biological tests

2.5.1. Evaluation of insecticidal efficiency of crude essential oils

The evaluation was done by contact-inhalation. A micropipette was used to remove 50 µl, 100 µl, 150 µl, 200 µl and 250 µl of each essential oil and diluted it in 2.5 ml acetone to formulate

insecticides. For each preparation, 0.5 ml was pumped and flowed regularly on a disk of filter paper (Wathmann n°1) of 9 cm diameter placed in a Petri dish. The dish was finally covered; a control with acetone alone, without essential oil was done. This permit to obtain the following concentrations: 125 ppm, 250 ppm, 375 ppm, 500 ppm and 625 ppm by dish. After this, 20 insects were introduced in each dish 4 min later. The dish was finally covered; a control with acetone alone, without essential oil was done. For each preparation, 4 replications were carried out. The amount of death insects was counted 24 h after the application.

2.5.2. Determination of lethal doses 50 and 90 (LD₅₀ and LD₉₀) of essential oils of *Xylopia aethiopica* and *Eucalyptus citriodora*

These experiments aimed to establish the lethal doses 50 and 90 (LD₅₀ and LD₉₀) of essential oils of *X. aethiopica* and *E. citriodora*. With a micropipette (Rainin Magnetic-assist), 8 volumes of *E. citriodora* 20, 40, 60, 80, 100, 120, 140 and 160 µl and 4 volumes of *X. aethiopica* 20, 40, 60 and 80 µl were pumped and each volume was diluted into 2.5 ml of acetone. For each preparation, 0.5 ml was pumped and flowed regularly on a disk of filter paper (Wathmann n°1) of 9 cm diameter placed in a Petri dish. After this, 20 insects were introduced in each dish 4 min later. The dish was finally covered; a control with acetone alone, without essential oil was done. For each preparation, 4 replications were carried out. The amount of death insects was counted 24 h after the application.

2.5.3. Toxicity of powdery formulation with the essential oils of *Xylopia aethiopica*, *Eucalyptus citriodora* and flour of *Phaseolus vulgaris*

In the pots in glass of 1800 ml of capacity containing 99, 95 and 90 g of sorghum grains, 1, 5 and 10 g of each aromatized flours were added respectively in sort that each pot had 100 g of content. The content of each pot was homogenized by stirring it 20 times using a spatula. Six treatments without control were made. The pots that contain only the sorghum grains constitute the control. 20 young adults of *S. oryzae* were introduced in these pots for rearing. After each week on 7 weeks, all the pots were checked; the amounts of dead adults were evaluated. For each preparation, 5 replications were made.

2.6. Statistical analysis

Data on mortalities of this insect considered were subjected to one-way variant analysis (ANOVA I) in the aim to determine the most insecticidal formulations of essential oils and black beans' flour. To complete this analysis, the Duncan's multiple range test (DMRT) was used to group together the mean values that are not significantly different.

3. RESULTS

3.1. Insecticidal efficacy of essential oils of *Xylopia aethiopica*, *Eucalyptus citriodora* and *Echinops giganteus* on *Sitophilus oryzae* alone and in equal combinations

In the control (0 ppm), no mortality is observed. Similarly, for all the concentrations tested, the essential oil of *E. giganteus* does not induce any mortality; this essential oil therefore has no insecticidal property on *S. oryzae* for the doses tested. On the other hand, the essential oils of *X.*

aethiopica and *E. citriodora* taken alone, show, to varying levels, insecticidal properties on *S. oryzae* (Table 1).

Table 1. Insecticidal activity of the three essential oils *Xylopi aethiopica*, *Eucalyptus citriodora* and *Echinops giganteus* and their combinations towards *Sitophilus oryzae*

