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## Evaluation of the insecticidal efficacy of four plants to protect stored Bambara groundnuts [*Vigna subterranea* (L.) Verdc. (Fabaceae)]

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### ABSTRACT

Bambara groundnuts [*Vigna subterranea*] is one of the main legumes consumed in Northern Cameroon. However, stored nuts by farmers are quickly destroyed by pests leading to low yields. To overcome this constraint, the efficient control of stored grains from insect pests is dependent on synthetic insecticides. To reduce post-harvest losses of stored voandzou seeds, four botanical insecticides have been developed and evaluated on the main pest. Plant powders prepared from *Piper nigrum* Linn. (Piperaceae), *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae), *Xylopia aethiopica* Dunal (Annonaceae) and *Phaseolus vulgaris* L. (Fabaceae), at different doses were tested on *Callosobruchus maculatus* Fabricius under laboratory conditions. The rest of the study involved combining black bean powder with the three condiment species in different proportions. The analysis shows a highly significant difference ( $p < 0.0001$ ) between the mortality induced by formulations on day two of exposure with the lowest dose (0.5 g). These mortalities are 100%,  $94.44 \pm 3.79\%$ ,  $72.5 \pm 96$  and  $8.33 \pm 4.77$ , respectively for *S. aromaticum*, *P. nigrum*, *X. aethiopica* and *P. vulgaris* powders. In relation to the formulation, the results show *S. aromaticum* powder induced 100% mortality compared with 94.44%, 72.59% and 8.33% respectively for *P. nigrum*, *X. aethiopica* and *P. vulgaris* plant powder at a dose of 5%. The results show that the natural substances used had a good insecticidal action against bruchid. The persistent compounds present in the studied spice plant powders are not toxic for human consumption at the concentrations used, but rather a beneficial effect.

**Key words:** botanical insecticide, aromatic plants, powder, seeds, storage, insect pest

### RÉSUMÉ

#### Évaluation de l'efficacité insecticide de quatre plantes pour protéger les arachides Bambara stockées [*Vigna subterranea* (L.) Verdc. (Fabacées)]

Le voandzou [*Vigna subterranea*] est l'une des principales légumineuses consommées dans le Nord du Cameroun. Cependant, les grains stockés par les agriculteurs sont rapidement détruits par les ravageurs, ce qui entraîne de faibles productions. Pour surmonter cette contrainte, la lutte efficace contre les insectes ravageurs des grains stockés dépend des insecticides de synthèse qui sont pour la plus part dangereux. Pour réduire les pertes post-récolte des graines de voandzou stockées, quatre insecticides botaniques ont été développés et évalués sur le principal ravageur. Des poudres de plantes préparées à partir de *Piper nigrum* Linn. (Piperaceae), *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae), *Xylopia aethiopica* Dunal et *Phaseolus vulgaris* (L.) (Fabaceae) à différentes doses ont été testées sur *Callosobruchus maculatus* Fabricius en conditions de laboratoire. La suite de l'étude a consisté à combiner la poudre de haricots noirs avec les trois espèces de condiments dans différentes proportions. L'analyse montre une différence très hautement significative ( $p < 0,0001$ ) entre les mortalités induits par les quatre poudres insecticides au deuxième jour d'exposition avec la dose la plus faible (0,5 g). Ces mortalités sont de 100%,  $94,44 \pm 3,79\%$ ,  $72,5 \pm 96$  et  $8,33 \pm 4,77$  respectivement pour les poudres de *S. aromaticum*, *P. nigrum*, *X. aethiopica* et *P. vulgaris*. En ce qui concerne la formulation, les résultats montrent que la poudre de *S. aromaticum* a induit une mortalité de 100% contre 94,44%, 72,59% et 8,33% respectivement pour les poudres de *P. nigrum*, *X. aethiopica* et *P. vulgaris* à une dose de 5%. Les résultats montrent que les substances naturelles utilisées ont une bonne action insecticide contre les bruches. Les composés persistants présents dans les poudres de plantes aromatiques étudiées ne sont pas toxiques pour la consommation humaine aux concentrations utilisées, mais plutôt bénéfique.

