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Diversity of plants used to store cereals and leguminous and evaluation of the potential use of three aromatic plants against maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract
Study was conducted to inventory pesticidal plants used by smallholders to avoid attack of insect's stored products at northern Cameroon. Then, to evaluate the effectiveness of three most reported against maize weevil. To this end, a survey was conducted and samples of plants were collected and identified. Granaries were setting up and a dose 1% w/w powder of each plant was mixed to maize. It was recorded that plants listed by producers belong to 16 families. *Lippia rugosa* (L) (Verbenaceae) reduces significantly the impact of *S. zeamais* on maize stored in granary compared to *Hyptis spicigera* (Lamiaceae) ($X^2 = 1263.98$, $P<0.01$) and *Xylopia aethiopica* (Annonaceae) ($X^2 = 146.35$; $p<0.01$). These results highlight that *L. rugosa* and *X. aethiopica* keep their efficiency even in an open storage device like the granary. They can be used as alternative method to reduce application of chemical products on stored maize.

Keywords: Granary, insect pests, northern Cameroon, pesticidal plants, stored products

1. Introduction

The main challenges that the human being faces are not only the availability of food but also keeping it preserving quality and quantity [2]. In the Sahelian savannas of sub-Saharan Africa which included northern part of Cameroon, the dry season stand from six to nine months a year, while the wet season which is associated to farm production is very short, about three months [3]. To made food available along the year, farmers should succeed the storage. To achieve this goal, the most practice currently used is the applying of the chemical pesticides [12]. However, repeated use of chemical pesticides mostly of poor quality has raised health and environmental concerns [6] and they induce resistant strains or the resurgence of pests [8]. Furthermore, much mismanagement of these pesticides is observed at the peasant level in Central and West Africa [18, 15] aggravating the sanitary and environmental consequences. There is a need to urgently address this chemicals management problem. For this purpose, use of botanical products as an alternatives method was the subjects of multiples studies [4, 26, 21]. In fact, in the areas where the storage of food is a habit practice, the use of local biodiversity products, namely pesticidal plants, as a source of protection of foods is current [10, 25, 22, 17]. In sub-Saharan Africa, at least 30 pesticidal plants mentioned by farmers are regularly harvested and used to protect stored products [1]. Then year ago, in 2007, a survey conducted at the northern Cameroon, had revealed that these pesticidal plants belong to 17 botanical Families and the most important ones are Poaceae, Labiateae, Asteraceae, Annonaceae and Fabaceae [13]. Many studies conducted in laboratory conditions have shown that some of these plants have an insecticidal effect against various stored product insects. According to smallholders, the practice consists to introduce plants into the granaries in the same time with the product to store [1]. However, full documentation and scientific evaluation of this peasant practice, consisting to use plants directly into the granaries for pest management purposes is still lacking.

Hence, three local plants, included *X. aethiopica, H. spicigera* and *L. rugosa,* already being used by farmers as grain protectants were chosen. The aim of the present study is to investigate in order to validate or invalidate and optimize the use of these three cited potential pesticidal plants in reducing maize grain storage losses due to *S. zeamais*.

2. Materials and methods

2.1 Presentation of sampling sites and surveys procedures

The present study was conducted at the north part of Sudano-sahelian zone of Cameroon. This zone is characterized by annual average rainfall of 700 mm. Unimodal rainfall rules there with a short rainy season (June to September), major rain are recorded from July to August. Annual total precipitations vary between 400 mm to 1000 mm with a mean temperature around 28°C.

Concerning the inventory of plants used by farmers to protect stored products, a survey was conducted in 78 localities of North Cameroon and 476 smallholders were interviewed. Outcomes of this interview, some active plants were collected and kept in herbarium for further identification. These plants were identified by Professor Mapongmetsem Pierre Marie (botanist, laboratory of biodiversity and sustainable development, Faculty of Science, the University of Ngaoundere-Cameroon) and compared to the reference collection of the National Herbarium (Yaounde/Cameroon).

2.2 Evaluation of the insecticidal effect of three active plants

2.2.1 Biological material

In accordance with the results obtained after the survey, three most cited active plants were chosen and used as botanical pesticide on corn weevil *S. zeamais*. They were: *X. aethiopica, H. spicigera* and *L. rugosa*. Thus, the dried fruits of *X. aethiopica* were purchased on the Ngaoundere market; they are collected in Ngaoundal (06°31 N; 13°17 E; altitude 930m). The dry inflorescences of *H. spicigera* were collected in Malang (07°27 N; 13°03 E; altitude 1131m) in the district of Ngaoundere III (Adamawa - Cameroon). The fresh leaves of *L. rugosa* were collected at Mbé (07°51 N; 13°36 E; altitude 603m).

