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Bioactivity of flours of seeds of leguminous crops *Pisum sativum, Phaseolus vulgaris* and *Glycine max* used as botanical insecticides against *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) on sorghum grains

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ABSTRACT

Cereals occupy an important place in the solving of food insecurity problems in the northern Cameroon. Chemicals continue to be the main tools to protect cereals during storage in spite of the deleterious effects due to their current usage. Nowadays, entomotoxic proteins of seeds of leguminous are more and more considered as alternative insecticides. Efficacy of crude flours of four legumes seeds, applied to the sorghum grains, was evaluated against *Sitophilus oryzae* L. (Coleoptrea: Curculionidae) by contact-ingestion. This research revealed that flours of bean and pea are very active on *S. oryzae.* The lethal dose 100 (LD_{100}) and the lethal times 100 (LT_{100}) are respectively 5 g and 20 days for pea's flour and 10 g and 50 days for bean's flour. The toxicity of flours' seeds increased with the dose and the duration of application (F=169.734***, Df =6; 28). During a period of 90 days, the flours of *P. sativum* and P. vulgaris keep their insecticidal activities, showing constant mortality rates for bean (F= 2.325ns, Df =6; 28) and for pea (F=1,91ns, df= 34). The flours of seeds of *P. sativum* and *P. vulgaris* at the weight ratio of 5 % and 10 % respectively can provide protection of sorghum grains against the attack of *S. oryzae.* The legumes seeds are the alternative active ingredients to hazardous conventional insecticides.

Keywords: Cereals; leguminous plants; entomotoxic proteins; persistence; *Sitophilus oryzae.*

INTRODUCTION

One of the major problems with agriculture nowadays is to produce more and more or reduce significantly the post harvest losses in order to provide food for the population which number is in permanent increase (Ngamo Tinkeu et al., 2007). Cereals represent in poor countries nearly 75% of the food calories, brought in the least expensive form to the human populations. Cereals,

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principally sorghums and millets harvested once a year at a fixed period in Saharan Africa, constitute the daily major staple foods for the population of the northern Cameroon (Ngamo Tinkeu, 2000). To ensure the availability of cereals and legumes on the market throughout the year and the seeds for the next crop, the successful storage is unavoidable. During storage, many biotic and physical factors affect the quality and quantity of the stored foodstuffs products. The harmful insects are the major constraints of the storage of cereals and legumes grains (Mebarkia et al., 2012; Silva et al., 2013). Among these insect pests of stored products, several are the Coleopterans. The most destructive and redoubtable insects of these Coleopterans are tropical species which belong to the genus *Sitophilus* and *Tribolium*. These two genera attack principally the cereals grains and flour. *Sitophilus oryzae* L (Coleoptera: Curculionidae) is the main pest of cereals in the subtropical Africa (Dal Bello et al., 2001; Ngamo Tinkeu et al., 2007; Hasan Iqbal et al., 2013). In the northern Cameroon, *S. oryzae* infests all cereals and their manufactured products. During storage, the damages due to insects especially the genus *Sitophilus* affect the quality, the quantity, the commercial and agronomic value of the products (Dal Bello et al., 2001; Athanassiou et al., 2011).

To prevent these losses, local farmers currently use chemical insecticides which nowadays are the most popular tools to achieve any pest control in the area. Despite the strong growth of the industries of chemical insecticides, the losses due to insect pests would have doubled. This abusive utilization of chemicals endangers the environment which is polluted and the consumers feeding on treated grains ingested great quantity of residues of pesticides (Dal Bello et al., 2001; Taponjou et al., 2002). These synthetic pesticides are expensive for the small-scale farmers in the northern Cameroon and pose potential risks due to the lack of technical knowledge on their safe use.

Due to the side effects of chemical insecticides on the environment, human health and useful organisms, recent research focus on methods to reduce the application of systematic insecticides and to replace them with other methods (Louis, 2004; Karimi et al., 2010). It becomes, therefore, useful to build up alternative methods of controlling pest by methods that are user-friendly as the use of agents with high efficacy on the pest and low persistence in the food. There are needs to develop and popularize such control techniques that are clean and environmentally-friendly method as the use of natural products from plants. These natural products as entomotoxic proteins from seeds of leguminous are often highly specific, biodegradable and of low persistence (Gupta et al., 2013). Before the advent of chemicals, the natural products used as pesticides constituted the only way to control pests during storage in the world; some of these natural products protect grain without any observed effects on their germination, their smell and their taste. Ethno botany has therefore, played a very important role in the protection of crops against pests in many countries in Africa and Asia (Belmain et al., 2001; Regnault-Roger et al., 2002); plants were used at the time in granaries by the farmers naturally to protect their produce.