Essential oils Doses (ppm)	<i>X. a</i>	<i>E. c</i>	<i>E. g</i>	<i>X. a</i> + <i>E. c</i>	<i>X. a</i> + <i>E. g</i>	<i>E. c</i> + <i>E. g</i>
0	0±0 ^f	0±0 ^f	0±0 ^f	0±0 ^f	0±0 ^f	0±0 ^f
125	55±12.2 ^c	52.5±10.4 ^c	0±0 ^f	0±0 ^f	40±10.4 ^{de}	31.25±4.8 ^e
250	100±0 ^a	83.75±17.0 ^{ab}	0±0 ^f	53.75±4.8 ^c	76.25±6.3 ^b	76.25±7.5 ^b
375	100±0 ^a	98.75±2.5 ^a	0±0 ^f	88.75 ^a	90±3 ^{ab}	78.75±4.8 ^{ab}
500	100±0 ^a	100±0 ^a	0±0 ^f	100±0 ^a	100±0 ^a	80±0 ^{ab}
625	100±0 ^a	100±0 ^a	0±0 ^f	100±0 ^a	100±0 ^a	100±0 ^a
F(108;35)			236.384***			

The mortality values followed by the same letter do not differ significantly, $p \leq 0.001$; Legends : *X. a* : *Xylopi aethiopica* ; *E. c*: *Eucalyptus citriodora* et *E. g*: *Echinops giganteus*

It appears that the effectiveness of insecticidal properties of essential oils varies depending on the plant species, the dose used and the combinations made. The results confirm the toxicity of the essential oils of *E. citriodora* and *X. aethiopica* alone, and that of the combination of *X. aethiopica*+ *E. giganteus*, *E. citriodora* + *X. aethiopica* and *E. citriodora* + *E. giganteus* on *S. oryzae*; but this toxicity varies according to the essential oils and their combinations.

At the 125 ppm dose, the ANOVA I shows there is a very highly significant difference between the mortalities induced by the essential oils of *E. citriodora* + *X. aethiopica* and *X. aethiopica* + *E. giganteus* on the one hand , and between the ones of *X. aethiopica* + *E. giganteus* and *E. citriodora* + *E. giganteus* on the other hand. Except this dose, a comparison by ANOVA I shows no significant difference between the different balanced combinations (Table 1). Although the mortalities induced by the 250 ppm to 625 ppm doses of the essential oils separately are high than those of the mixtures, the ANOVA I does not indicate any difference. As the control treatment, the mixture *E. citriodora* + *X. aethiopica* does not induce mortality at 125 ppm. The mixture *X. aethiopica* + *E. giganteus* is more effective than the mixture *E. citriodora*+ *X. aethiopica* which is also more insecticidal than *E. citriodora* + *E. giganteus*.

For all combinations made, it appears a negative interaction between the constituents of the essential oils put together. The phenomenon of synergy is not observed for *X. aethiopica* + *E. giganteus* and *E. citriodora* + *E. giganteus* mixtures; this suggests that the essential oils of *X. aethiopica* and *E. citriodora* would be very rich in antagonistic compounds. At doses 125 and 250 ppm, the essential oil of *X. aethiopica* induces respectively 55% and 100% of mortality whereas that of *E. citriodora* induces a mortality of 52% and 83.75%, the mortality of 100% is being obtained only from concentrations of 500 ppm. Although the Duncan test does not show a significant difference at 1 % for the doses used between these two products; these results show that the essential oil of *X. aethiopica* has a stronger insecticidal potential than that of *E. citriodora* (Table 1).

3.2. Expression of the insecticidal efficiency of the two essential oils towards *Sitophilus oryzae* by the estimation of their LD₅₀ and LD₉₀

The essential oil of *X. aethiopica* is more effective with LD₅₀ and LD₉₀ respectively equal to 37.1 and 53 ppm than that of *E. citriodora* with LD₅₀ and LD₉₀ respectively of 57.4 and 112.8 (Table 2).

Table 2. Expression of the insecticidal efficiency of the essential oils of *Eucalyptus citriodora* and *Xylopiya aethiopica* towards *Sitophilus oryzae* by the estimation of their LD₅₀ and LD₉₀