**Mots clés :** insecticide botanique, plantes aromatiques, poudre, graines, stockage, insectes nuisibles.

### INTRODUCTION

The latest United Nations projections suggest that the world's population could reach around 8.5 billion by 2030 and 9.7 billion by 2050, and most of this growth is

expected to occur in developing countries (DESA, 2022). Although more food will be required to be produced, competing interests, such as rapid urbanization, prompt

the need for sustainable intensification of agriculture on land that is available (Mulazzani et al., 2020). Unfortunately, food production lags behind with undernourishment being rampant with a prevalence rate of over 42% corresponding to 783 million underfed people (FAO, 2023) who mostly depend on cereal-based protein-deficient staple foods which are not even always available. In Cameroon, 11% of the population is facing acute food insecurity. This represents 3 million people from national population. The results of the March 2023 Harmonised Framework analysis indicate that the Far-North, North-West and South-West regions are the worst affected (UN-OCHA, 2023). It is therefore imperative to solve the problem of food insecurity. Faced with this need, the integration of legumes in general and in food habits of underfed population can be a fundamental element to strengthen this resilience. Groundnuts (*Arachis hypogea* L.), common beans (*Phaseolus vulgaris* L.), cowpeas (*Vigna unguiculata* (L.) Walp), Bambara groundnuts and soybeans (*Glycine max* (L.) Merrill) [Fabaceae] constitute the main legumes cultivated and consumed in Cameroon because of their importance in the eating habits of populations. Among these legumes are Bambara groundnuts [*Vigna subterranea* (L.) Verdc.] also called Bambara peas or ground pea which is a minor legume whose culture has remained within the limits of sub-Saharan Africa where it has adapted under various climatic and ecological conditions (Ngamo et al., 2016; Fatimé et al., 2018; Temegne et al., 2018). The consumption of its seeds contributes to improve the quality of the diet as a protein supplement. They are highly caloric plant (387 kcal / 100 g) rich in vitamins minerals and very balanced in proteins (Madou et al., 2024; Goudoum et al., 2016). These nuts can help to maintain good health due to its possession of antioxidants (Arise et al., 2016). Unfortunately, despite its many advantages, Bambara groundnuts is still one of the most neglected and less used species in variety breeding programs (Gbaguidi et al., 2016; Assoumane et al., 2020). However, this speculation faces certain constraints amongst which are low productivity and lack of knowledge on conservation of stored grains. Several authors have showed that post-harvest losses and quality deterioration of the legume is majorly caused by the insect pest *Callosobruchus maculatus* (Chougourou et al., 2016; Madou et al., 2018; Issa et al., 2018). In addition, previous surveys carried out in this study area revealed that *C. maculatus* and *Callosobruchus subinnotatus* (Pic.) were the most frequently encountered insects in farmer stocks (Ngamo et al., 2016; Fotso et al., 2019). Faced with the extent of their attacks and the consequences on the quality of Bambara groundnuts, these pests undoubtedly constitute one of the major constraints on the productivity of leguminous crops. Despite being a worldwide constraint, these insect pests are more rampant in developing countries in general and those of Africa in particular due to the climatic conditions of Africa which favor their growth (Sanon et al., 2018). Several methods are recommended for the control of pests of stored legumes products among which is the chemical control with synthetic insecticides being the most used strategy

for Bambara groundnuts pest control and many other legumes (Sankara et al.2016, Madou et al., 2018). The excessive, repeated and unreasoned application of the latter against insect pests in recent decades has led to harmful effects on both the consumers, environment and biodiversity (Cruz et al., 2016; Zaki et al., 2020). Increasingly, studies are also showing a link between exposure to synthetic chemical pesticides exposure to synthetic chemical pesticides cancer, cardiovascular disease, diabetes cardiovascular disease, diabetes, neurodegenerative neurodegenerative disorders such as Parkinson's, Alzheimer's and as well as amyotrophic lateral sclerosis, congenital congenital malformations and reproductive reproductive disorders (Anakwue, 2019) However, research into alternative methods of protecting stored foodstuffs by using plant substances with an insecticidal effect is promising in the fight against insect pests of stored foodstuffs (Ngamo et al., 2020; Ayiki et al., 2019). The use of pesticide plants is proving to be an ancestral practice practice in Africa. In fact, many plants are known and used for their biocidal activities (toxic, repellent, anti-appetant) against a wide range of pests range of pests (Lengai et al., 2018). These substances are obtained from the various parts of the plant, such as flowers, leaves, bark, sap, wood, roots, pods, bulbs, rhizomes, fruits and seeds in a dry or fresh state dry or fresh (Muthomi, 2018; Werrie et al., 2020). Faced with this worrying situation of chemical pesticides and given the need to preserve food resources, it becomes urgent to think of alternative insecticides. Bearing in mind the role of Bambara groundnuts in eradicating hunger and improving food security, the present study was carried out with the aim to develop a bio-insecticide based on spice plant powders for post-harvest protection of Bambara groundnuts.