Among the methods which are explored since the awareness, the biological methods by usage of several different classes of plant proteins have shown their insecticidal activities towards a range of economically important insect pests by direct assay or by expression in transgenic plants (Ussuf et al., 2001; Louis, 2004; Tamgno, 2009; Karimi et al., 2010; Mebarkia et al., 2012; Gupta et al., 2013).

The present work aimed at considering the case of flours of 4 edible seeds leguminous crops: two varieties of soybean *Glycine max* (SJ320 and DOCKO), one variety of common bean *Phaseolus vulgaris* (little black) and the pea *Pisum sativum* which will be used for the control of major pest of cereals grain *Sitophilus oryae* L. during storage in the northern Cameroon. Coming back to natural products as plant extracts could be an alternative to dangerous synthetic insecticides which continue to be used in both licit and illicit way because of few products proposed by science to their replacement.

MATERIALS AND METHODS

Leguminous seeds used and their origins

The leguminous seeds used in this study are the little black bean, one of the varieties of common bean *Phaseolus vulgaris* coming from Bandjoun in West Region, Cameroon, the two varieties of soybean *Glycine max* SJ320 and DOCKO coming from the Wakwa Institute of Agricultural Researches for the Development Centre of the Adamawa Region, Cameroon and the pea *Pisum sativum* were bought at Ngaoundéré in Adamawa Region-Cameroon. These seeds were reduced to flour by grinding in the Laboratory of Entomological Research of the University of Ngaoundéré. The flours obtained were sieved using a sieve of mesh-size lower than 0.5 mm. The different fractions of flours obtained were weighed using a SARTORIUS balance, type 126400 with precision of 0.01 g. Flours were mixed directly with sorghum grains for biological assays.

Stored product insect pest used

The individuals of *S. oryzae* used for the test were reared on sorghum in incubators at 28 ± 2.2 °C and 65 ± 5.7% r.h. in the Unity of Entomology Researches at the University of Ngaoundéré. They were used at adult stage and the two weeks old. The millet weevil belonged to the strain collected in november 2003 in the granary of a peasant at Beka Hosséré (Ngaoundéré, Cameroon). For the experiments, adults of *S. oryzae* used were two weeks old. Before being tested, these insects were starved for a 48 hours period.

Research of the efficacy of the crude flours of seeds of leguminous crops

The formulations tested were prepared by mixing the flours of the insecticidal legume grains at a proportion which ranged from 0 g to 100 g made with the SARTORIUS balance by a step of 20 and were supplemented with the sorghum grains in opposite direction so that each pot of 1200 ml capacity had a total content of 100 g. The contents of each pot were homogenized by stirring it 20 times using a spatula. Each pot was infested with 10 couples of *S. oryzae* of two weeks old. For each treatment, 5 replications were made. 50 days after the application, all the pots were checked and the number of died insects was counted in each pot.

Determination of the lethal dose 100 (LD_{100}) and the lethal time 100 (LT_{100}) of the flours of *Pisum sativum* and *Phaseolus vulgaris* on *Sitophilus oryzae*

In the 1200 ml capacities glass pots disposed in two lots; a precise weighing of the sorghum grains (80 g, 90 g, 95 g and 100 g) weighed with the same balance were introduced in each lot. Every lot was powdered and supplemented by pea’s flour or bean's flour also weighed with the same balance in opposite direction so that each pot had a content 100 g; then the content of each pot was homogenized. For each trial, 5 replications were made. In each pot, 10 couples of *S. oryzae* of two weeks old were introduced. After every 10 days till 50 days, all the pots were checked and the mortalities of insects were noted.

Persistence of the insecticidal efficiency of the LD_{100} of flours of *Pisum sativum* and *Phaseolus vulgaris* on *Sitophilus oryzae*

The study of the persistence activity of 2 flours was made by introduction of insects on treated sorghum grains with the LD_{100} put in glass pots. The mortality was noted 50 days later. Two batches of pots were prepared: one with the LD_{100} of pea (5 g) and the other with the LD_{100} of black bean (10 g). All the pots treated that the persistence was to be evaluated were prepared the same day, this day was called "day 0" and kept in pots. On the day 1 and on every 15 days till the day 90, 10 couples of *S. oryzae* were introduced in the pots. For every trial, five replications were made each time. Dead insects were counted 50 days after application.

Statistical analyses

Data on the mortality rates of the insect were subjected to one-way variant analysis (ANOVA I) test followed by a classification by the Duncan's multiple range test in the aim at determining the most insecticidal legume flour and the most efficient treatment on the adult of *S. oryzae*.