The plant materials collected were shade-dried during two weeks then crushed in a wooden mortar separately and sieved using a 5 mm sieve to obtain powders. It is in this form that the pesticidal plants were applied on maize grain as admixtures when filling the storage modules.

The commodity considered in this experiment was white CMS 8504 maize collected from producer groups in the Mbe area (Adamawa, Cameroon). This maize have been sorted, vanned and weighed before its introduction into the granary.

2.2.2 Experimental design and bio essay

An experimental design consisting of 12 experimental granaries was set up. Experimental granaries were built à Ngada Mabanga (Ngaoundéré, Cameroun) according to farmer's design. The model used were the earthen bottle type with 4 compartments (Fig. 1 a) which is the widely spread and commonly used by smallholders in the study area. Granaries were filled and each compartment received a bag of 100kg of corn. Powders of plants were used at doses 1% w/w. Each treatment was replicated 3 times. Three untreated granaries served as a control. To fill these 12 granaries, it took 4800kg of corn and 12kg of powder of each of the three aromatic plants.

2.2.3 Granaries infestation follow-up

One week after filling the twelve granaries, each compartment was infested with ten couples of the same strain of maize weevil standardized in the laboratory, which aged varied from 10 to 15 days. In each compartment, a pitfall trap (Fig. 1 c) with an opening at 50cm from the surface was installed. The traps were located in the central position of the compartment and were removed every 15 days through the observation period which was 170 days. At each extraction of the pitfall, the contents of traps was grouped in a tube and carried to the laboratory. At the laboratory, trapped insects were identified and counted. Cane probe (Fig. 1 b) were used to extract grains from granaries every 15 days. These collected grains were placed in 1200 ml glass jars at laboratory for observation. After 45 days, the contents of jars were sieved in order to observe emergence of insects and to assess level of infestation of the maize stored in granaries.

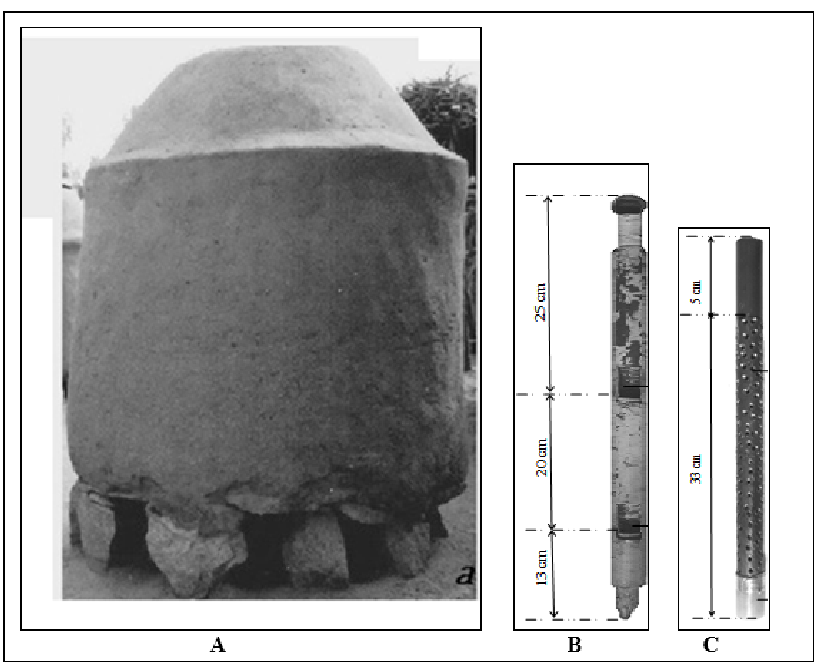

Fig 1: a-Earthen bottle granary type; b- trap probe; c- pitfall trap.

2.2.4 Grain damage assessment
At the laboratory, the effect of the three plants *L. rugosa*, *H. spicigera* and *X. aethiopica* on *S. zeamais* were evaluated in glass jars of 1200ml of capacity served as mini granary for the storage of 500g of maize. The treatments used were 5%, 10% and 15% w/w of each pesticidal plant then an untreated control. For each treatment five replications were done. After mixed maize and plant product in the jar, five pairs (males and females) of young weevils aged between 10 to 15 days are added into the jar. Thus, the contents of jars were monitored during 100 days and destroyed afterwards. The parameters noted are: the number of grains presenting the attack symptoms, the weight loss of the grains, the percentage of attack and the number of insects alive.