## MATERIALS AND METHODS

### Plant and animal materials

Dried fruits of *Xylopiya aethiopica* (Figure 1A), fruits of *Piper nigrum* also called black or wild pepper (Figure 1B), the inflorescences of *Syzygium aromaticum* commonly known as cloves (Figure 1C) and the black bean seeds *Phaseolus vulgaris* (Figure 1D) were purchased at the small market in Ngaoundéré, Adamawa region (Cameroon) and brought to the laboratory for further use. These plant spices were each cleared of all impurities then ground into powders using a mill with movable hammers (CULATTI). The different powders obtained were weighed with an electric balance (SARTORIUS) to the nearest 0.01 g and directly used for biological tests. The adults of *C. maculatus* was a strain collected from Maroua beetle and raised on Bambara groundnuts at the Applied Entomology Laboratory of the University of Ngaoundéré. The breeding consisted of extracting 30 pairs of newly emerged insects and placing them in pots containing 200 g of healthy Bambara groundnuts seeds and then incubating them in an oven regulated at 28 °C and a relative humidity of 60%, until the emergence of new bruchids (30-35 days).



Figure 1. Dry pod of *Xylopiya aethiopica* (A); seed of *Phaseolus vulgaris* (B); inflorescences of *Syzygium aromaticum* (C); the fruits of *Piper nigrum* (D)

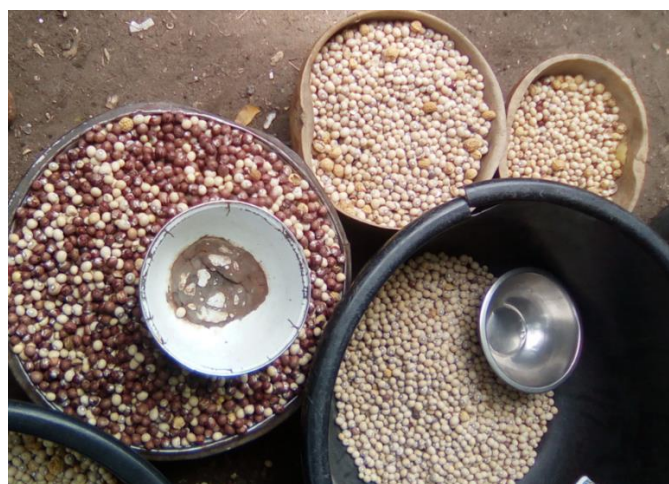


Figure 2. Dried Bambara groundnuts seed on display at a local market in the far north of Cameroon

#### Determination of dose-mortality variation in adults of *Callosobruchus maculatus*

The powders obtained were introduced separately into the test tubes at different proportions: 3.5 g; 3g; 2.5g; 2g; 1.5g; 1g; 0.5 g and 0 g (control) corresponding to eight treatments labelled according to doses. For each treatment four replicates were performed and in each replicate 10 individuals of mixed sex of adult *C. maculatus* of 48 hours of age were introduced. The tubes were manually stirred for 30 seconds and corked with cotton balls for good ventilation and placed in an oven (FRANCE ETUVE) set at 28 ° C. All the tubes were checked every two days for a period of 6 days. The observed parameter was the number of dead individuals which was verified by exercising delicate touches using the tip of the entomological forceps on the appendages of the insects and no movement of the appendages exhibited death of insect (Ileke & Oni, 2011).

**Evaluation of the efficacy of the combined powders of *P. nigrum*, *S. aromaticum* *X. aethiopica* with *P. vulgaris***  
The second stage of this study consisted in combining the black bean powder with the three other studied plant

species. The powdery formulations in equal proportions were obtained by a simple combination of a mass X of the powder of the spice plants with another mass Y of the black bean flour so as to obtain a portion of 100% (Tamgno and Ngamo, 2018b). Therefore the powder of *S. aromaticum* (SA) was withdrawn in the following proportions 0%; 1%; 2%; 3%; 4%; 5%; 10%; 15%; 20% and 25% and those of *P. nigrum* (PN) and *X. aethiopica* (XA) in the proportions of 0%; 5%; 10%; 15%; 20%; 25%; 30%; 35%; 40%; 45%; 50%; 55%; 60%; 65%; 70%; 75%; 80%; 85%; 90 and 95% and supplemented with Y% black bean powder (*P. vulgaris*) so as to obtain 1g of mass. The difference in proportion between the species is a function of the effectiveness of each of the powders.

**Evaluation of the persistence of the insecticidal activity of the formulations of *P. nigrum*, *S. aromaticum*, *X. aethiopica* associated with *P. vulgaris***  
After determining the lowest effective dose of each of the combinations, we tested them afterwards. The new three formulations were repeated four times and for each replicate 10 individuals of mixed sex of adult *C. maculatus* of 48 hours of age were introduced into test



tubes. The test tubes were checked every 2 days for a period of 20 days and the mortalities evaluated.