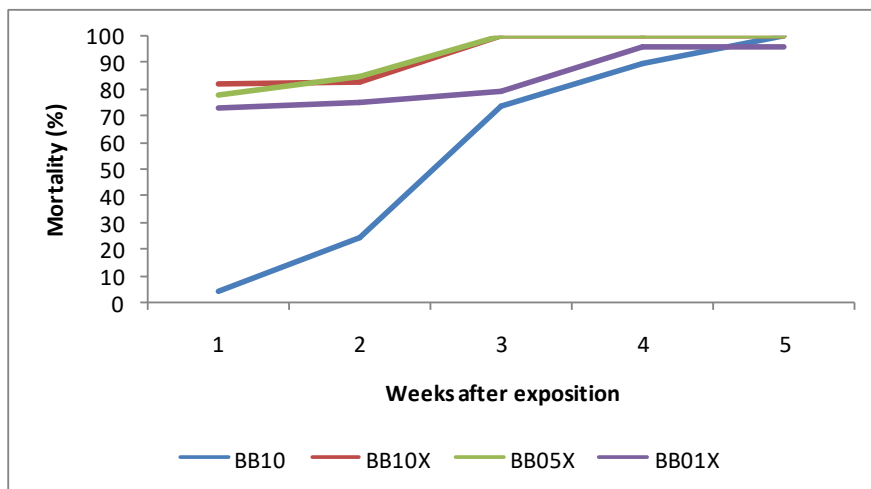
	LD ₅₀ (ppm)	LD ₉₀ (ppm)	Equation of Regression	correlation	df
<i>E. citriodora</i>	57.4 ^a	112.8 ^a	y=0.0231x +3.6732	0.9***	7
<i>X. aethiopica</i>	37.1 ^b	53 ^b	y=0.0804x +2.02	0.98***	3

In the same colon, the letter affected by the same letter do not different significantly ($p \leq 0.001$).

Moreover, the statistical analysis between the different LD₅₀ and LD₉₀ shows a very highly significant difference between the two essential oils. The origin of these variations of efficiency between the essential oils can be explained by the difference in the chemical composition of the different plant species. For these essential oils of *X. aethiopica* and *E. giganteus*, the correlation is strong and positive between the induced mortalities and the doses of essential oils ($r=0,9^{***}$).

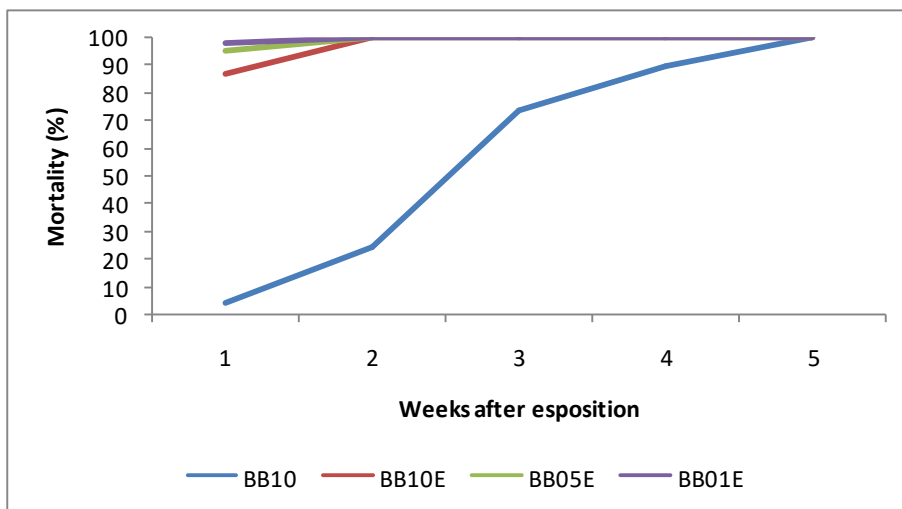
3.3. Effects of *Xylopiya aethiopica* and *Eucalyptus citriodora* essential oils formulated with flour of black beans on *Sitophilus oryzae*

The different treatments have insecticidal properties, and the toxicity of the different formulations on *S. oryzae* varies according to the proportions of black bean meal, the essential oil and the duration of exposure. BB01E treatment induces almost 100% of mortality compared to 73% of mortality induced by BB01X treatment at the end of the same first week of exposure (Figures 1, 2).