2.3 Statistical analysis
The data collected were processed, expressed as mean and standard deviation. The Graph Pad Prism 5 software permits to perform an analysis of variance and to classify the averages according to their significances.

3. Results and discussion
3.1 Diversity of plants used to protect stored products at the sudano sahelian zone of Cameroon
It is come out from analysis of farmer's responses; and identification of samples of plant collected from field that plants potentially insecticide used to protect stored foods at the northern part of Cameroon belong to 16 botanical Families. These results are in accordance with those of [17] who identified 17 botanical Families in the same area. These observations suggest that in this zone, regardless of massive utilization of chemical pesticides due to the liberalization of phytopesticide market, use of plants as protectant product remains a current practice at the smallholder's level. The most commonly plants cited are identified as belonging to the Family of Lamiaceae with 336 citations, Family of Meliaceae with 262 citations Family of Rutaceae with 157 citations and Family of Solanaceae with 113 citations. Those cited between 50 to 100 times are Family of Fabaceae with 81 citations, Moraceae (57 citations), Sapindaceae (51 citations) and Balinitaceae (65 citations). The least cited, less than 50 times are the Families of Vernonaceae (42 citations), Anacardiaceae (26 citations), Asteraceae (6 citations), Polyaceae (2 citations), Poaceae (38 citations), Myrtaceae (10 citations), Cucurbitaceae, Caesalpiniaceae (23 citations) and Euphorbiaceae (1 citation).

According to table 1, it highlights that in the northern part of Cameroon, various plants are introduced into the storage modules to kill or repel insect pests from stored commodities. Among these plants, *H. spicigera* which is cited 283 times is the best known and the most used, followed by *Azadirachta indica* A. Juss (Meliaceae), cited 213 times. The third plant was *Vepris heterophylla* (Engl.) Letouzey (Rutaceae) cited 157 times. It is also recorded that various part of plants were collected by producers for this purpose including: barks, leaves, seeds, roots or fruits. The whole plant, in the case of annual herbaceous was also collected and introduced into the granaries.

The plants used as protective agents are introduced into the stored food whole or after been reduced into powder, ash or as extracts. Major parts of these protective plants are seasonal and thus only available during the wet season or during the dry season. However, some of them such as *V. hetrophylla, A. indica* are perennials and are present year round.

H. spicigera and *V. heterophylla* which are aromatic plants were studied and it is revealed that it is certain components present in their essential oil which induce toxic effect against pests. According to [20], the essential oil of *H. spicigera* had two main components: 1,8-cineol and (E) caryophyllene. Other active compounds found in this essential oil were α-pinene, β-pinene, α-terpineol and linalool. The essential oil of *V. heterophylla* contained elemol, sabinene, (E)-β-ocimene, guiaol, limonene, (E)-caryophyllen and additional compounds such as myrcene and terpinolene. Concerning *A. indica,* its insecticidal properties are well documented [27]. The main compound which has adverse effect on insect is azardirachtin [23, 11].

Table 1: Diversity and frequency of use of plants potentially insecticide by northern Cameroonian's smallholders.