**RESULTS**

**Evaluation of insecticidal effect of *S. aromaticum*, *P. nigrum*, *X. aethiopica* and *P. vulgaris* powders at different doses on mortality of *C. maculatus***

Bioassays on the insecticidal efficacy of the powders of *S. aromaticum*, *P. nigrum*, *X. aethiopica* and *P. vulgaris* were tested at different doses are illustrated in Figure 2. It appeared that mortality increased with concentration and the period of exposure. The insecticidal properties of the

powders tested manifest themselves in different ways either by inhalation toxicity for powders of aromatic plants or by ingestion for powders of legumes in adults. Mortalities of beetles range from  $8.33 \pm 4.77\%$  to  $100 \pm 0.00\%$  (Figure 2). Significant difference on the mortality of beetles was ( $p < 0.0001$ ) observed among the four insecticidal powders on day two of exposure with the smallest dose (0.5 g). The mortalities induced were 100%,  $94.44 \pm 3.79\%$ ,  $72.5 \pm 96\%$  and  $8.33 \pm 4.77\%$  respectively *S. aromaticum*, *P. nigrum*, *X. aethiopica* and *P. vulgaris*. Highest mortality from *P. vulgaris* was not reached until the sixth day of exposure with the smallest dose 0.5 g.

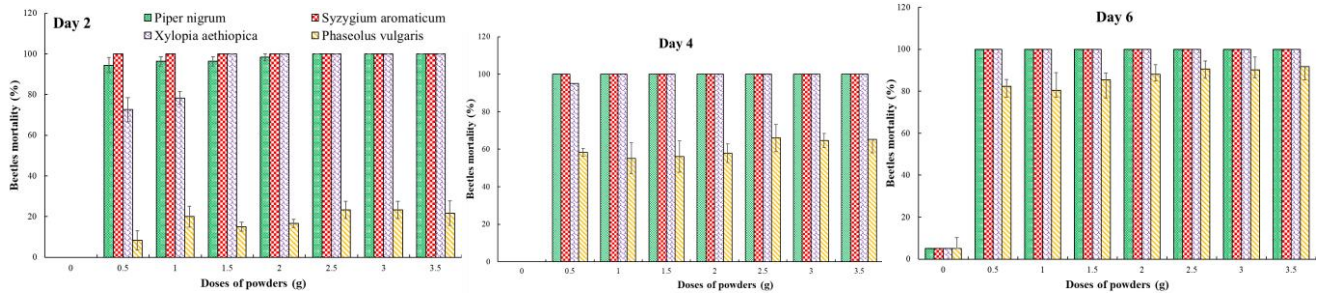


Figure 3. Mortality of *C. maculatus* during a six-day treatment with *S. aromaticum*, *P. nigrum*, *X. aethiopica* and *P. vulgaris* powders

**Insecticidal efficacy of powdery combinations of *S. aromaticum*, *P. nigrum*, *X. aethiopica* with *P. vulgaris* on *C. maculatus***

The insecticidal effect of three powdery combinations (*S. aromaticum* + *Phaseolus vulgaris*; *P. nigrum* + *P. vulgaris* and *X. aethiopica* + *P. vulgaris*) on mortalities of *C. maculatus* during 6 days is presented in Table 1. The analysis showed a highly significant difference ( $p < 0.0001$ ) between the mortality induced by the formulations based on *S. aromaticum*  $F = 33.308$ . *P. nigrum* = 51.488 and *X. aethiopica*  $F = 40.32$  two days after exposure. The powder of *S. aromaticum* induced a

mortality of 100% against 50% and 30% respectively for the plant powder of *P. nigrum*, *X. aethiopica* and *P. vulgaris* at the dose of 5% after 24 hours and 100%, 70% and 52% after 48 hours for same species respectively. The application of the effective doses of the insecticide formulations derived from the three condiment powders associated with the *P. vulgaris* powder in the presence of *C. maculatus* has shown an efficacy of 100% mortality for the following combinations: *S. aromaticum* (4%) + *P. vulgaris* (96%); *P. nigrum* (70%) + *P. vulgaris* (30%) and *X. aethiopica* (85%) + *P. vulgaris* (15%).