RESULTS

Efficacy of the crude flours of seeds of leguminous crops towards *Sitophilus oryzae*

The toxicity by the contact-ingestion on the adults of *S. oryzae* exposed to the crude flours used 50 days after application was recorded in Table 1. The results clearly showed the flours of pea *P. sativum* and black bean *P. vulgaris* had insecticidal effect towards rice weevil *S. oryzae*. The flours of two varieties of soybean *G. max* (SJ320 and DOCKO) were not insecticidal on this insect pest. After one-way ANOVA test followed by a classification by the Duncan's multiple range test, there is a very high significant difference between the treatments (F=541.109, df= 20; 84); it is also evident that the values of mortality rates of the two varieties of soybean excepted the treatments at the 100% concentration do not differ significantly for the control (F=1.583 ns, df=44). All the treatments of bean and pea have induced 100% of mortality. Pea and black beans are the most active leguminous crops towards *S. oryzae.*

Determination of the lethal dose 100 (LD_{100}) and the lethal time 100 (LT_{100}) of flours of *Pisum sativum* and *Phaseolus vulgaris* on *Sitophilus oryzae*

The killing activities of the two flours towards the targeted insect pest *S. oryzae* were evaluated by the calculation of the lethal dose (LD) and the lethal time (LT). The presided LD and LT were determined by the proportion of the experimental population killed. The LD_{100} and LT_{100} were not the same for both flours on the rice weevil *S. oryzae* (Table 2). In the Table 2, the flour of pea was more active than the flour of black bean. The LD_{100} of pea and its LT_{100} were respectively 5 g and 20 days against those of black bean which were respectively 10 g and 50 days. The sensibility of *S. oryzae*, the main harmful insect in the subtropical Africa is function of plants, doses and duration of exposition.

Persistence of insecticidal activity of the tested flours of *Pisum sativum* and *Phaseolus vulgaris* on *Sitophilus oryzae*

The results from Table 3 show that the mortality rates remained constant with time following introduction of insects. After one-way ANOVA test followed by a classification by the Duncan's multiple range test, the values of mortality rates do not differ significantly for the pea (F=1,91 ns, df= 6; 27, P≤ 0.05) and the black bean (F=2,325 ns, df= 6; 27, P≤ 0.05).

Table 1: Mortality rates induced by contact-ingestion of different doses of four flours leguminous crops against *Sitophilus oryzae* 50 days after application.

	0 g (control)	**20 g**	**40 g**	**60 g**	**80 g**	**100 g**
Pisum sativum	1,92±0,88^{a}	100±0^{b}	100±0^{b}	100±0^{b}	100±0^{b}	100±0^{b}
Phaseolus vulgaris	1,92±0,88^{a}	100±0^{b}	100±0^{b}	100±0^{b}	100±0^{b}	100±0^{b}
***Glycine max* DOCKO**	1,92±0,88^{a}	9±5,83^{a}	6±5,83^{a}	3±2^{a}	3±2^{a}	100±0^{b}
***Glycine max* SJ320**	1,92±0,88^{a}	4±3,70^{a}	1±2^{a}	2±2,4^{a}	5±5,5^{a}	100±0^{b}

The values followed by the same letter don't differ significantly (F=541.109, Df= 20; 84), P ≤ 0.01.

Table 2: Insecticidal efficiency through determination of lethal doses and lethal times of seeds' flour of *Pisum sativun* and *Phaseolus vulgaris* used against *Sitophilus oryzae.*

	LD100 (g)	**LT100 (days)**	**r (n=5)**
P. sativum	5	20	0,7***
P. vulgaris	10	50	0,9***

r= coefficient of correlation

Table 3: Persistence of insecticidal activity of pea and black bean every 15 days till day 90 with the LD100 of each flour on *Sitophilus oryzae*.

Age (days)	**1**	**15**	**30**	**45**	**60**	**75**	**90**	**F**
Black bean	100±0	95±5	98±1.5	96±2.9	100±0	100±0	100±0	2.325ns
Pea	100±0	97±4	100±0	98±2.4	100±0	100±0	100±0	1.91ns

F= values of Fisher, ns= not significant, degree of freedom (6; 27), P ≤ 0.05.