Species	Family	% of use	Targeted stored Products
Hyptis spicigera	Labieae	59,45	sorghum, cowpea, bambara groundnut
Azadirachta indica	Meliaceae	44,74	All kind of product (seed)
Vepris heterophylla	Rutaceae	32,98	sorghum, cowpea, bambara groundnut, maize, groundnut
Capsicum annuum	Solanaceae	19,95	sorghum, cowpea, bambara groundnut
Balanites aegyptiaca	Balanitaceae	13,65	sorghum, cowpea, groundnut
Ficus abitulifolia	Moraceceae	11,97	sorghum, cowpea, bambara groundnut, maize, groundnut
Ocimum canum	Labieae	11,13	sorghum, cowpea, bambara groundnut, maize, groundnut
Allophylus africanus	Sapindaceae	10,71	sorghum, cowpea, groundnut
Kaya senegalensis	Meliaceae	10,29	sorghum, cowpea, maize, groundnut
Daniellia oliveri	Fabaceae	9,03	sorghum, cowpea, millet, maize, groundnut
Lippia rugosa	Verbenaceae	8,82	sorghum, cowpea, millet, maize, bambara groundnut
Rottboellia cochincinensis	Poaceae	7,98	sorghum, cowpea, millet, maize, bambara groundnut
Cissus populnea	Vitaceae	6,51	sorghum, cowpea, millet, maize, groundnut
Sclerocaria birrea	Anacardiaceae	5,46	groundnut, cowpea, millet, maize, bambara groundnut
Cassia sieberiana	Caesalpiniaceae	4,83	groundnut, cowpea, millet, maize, bambara groundnut
Arachis hypogea	Fabaceae	4,41	cowpea, groundnut
Nicotinia tabacum	Solanaceae	3,78	groundnut sorghum, cowpea, millet, maize, bambara groundnut
Prosopis africana	Fabaceae	3,57	cowpea, bambara groundnut
Eucalyptus sp	Myrtaceae	2,10	cowpea, groundnut, bambara groundnut
Momordica charantia	Cucurbitaceae	1,68	groundnut cowpea, bambara groundnut
Vernonia amygdalina	Asteraceae	1,26	All kind of stored product
Securidaka longipedunculata	Polygaceae	0,42	cowpea, bambara groundnut groundnut
Recinus communis	Euphorbiaceae	0,21	cowpea, groundnut

3.2 Diversity of insects in granaries and efficacy of three aromatic plants on maize weevil

3.2.1 Entomofauna of granaries

The identification of insects trapped in the various granaries revealed the presence of six insects' species, among them; three belonged to Order of Coleoptera. It was *S. zeamais* (Curculionidae), *Tribolium castaneum* (Tenebrionidae) and *Oryzaephilus surinamensis* (Silvanidae). There was found one specie belonging to Order of Lepidoptera identified as *Ephestia kuehniella* (Pyralidae) and *Anisopteromalus calandrae* (Pteromalidae) which is a specie belonging to Order of Hymenoptera (Table 2). The relative abundance of theses insect's species and their status are reported at Table 2.

Table 2: Insects trapped in granaries during the essay period.

Order	Family	Genera	Specie	Status	Year 1 (%)	Year 2 (%)
Coleoptera	Curculionidae	*Sitophilus*	*S. zeamais*	Primary pest	82,78	2,45
Coleoptera	Tenebrionidae	*Tribolium*	*T. castaneum*	Secondary pest	4,85	43,68
Lepidoptera	Pyralidae	*Ephestia*	*E. kuehniella*	Secondary pest	10,09	0,73
Coleoptera	Silvanidae	*Oryzaephilus*	*O. surinamensis*	Secondary pest	/	53,12
Hymenoptera	Pteromalidae	*Anisopteromalus*	*A. calandrae*	Prasitoid	2,28	/

According Table 2, the number of insects trapped in granaries has varied qualitatively and quantitatively within the first and second year of study. Thus in the first year of study, *S. zeamais* was dominant and constitute 82.78% of trapped insects and its parasitoid *A. calandrae* was present and represent 2.28% of insect captured. According to [24] and [14], *S. zeamais* is the main pest of stored maize in granary in northern Cameroon. The second year of experiment revealed the dominance of *O. surinamensis* and *T. castaneum,* no parasitoid was found.

3.2.2 Bio efficacy of plant powders against *Sitophilus zeamais* in the granaries

Efficiency of the three active plants used was evaluated through the number of *S. zeamais* emerged in different granaries treated with plants. According to the figure 2, significant infestation of this main pest was observed only 100 days after its inoculation in the granaries without plants and treated whith *H. spicigera*. Less than two months, the storage of maize does not require treatment. According to this observation, smallholders are advised to treat their stock of maize in granaries only if they want to store it up to three months. *L. rugosa* and *X. aethiopica* protect corn up to 120 days. *H. spicigera* beyond this period loses all its effectiveness. There was not difference between number of *S. zeamais* trapped in granaries treated with *H. spicigera* and granaries which serve as control. The loss of activity of this plant results from of degradation of the active compounds present in this aromatic plant [7, 5]. Furthermore, Jirovetz *et al* [9] and Ngassoum *et al* [19] showed that essential oil of *L. rugosa* is rich in oxygenated monoterpenes and sesquiterpenes which are relatively less volatiles aromatic compounds than hydrocarbon monoterpenes which majors compounds found in essentials oil of *H. spicigera*. In the granary which constitutes a non-hermetic storage device, *H. spicigera* will lost its effectiveness very quickly. These results suggest that, *H. spicigera* must not be recommended to protect maize in granaries.