Table 1. Evaluation of the insecticidal effect of the three powdery formulations of *P. nigrum*, *X. aethiopica*, *S. aromaticum* associated with *P. vulgaris*

DIFFERENTS POWDERS	Percentage aromatic plant (%)	DAY 2	DAY 4	DAY 6
x% <i>S. aromaticum</i>	0	0.00±00 <sup>b</sup>	0.00±00 <sup>b</sup>	00±00 <sup>b</sup>
+ y% <i>P. vulgaris</i>	1	86.66±6.31 <sup>a</sup>	95.00±2.88 <sup>a</sup>	100±00 <sup>a</sup>
	2	93.33±2.84 <sup>a</sup>	97.50±1.79 <sup>a</sup>	100±00 <sup>a</sup>
	3	97.50±2.50 <sup>a</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	4	100±00 <sup>a</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
F		33.308***	153.268***	-
x% <i>P. nigrum</i> +	0	0.00±00 <sup>F</sup>	0.00±00 <sup>E</sup>	00±00 <sup>b</sup>
y% <i>P. vulgaris</i>	5	50.00±4.08 <sup>c</sup>	70.00±4.08 <sup>d</sup>	100±00 <sup>a</sup>
	10	60.00±4.08 <sup>de</sup>	82.50±2.50 <sup>c</sup>	100±00 <sup>a</sup>
	15	75.00±5.00 <sup>cd</sup>	87.50±6.29 <sup>bc</sup>	100±00 <sup>a</sup>
	20	77.50±6.29 <sup>bcd</sup>	97.50±2.50 <sup>ab</sup>	100±00 <sup>a</sup>
	25	90.00±4.08 <sup>abc</sup>	97.50±2.50 <sup>ab</sup>	100±00 <sup>a</sup>
	30	87.50±2.50 <sup>abc</sup>	99.50±4.78 <sup>a</sup>	100±00 <sup>a</sup>
	35	90.00±4.08 <sup>abc</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	40	92.50±4.78 <sup>abc</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	45	92.50±4.78 <sup>abc</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>

DIFFERENTS POWDERS	Percentage aromatic plant (%)	DAY 2	DAY 4	DAY 6
	50	95.00±2.88 <sup>ab</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	55	95.00±2.88 <sup>ab</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	60	97.50±2.50 <sup>a</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	65	98.00±2.88 <sup>a</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	70	100±00 <sup>a</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
F		51.488***	105.048***	-
x % <i>X. aethiopica</i> + y% <i>P. vulgaris</i>	0	0.00±00 <sup>h</sup>	0.00±00 <sup>g</sup>	00±00 <sup>c</sup>
	5	30.00±7.07 <sup>g</sup>	52.50±2.50 <sup>f</sup>	77.50±4.7 <sup>b</sup>
	10	32.50±6.29 <sup>fg</sup>	62.50±4.78 <sup>ef</sup>	77.50±2.50 <sup>b</sup>
	15	40.00±4.08 <sup>efg</sup>	67.50±4.78 <sup>def</sup>	87.50±4.78 <sup>ab</sup>
	20	42.50±4.78 <sup>efg</sup>	70.00±4.08 <sup>defg</sup>	90.00±4.08 <sup>ab</sup>
	25	50.00±5.77 <sup>efg</sup>	72.50±4.78 <sup>bcdef</sup>	92.50±4.78 <sup>a</sup>
	30	50.00±4.08 <sup>efg</sup>	82.50±4.78 <sup>abcde</sup>	95.00±5.00 <sup>a</sup>
	35	52.50±4.78 <sup>def</sup>	82.50±11.81 <sup>abcd</sup>	95.00±00 <sup>a</sup>
	40	55.00±6.45 <sup>de</sup>	85.00±5.00 <sup>abcd</sup>	100±00 <sup>a</sup>
	45	55.00±2.88 <sup>de</sup>	90.00±4.08 <sup>abc</sup>	100±00 <sup>a</sup>
	50	57.50±2.50 <sup>de</sup>	92.50±4.78 <sup>ab</sup>	100±00 <sup>a</sup>
	55	60.00±4.08 <sup>cde</sup>	95.00±2.88 <sup>a</sup>	100±00 <sup>a</sup>
	60	60.00±00 <sup>cde</sup>	95.00±2.88 <sup>a</sup>	100±00 <sup>a</sup>
	65	72.50±4.78 <sup>bcd</sup>	97.50±2.50 <sup>a</sup>	100±00 <sup>a</sup>
	70	80.00±4.08 <sup>abc</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	75	90.00±4.08 <sup>ab</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	80	92.50±4.78 <sup>ab</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
	85	100±00 <sup>A</sup>	100±00 <sup>a</sup>	100±00 <sup>a</sup>
F		40.32***	33.45***	71.80***

\*\*\*: p < 0.0001. NB: The values bearing the same letters in a column are not significantly different at the 5% threshold.