DISCUSSION

With all the disadvantages of use of chemicals, it becomes an emergency to build up storage tools that are user-friendly methods with low adverse or no effect on environment, on consumers and on useful organisms. One of the most important deficiencies of industrial pesticide is their persistence in the environment and their stability throughout trophic chains (Ngamo Tinkeu, 2000; Dal Bello et al., 2001; Ngamo Tinkeu et al., 2007). In the past, peasants used local plants that they introduced in their granaries with crops in order to kill insects present or to repel those coming to infest their stored products; nowadays, with the development of phyto-pharmaceutical industries and liberalization of chemical insecticide markets this traditional know-how popular is disappearing (Tapondjou et al., 2002). In spite of the restrictions of the Montreal Protocol, few original alternative products are proposed by research for their replacement.

The utilization of flours of seeds of leguminous crops as protectant for stored cereals for human and animal consumption is important as an alternative to chemical pesticide. The seeds of these leguminous crops are edible by the population and the animals that indicate their safety and potential contribution in protection against attack of *Sitophilus* species. Indeed, Kim et al. (2003) indicated that the entomotoxin proteins are target specific and non toxic to the mammals. Therefore, such bioactive substances can be effectively used in the integrated pest management. Thanks to their consumption by the human beings and useful animals and the fact which are very biodegradable in the environment, the utilization of flours of seeds of leguminous crops as protectant for stored cereals for human and animal consumption is important as an alternative to chemical pesticide. They reduce consequently the risk of food poisoning and water polluting.

The difference of mortality rates (LT_{100}, LD_{100}) between the flours would be due to the qualitative and quantitative specific composition of the leguminous plants from which they were extracted (Boeke et al., 2004); also, the presence or not of anti-feeding, indeed, the pea's seeds are deprived of anti-feeding, which supports the food catch of the insect pest whereas those of common bean are rich, which limits the alimentation of *S. oryzae* (Boeke et al., 2004; Louis, 2004). This difference is especially linked to their biochemical constituents principally the entomotoxic proteins which are inherent to their taxonomy (Ussuf et al., 2001; Louis, 2004). Indeed, the plant species can present some variations in the concentration and the quality of their secondary metabolites (Vasconcelos and Oliveira, 2004). In fact, *P. sativum* belongs to the tribe of Viciea and *P. vulgaris* to the tribe of Phaseolea (Louis, 2004). The leguminous plants are the plants rich in entomotoxic proteins (Karimi et al., 2010; Mebarkia et al., 2012, Gupta et al., 2013). These proteins have the biological activities towards several insect taxa such as Coleopteran, Lepidopteran and Homopteran (Ussuf et al., 2001; Regnault-Roger et al., 2002 Louis, 2004; Karimi et al., 2010). In the pea, the entomotoxic protein is called albumin PAb1; there is also a lectin PSA; in bean, the entomotoxic factor is arcelin and lectin PHA (Iulek et al., 2000; Louis, 2004; Silué, 2009; Karimi et al., 2010). Other researchers have shown the anti-insect activities for these and other sources of the flours of leguminous seeds (Carlini and Grossi-de-Sa, 2002; Louis, 2004; Wang et al., 2006; Fatimé, 2007; Tamgno, 2009; Mebarkia et al., 2012).

About the persistence of insecticidal activities of two flours, it is clearly evident that the mortality rates stay practically constant with the time before introduction of *S. oryzae*. The constant persistence of the efficacy of these flours would be linked to their biochemical and chemical constituents that are not volatile, nor photo labile (Regnault-Roger et al., 2002). The only inconvenient of the very long storage of these legumes under dried seeds forms is the necessity of anchorage before cooking (Castro et al., 2005). Indeed, the legumes are able to retain their biological activity for several years in dry seeds (Petit et al., 2005; Mebarkia et al., 2012).

Conclusion

With the dominance of dangerous synthetic insecticides in the northern Cameroon, the search of alternative methods for the protection of stored products against harmful insect pests is imperative. The edible legumes are well known by the local farmers and can be welcomed as natural insecticides. The present study examined the persistence of crude flours of seeds of *P. sativum* and *P. vulgaris* (Fabaceae) after the determination of the insecticidal activities of *G. max*, *P. vulgaris* and *P. sativum* towards *S. oryzae* for use as botanical or biological insecticides on cereals grains generally and sorghums or millets grains particularly in northern Cameroon. This study showed the LD_{100} and LT_{100} are respectively 5 g and 20 days for pea against 10 g and 50 days for black bean. The flour of pea is more active than bean's one. As concern persistence of insecticidal activity, the two flours leguminous crops don't lose their activity during 90 days. These two flours of seeds can be exploited for treatment of stored post-harvest cereals to reduce pest losses.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

LSNT is the supervisor of the work, he defines the main lines of the study; BRT has done a realization of the study, he makes all the bioassays; MG supplies the soybeans that have been used in the bioassays.

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