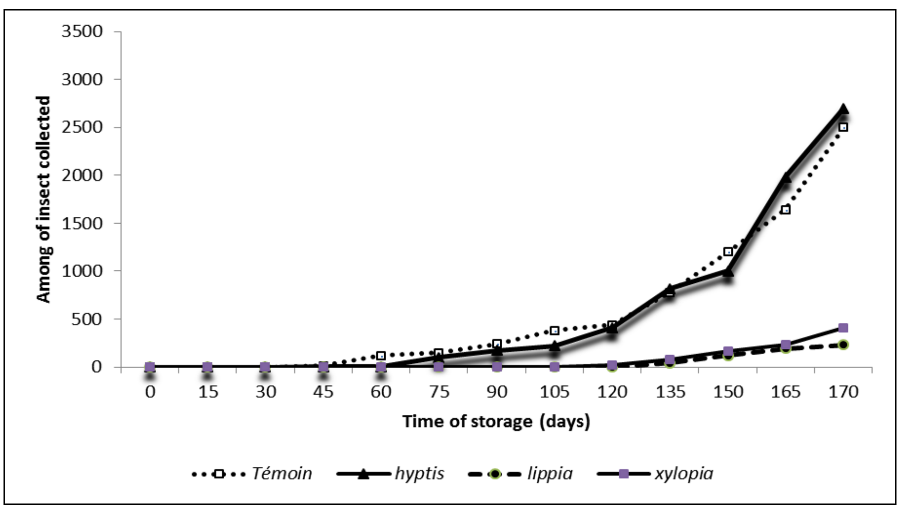

Fig 2: Effect of plants' powders on degree infestation of corn by *Sitophilus zeamais* during 170 days of storage.

3.2.3 Effect of plants on the parasitoids *Anisopteromalus calandrae* in the granaries

At first year of study where *A. calandrae*, the natural enemy of *S. zeamais,* were found into different granaries and individuals trapped are counted. It is reported that Hymenopteran parasitoids are frequently used as natural enemies in various biological control programs [30, 29, 28]. The results presented in Figure 3 shown the evolution of number of this insect during the study. It's appears that the parasitoids emerge witnesses from the 30th day in granaries treated with *L. rugosa* and in those which represent the control. This number increases along the storage period. However, in granaries treated with *H. spicigera* and *X. aethiopica*, the emergence of parasitoid was observed from 90 days. At the end of the experimental period of storage, among of individuals trapped in granaries treated with *H. spicigera* remains low. These results suggest that the powder of *H. spicigera* has more adverse effect on *A. calandrae* than powder of *X. aethiopica* or *L. rugosa*. In fact, parasitoid insects as *A. calandrae* localize and recognize their hosts by their odors [16]. In this process of recognition, the odors emitted by plants, which inconvenience pests and away them by protecting the grains can have an effect on them. According to these observations, it can be concluded that of *H. spicigera* must not be recommended to use into granary to protect maize because it have strongly adverse effect on *A. calandrae* which a benefit insect.

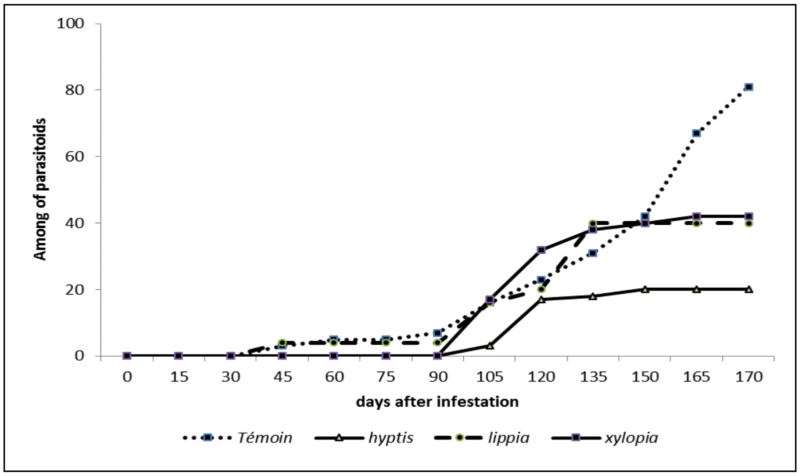

Fig 3: Effect of plants' powders on development for 170 days of parasitoids

3.2.4 Effect of powders of plants on degree of infestations of white corn CMS 8504 by the *Sitophilus zeamais* in laboratory essay

The efficacy of powders of *X. aethiopica*, *L. rugosa* and *H. spicigera* was evaluated in laboratory during 100 days. Table 3 shows the number of grains attacked by *S. zeamais* for 100 days in the presence of varying proportions of the powders of three plants.