### Evaluation of the LD<sub>50</sub> and LD<sub>95</sub> (lethal doses) of single powder doses of spice plants on mortality of *C. maculatus* adults

The lethal doses of LD<sub>50</sub> and LD<sub>95</sub> of the single doses of the studied spice plant powers are presented in Table 2. All the single doses of the studied spice plant powers used were toxic to *C. maculatus* and the lethal dose values decreased with exposure period for all doses tested. After

two, four and six days of exposure of *C. maculatus* to *P. nigrum* the LD<sub>50</sub> was between 4.63 and 0.09 and the LD<sub>95</sub> between 53.37 and 1.03. The lethal doses for the second fourth and sixth day of exposure for *S. aromaticum* were between 0.91 and 0.15 for the LD<sub>50</sub> and 3.23 and 0.55 for LD<sub>95</sub>. The values obtained are lower than the smallest dose used at 4 and 70% respectively for *S. aromaticum* and *P. nigrum*.

Table 2. Lethal doses of powder preparations resulting in 50% to 95% mortality of *C. maculatus* adults after 2, 4 and 6 days of exposure.

Powder formulation	DL50	DL95	Pente ± ES
Day2			
<i>Piper +phaseolus</i>	4.63	53.37	1.03±0.0
<i>Syzygium+phaseolus</i>	0.91	3.23	0.123±0.054
<i>Xylopi+phaseolus</i>	19.85	342.97	-
Day4			
<i>Piper +phaseolus</i>	1.616	18.631	0.323±0.06
<i>Syzygium+phaseolus</i>	0.48	1.715	0.945±0.06
<i>Xylopi+phaseolus</i>	6.672	115.27	-
Day 6			
<i>Piper +phaseolus</i>	0.09	1.037	1.620±0.23
<i>Syzygium+phaseolus</i>	0.15	0.55	2.419±0.205
<i>Xylopi+phaseolus</i>	1.271	21.96	-

### Determination of the effective insecticidal dose of the combinations of *S. aromaticum* + *P. vulgaris* *P. nigrum* + *P. vulgaris* and *X. aethiopica* + *P. vulgaris* applied to Bambara groundnuts seeds in the presence of *C. maculatus*

The insecticidal efficacy of the combinations of spice plant powders depends on the species used. To the various treatments T0, T1, T5, T10 and T15 which are respectively the treatments at the dose 0, 1, 5, 10 and 15 g of each of the formulations. The results show a highly significant difference (p < 0.0001), emergence of *C.*

*maculatus* induced in the presence of the different powdery insecticide formulations. 35 days after application (Figure 3). In the T10 and T15 treatments the formulations based on *S. aromaticum* + *P. vulgaris* and *P. nigrum* + *P. vulgaris* induced 100% mortality, thus not emergence against the control treatment which recorded 2115 individuals of *C. maculatus* collected. In addition, the same formulations induce respectively for

T1 treatments an emergence of 96 and 141 individuals, as well as T5 treatments causing 119 and 160 emerging individuals. The formulation based on *X. aethiopica* + *P. vulgaris* generates for the T5 and T1 treatments an emergence of 966 and 318 individuals of *C. maculatus*. Formulations based on *S. aromaticum* and *P. nigrum* were more effective.

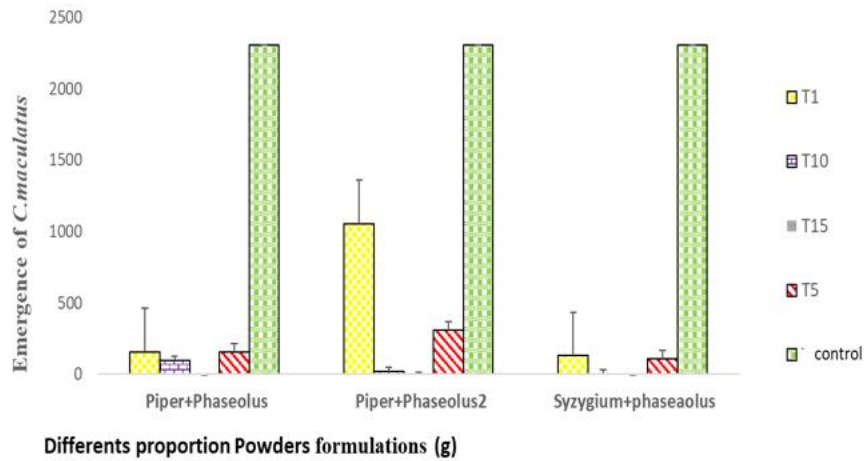


Figure 4. Emergence of *C. maculatus* according to the three formulations 35 days after application

**Persistence of the insecticidal activity of the formulations tested**

Mortalities of *C. maculatus* varied over time for all three combinations (*S. aromaticum* (4 %) + *P. vulgaris* (96 %); *P. nigrum* (70%) + *P. vulgaris* (30 %) and *X. aethiopica* (85 %) + *P. vulgaris* (15%)). Regarding the effectiveness of the three combinations the effective doses caused 100% mortality of *C. maculatus* after 6 days of exposure (Figure 4). Beyond 6 days, mortality decreased gradually until the 20<sup>th</sup> day for the

combination *X. aethiopica* + *P. vulgaris*. After 15 days of exposure, the mortality was 100% for the combinations *S. aromaticum* + *P. vulgaris* and *P. nigrum* + *P. vulgaris*. The same proportion induced respectively 100% and 85% mortality after 20 days exposure compared to the combination of *X. aethiopica* + *P. vulgaris* which resulted in only 10% mortality of the population of *C. maculatus* after this same period of exposure.