Legends: BB10: Black bean at 10 g, BB10X, BB05X and BB01X: Black bean at 10, 5 and 1 g formulated with the essential oil of *X. aethiopica*

Figure 1. Mortalities of *Sitophilus oryzae* by contact -inhalation and ingestion of aromatized flours with *Xylopiya aethiopica*



Legends: BB10: Black bean at 10 g, BB10E, BB05E and BB01E: Black bean at 10, 5 and 1 g formulated with the essential oil of *E. citriodora*

Figure 2. Mortalities of *Sitophilus oryzae* by contact -inhalation and ingestion of aromatized flours with *Eucalyptus citriodora*

The comparison of the results obtained with the different formulations shows that those based on the essential oil of *E. citriodora* are more toxic than those based on *X. aethiopica*. Indeed, the χ^2 test shows that there is a statistically significant difference ($p \leq 0.05$) between the mortalities induced by these 2 groups of formulation 2 weeks after infestation for treatments BB10X and BB10E, BB05X and BB05E of a part and 3 weeks after infestation for treatments BB01X and BB01E (Table 3).

It should also be noted that treatments BB10E, BB05E and BB01E induce 100% mortality 2 weeks after infestation, whereas this rate is reached with BB10X and BB05X only after four weeks (Figures 1, 2). At this time, treatment BB01X induces 96% mortality. In acute toxicity tests, the essential oil of *X. aethiopica* was found to be more toxic than that of *E. citriodora* (Tables 1, 2). After the first week, the χ^2 value shows there is no difference between mortality rates induced by the treatments BB10E and BB10X ($\chi^2=5ns$); on contrary, the difference is very highly significant between ($\chi^2= 94***$) the other treatments except BB05E and BB05X ($\chi^2=17*$) (Table 3).

Table 3. Comparative values of square Khi on the mortalities rate according to weeks

Weeks	BB05 - BB05E	BB05- BB05X	BB05E- BB05X	BB10- BB10E	BB10- BB10X	BB10E- BB10X
1st	94***	77***	17*	83***	78***	5ns
2nd	81***	66***	15*	78***	61***	17*
3rd	36**	36**	0ns	26**	26**	0ns
4th	14*	14*	0ns	10ns	10ns	0ns
5th	0ns	0ns	0ns	0ns	0ns	0ns

*ns: not significant, * : significant, ** highly significant, ***: very highly significant*

At the fifth week, there is no significant difference between all the treatments. After 3 weeks, the treatments BB05E, BB05X, BB10E and BB10X induce 100% of mortality (Figures 1, 2, Table 3). There is the opposite effect, which suggests that there are synergists in black bean flour. ANOVA I also reveals that there is a very highly significant difference between essential oil treatments and control treatments (Table 3). A comparison between the different treatments of the same group of formulation using the Chi squared test shows that there is no statistically significant difference, that is to say that BB01E = BB05E = BB10E or else BB01X = BB05X = BB10X.

4. DISCUSSIONS

The use of chemicals poses to human health and their environment problems of persistence and undesirable effects on non-target organism and the insect pest the phenomenon of resistance.

Nowadays, many researches demonstrate the value of utilization of natural products as the safe alternatives to control storage pest. The bioassays made in this study were principally experimental and are focused on *S. oryzae*, major insect pests of cereals in Cameroon. The utilization of flours of black beans' as support of essential oils for the protection of stored cereals and their persistence is important as an alternative to chemicals.

The differences observed in the insecticidal efficiency of these essential oils (Table 1) are linked to their chemical composition that the amount and the diversity of the active molecules are not the same (Kanko et al, 2018, Nguemtchouin Mbouga, 2012, Regnault-Roger et al, 2008). The absence of mortality induced by the essential oil of *E. giganteus* can explain by the fact that this oil is exclusively rich in sesquiterpenoids (Menut et al, 1996); or the composition of essential oils with a strong insecticidal power are monoterpenoids (Isman, 2000, Prates et al, 1998). For all combinations made, it appears a negative interaction between the constituents of the essential oils put together. Despite of the presence of the lignans mentioned in the essential oil of *E. giganteus* which are generally inactive alone, but brought into contact with other molecules whose biological effect is known and established, significantly increase their toxicity (Regnault-Roger et al, 2008, Tene et al, 2004). The phenomenon of synergy is not observed for *X. aethiopica* + *E. giganteus* and *E. citriodora* + *E. giganteus* mixtures; this suggests that the essential oils of *X. aethiopica* and *E. citriodora* would be very rich in antagonistic compounds. The mixture *E. citriodora* + *X. aethiopica* gives a tangible evidence of this antagonistic effect, because the mortalities observed for this mixture are much lower than those expected precisely at the 125 ppm and 250 ppm doses. The mixture of essential oils permits the formation of new compounds or molecules by interaction (Ngassoum et al, 2007). In fact, similar results were obtained with the combination of the essential oils of *Ocimum gratissimum* and *L. rugosa* on *Sitophilus zeamais* (Aoudou, 2006). These results contrary those revealed for the balanced combinations of *Vepris heterophylla* with *Ocimum canum* or *Hyptis spicigera* toward *S. oryzae* where the phenomena of synergy were observed (Ngamo Tinkeu et al, 2007). The synergy between *O. canum* and *H. spicigera* has increased their repellent effects (Ngassoum et al, 2007).