It appears that the number of grains attacked varies according to doses and plants used. At 5%, the number of grains attacked is high in each treatments compared to 10% and 15%. With all treatments, there is a significant difference between treated and untreated maize excepted maize treated with *L. rugosa* where it was no significant difference between the three doses used. With *X. aethiopica* and of *H. spicigera*, the number of grains attacked decreased from 5% to 15% (Table 3) and there was significant difference between jar treated with plants and the control. These results suggest that in hermetic storage device, *H. spicigera* is most efficient on *S. zeamais* than *Lippia rugosa*. However, in relative open storage device like a granary, it appears that the efficacy of *H. spicigera* is rapidly loose.

Table 3: Effect of variation of doses of *Xylopia aethiopica, Lippia rugosa* and *Hyptis spicigera* on the level or percentage of attack of maize grains observed during 100 days in laboratory.

	X. aethiopica	***L. rugosa***	***H. spicigera***
Control	301 ± 10.69 a	301 ± 10.69 a	301 ± 10.69 a
5%	140.6 ± 29.14 b	255 ± 97.3 ab	184.3 ±43.6 b
10%	103 ± 22.8 bc	198 ± 41.7 b	113.6± 14 c
15%	70 ± 21.6 c	216 ± 48.1 ab	63.3 ± 14.7 d
F (ndl : 3 ; 11)	66.42***	2.17ns	69.94***

In the same column the values followed by the same letter are not significantly different.

3.2.5 Yield losses of maize stored in laboratory conditions due to *Sitophilus zeamais* attacks

According to table 4, it is appears that the level of attack and yield of weight losses depend of the quantity of the material used and the plant species. With *X. aethiopica,* at the mass ratio 5%, 10% and 15%, the percentage of seeds damaged is 5.37%, 3.86% and 2.57% respectively. However, there was no significant difference ($P \leq 0.05$) between effects of the three doses according to *L. rugosa*. In the presence of *X. aethiopica* and *H. spicigera, S. zeamais* is less aggressive and causes fewer losses. Registered attacks at the dose 15% are 2.57% and 2.34%, respectively.

Table 4: Variation of yield attack (A%) and weight losses (B%) of maize after 100 days of infestation by *Sitophilus zeamais* according to various doses of the three plants used.

	X. aethiopica		***L. rugosa***		***H. spicigera***	
	A%	**B%**	**A%**	**B%**	**A%**	**B%**
Control	10.15 ± 0.92 a	1.17 ± 0.1 a	10.15 ± 0.92 a	1.17 ± 0.1 a	10.15 ± 0.92 a	1.17 ± 0.1 a
5%	5.37 ± 0.91 b	0.62 ± 0.1 b	9.49 ± 3.6 a	1.09 ± 0.42 a	7.05± 1.59 b	0.88 ± 0.19b
10%	3.86 ± 0.82 b	0.44 ± 0.09 c	7.43 ± 1.58 a	0.86 ± 0.18 a	4.24 ± 0.56c	0.53 ± 0.05c
15%	2.57 ± 0.86 c	0.29 ± 0.09 c	8.1 ± 1.81 a	0.93 ± 0.29 a	2.34 ± 0.54 d	0.29 ± 0.05d
F (ndl :3 ; 11)	50.99***	46.42***	1.95ns	1.42ns	37.05***	34.88***

In the same column the values followed by the same letter are not significantly different.

4. Conclusion

It's appeared that smallholders of northern Cameroon know and use various plants to protect their product during the storage period. Sixteen families of plants were identified. According to efficacy of plants, it was observed that L. *rugosa* and *X. aethiopica* regularly introduced into the granaries by the farmers protect maize up to 120 days against weevil. They are active longer and protect maize more than *H. spicigera*.

Under the granary storage conditions, it would be better to protect maize by *L. rugosa* because this plant is more effective against maize weevil and have less adverse effect on parasitoids. On the other hand, the use of *H. spicigera* into the granary to protect maize must be discouraged because it has low effect on the pest but strongly inhibits the emergence of parasitoids. However, *H. spicigera* is best when it is used in closed storage device.

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