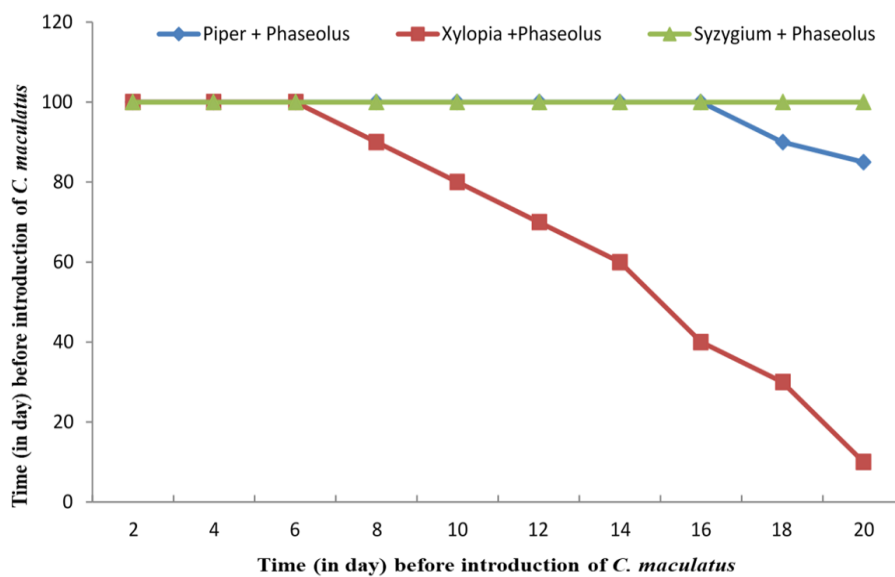


Figure 5. Persistence of the three powdery formulations combinations of *S. aromaticum* + *P. vulgaris*; *P. nigrum* + *P. vulgaris* and *X. aethiopica* + *P. vulgaris* 20 days after application

## DISCUSSION

Several legume varieties including Bambara groundnuts cultivated in Cameroon are attacked by weevils such as *C. maculatus* in storage systems. Research on insecticidal effect of plant extracts on the control of pests of Bambara groundnuts have been carried out (Fatmé *et al.*, 2018). Toxicity tests conducted in the laboratory with plant powders of *S. aromaticum*, *P. nigrum*, *X. aethiopicum* and *P. vulgaris* reported variable toxicities depending on the powders used and the duration of exposure (Tamgno *et al.*, 2018a). It appeared that except for *Phaseolus vulgaris*, the three other plant powders presented insecticidal effect on *C. maculatus* through inhalation. The mortalities recorded were 100%; 94.44%; 72%; 8.33% respectively for the *S. aromaticum*, *P. nigrum*, *X. aethiopicum* two days after exposure to 0.5 g dose. On insects, the three powders act by repulsion, contact and fumigation but also act as neurotoxic compounds (Jankowska *et al.*, 2018). The mortality caused by *Phaseolus vulgaris* increased on day six and reached 82.36% with a mode of action by ingestion. Biological tests on the insecticidal efficacy of legume seeds were explored and appeared that these seeds are a source of entomotoxic proteins against insect pests of the genera *Sitophilus* and *Tribolium* (Tamgno and Ngamo, 2014; Karbache Fatima, 2018). Several authors have indicated the presence of several entomotoxic molecules involved in the insecticidal effect of the powder: terpenes, alkaloids, cyanogenic glycosides and proteins (Werrie *et al.*, 2020). The difference in mortalities induced by the studied plant powders was significantly high and is believed to be due to the effect of the numerous insecticidal components found in the plant powders (Sanon *et al.*, 2018). Research on the insecticidal effect of certain plants on many insects of economic importance has revealed destructive, growth-inhibition (contact ingestion and inhalation) and repellent effects (Ngatanko *et al.*, 2017). Ileke *et al.* (2014) worked on the effect of *S. aromaticum* powders on *Sitophilus Zeamais* Motsch. and revealed that *S. aromaticum* powder at a dose of 0.5 g resulted in 32.5% mortality of *Sitophilus oryzae* Linnaeus after 96 hours' exposure. This was contrary to the 100% mortality observed in this study in 24 hours and could mean that the effectiveness of this powder depends on the type of insect pest treated. In addition to its insecticidal effect, cloves have been mentioned as one of the spices most frequently used during the current pandemic of COVID-19 pandemic along with other plants such as cinnamon, ginger, black pepper, garlic, and basil (Singh *et al.*, 2021; Pandey *et al.*, 2020). Similarly, the first studies on extracts of *P. nigrum* indicated that piperine and other active substances such as piperamides had toxic effects on *Callosobruchus chinensis* L. (Scott *et al.*, 2005). That 100% mortality of *C. maculatus* was observed with *X. aethiopicum* at 0; 2.0 and 3.0g as from day four exposure agrees with the works of Edwin (2018) who observed 63.3% mortality of *C. maculatus* over a 48-hour exposure period at similar doses. This mode of action can be largely attributed to great diversity of chemical structures (Farhana *et al.*, 2020), fumigant (Boeke *et al.*, 2004) and toxic properties of *X. aethiopicum* (Kim *et al.*, 2003). According to Sourabie *et al.* (2020),