The difference of the LD₅₀ and LD₉₀ of the essential oils of *X. aethiopica* and *E. citriodora* towards *S. oryzae* (Table 2) would be explained by a difference in the concentration of the compounds responsible for the insecticidal effect in the two essential oils. Indeed, the chemical composition of these two essential oils is different; *X. aethiopica* contains 38.2 % of sabinene and β-pinène and *E. citriodora* contains 85% of citronellal and 4.5% of citronellol (Kanko et al, 2018, Nguemtchouin Mbouga, 2012). In general, the toxicity of essential oils varies not only according to the concentrations used, but also according to the plant and animal species used (Tamgno, 2016, Lee et al, 2004, Kim et al, 2003, Taponjou et al, 2002, Pamo et al, 2002, Shaaya et al, 1997). The variability of the LD₅₀ of several essential oils on many stored grain pests was showed (Fatimé, 2007, Aoudou, 2006).

The analysis of mortalities induced by *X. aethiopica* and *E. citriodora* essential oils formulated with flour of black beans and the control on *Sitophilus oryzae* (Figures 1, 2, table 3) suggests different origins. Indeed, mortalities induced by the BB10 treatment would be the result of a food poisoning whereas those of formulations that of a respiratory and digestive poison; that essential oils generally have low to very low oral toxicity (Bruneton, 1999). The advantage of these

formulations is related to the fact that in addition to the insecticidal powers of the essential oils, the survivors of bios tests would be affected in their development cycle. In fact, some essential oils affect the life cycle by acting on female spawning, or obviously by reducing the emergence rate of the F1 generation (Kouninki *et al.*, 2007, Belmain *et al.*, 2001, Regnault-Roger and Hamaraoui, 1995).

Very few studies exist of the possibility of increasing the efficacy of use of essential oils in real conditions in the protection of stored products during storage. The essential oil of *O. gratissimum* losses their efficacy after 8 days on maize, but when this essential oil passes to 45 and 107 days after fixation according to the treated fractions of clay (Nguemtchouin Mbouga, 2012). In the same way, the powder of *X. aethiopica* mixed with the essential oils of the same plant has the potential to give longer-term protection of grains against *Callosobruchus maculatus* (Kouninki et al, 2007). Lipids or others compounds present in the flour of black beans that can absorb on terpenoids and slow down increases probably the persistence of the insecticidal activity of combination of flour and essential oils on the insect pests. Another advantage of these formulations is that once the insecticidal activity of the essential oils has faded, the single flour continues the protection because it itself has entomotoxic properties.

5. CONCLUSION

It emerges from this present work that the essential oils of *X. aethiopica* and *E. citriodora* have insecticidal properties on *S. oryzae* and that of *E. giganteus* does not have one. From combinations of essential oils, the most toxic is that between of *X. aethiopica* with *E. giganteus*. The powdery formulations with *E. citriodora* are more toxic than those of *X. aethiopica*. In view of all these results, black bean can be used both as an insecticide and as a support for essential oil in the protection of cereals. The antagonistic effect observed in the combinations of substances shows that the traditional practice of combining plants in granaries is not always beneficial. At the end of this work, powdery formulation based on the essential oils of *X. aethiopica* or *E. citriodora* and black bean flour could contribute to the subsequent protection of post-harvest cereals against insect pest attacks.

6. AUTHOR CONTRIBUTIONS

All the authors contribute to define the main lines of the study. LSNT, DCL and MBN have supplied the materials of study. BRT has done a realization of the study, he makes all the bioassays.

7. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

8. ACKNOWLEDGEMENTS

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