plant species are used either directly or transformed into powder or ash for product protection. Leaves are used more than other organs, but these observations do not with that of the present study, which focuses on the fruit.

Speaking of powdery formulations made from spice plants and black bean powder, the doses of 4%, 70% and 80% of the powders of *S. aromaticum*, *P. nigrum* and *X. aethiopicum* all induce 100% mortality 2 days after application. The mortalities induced by the formulations at the various doses would be due to the combined effect of the active ingredients (Tamgno and Ngamo, 2018b). To optimize the protection of stored foodstuffs against harmful insects in northern Cameroon, farmers simultaneously introduce more than one plant species (2 to 6 species) into the granary (Ngatanko *et al.*, 2017; Ayiki *et al.*, 2019). The formulations tested show with regard to adults of *C. maculatus*, a much more toxicity by inhalation than by ingestion or direct contact given their composition which is very rich in monoterpene. The main chemical compounds of these spices are respectively: Phenols (70 to 85%, 1- nonene (40%) and terpinen-4-ol (30.8%) for *S. aromaticum*, *Piper nigrum* and *X. aethiopicum*. In addition to their anti-insect potential, essential oils extracted from aromatic plants play important biological roles (Goudoum *et al.*, 2009). Among these roles, their antioxidant potential, in inhibiting the formation of free radicals by oxidation of free fatty acids in the foods to which they are applied studies on the antioxidant activity of two essential oils were mentioned by Goudoum, (2010). Three possible situations can arise when at least two compounds are put together: synergy or additive effect, antagonism and neutrality (Traore *et al.*, 2015; Tamgno and Ngamo, 2018a). For these formulations tested, the responses produced were synergistic effects. Thus, the persistence of powdery formulations based on *S. aromaticum* and *P. nigrum* can last 20 days under laboratory conditions and their insecticide effectiveness could go to more than 6 months for treatments at T10 and T15. The persistence of the biological activity of the powders of the formulations of *S. aromaticum*, *P. nigrum* and *X. aethiopicum* would be due to their very aromatic compounds. The volatile compounds could have been sequestered in the powders of *P. vulgaris*, which would explain their prolonged action (Campolo *et al.*, 2018). The active compounds of essential oils are very volatile metabolites, of specific toxicity according to the species of Arthropods (Kim *et al.*, 2003). Biopesticides offer the advantage of being naturally occurring, biodegradable, more or less selective and harmless against non-target organisms (Koffi *et al.*, 2018). The compounds responsible for the insecticide activity will gradually lose their effectiveness, while leaving an odor characteristic of the essential oil used. These residues can be consumed in the treated food.

## CONCLUSION

The irrational use of synthetic pesticides has become a major risk to human health and the environment, leading to the consideration of the use of other natural options. The bioinsecticides formulation from aromatic plants have been recognised as an important natural resource of



insecticides and as an alternative to synthetic insecticides. Formulations based on *S. aromaticum*, *P. nigrum* and *X. aethiopica* have insecticidal activities which protect the grains of Bambara groundnuts against attacks by *C. maculatus* while preserving their technological and nutritional qualities. The persistent compounds present in these powders were not toxic to the consumer at the concentrations used but rather had bioactive compounds with antioxidant properties beneficial for the latter. The use of these formulations by the farmers of Northern Cameroon will allow a considerable reduction in post-harvest losses due to insect pests attacks during storage and therefore provided a healthy preservation of the food resource. Thus, a successful means of free pest stored Bambara groundnuts will boost/ increase the production/yield of this crop thereby reducing food insecurity in the study area.

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