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Use of NeemPro®, a neem product to control maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) on three maize varieties in Cameroon

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Abstract

Background: *Sitophilus zeamais* is a key pest of stored maize causing serious economic damage. The predominant control of this pest is the use of synthetic residual pesticides, which have adverse effects on consumers and environment. The use of phytochemicals for controlling storage pests constitutes an attractive alternative to synthetic products, since plant may be more biodegradable and safer. The aim of this study was to evaluate the activity of NeemPro® against the maize weevil on three maize varieties in the laboratory and the effect of the insecticide on seed germination.

Results: NeemPro® relatively killed all the exposed weevils at 6 g/kg within 14 days with LC_{50} of 0.04, 0.07 and 0.11 g/kg in CLH103, CMS8501 and SHABA varieties, respectively, as observed in Malagrain. Treatments completely hindered or significantly reduced progeny emergence, percentage grain damage, grain weight losses, but did not affect grain germination after 4 months of storage. However, these parameters were lesser in SHABA variety.

Conclusions: NeemPro® may be used as alternative to Malagrain for the protection of stored maize against the infestation of *S. zeamais*.

Keywords: NeemPro®, *Sitophilus zeamais*, Maize varieties, Pest management, Cameroon

Background

In sub-tropical and tropical regions, *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) is the main pest of stored maize grain [1]. In Cameroon, maize is the main food crop and *S. zeamais* alone has already caused 80 % of grain damage during the period of storage [2]. Demissie et al. [3] reported that once the grains are damaged, this will reduce the market value, the percentage of germination, the weight and the nutritional value. That is why in Africa, effective and cheap methods are needed to reduce the damage caused by *S. zeamais* and so, to reduce food insecurity [3]. Infestation control of stored grains pests is primarily achieved by the use of synthetic chemical insecticides. However, due to environmental concerns and human health hazards of chemical insecticides, plant materials with insecticidal properties remain one of the most important locally available, biodegradable and inexpensive methods for the control of pests of stored products [4, 5].

Azadirachtin, the active insecticidal ingredient of *Azadirachta indica* A. Juss, (Meliaceae) [6], is found to be an environment-friendly pesticide and has many desirable properties. It is also selective with short persistence, toxic to target pests, has minimal toxicity to non-target and beneficial organisms and caused less damage to the ecosystem [6–9]. For these reasons, it has generated enormous worldwide interest due to its potential as a new insect pest control agent [10].

One way of promoting neem is to develop proprietary products. The knowledge of the high potency of

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azadirachtin against a wide variety of crop pests has now resulted in the development of many commercial neem formulations (CNFs) such as Nimbecidine, Econeem Plus, Soluneem, Limonool, Neemgold, Fortune Aza, NeemAzal TM-F, Margocide-OK, Neemark, Neem Oil Emulsion, Neem Plus, Neemrich, Neemosan, Neemta 2100, Nimlin, Margosan-O, Bioneem, Suneem [11, 12]. These formulations provide two great advantages. Firstly, the fragile natural resource, azadirachtin, is highly unstable in the seeds and the potency of the seeds is lost exponentially upon storage. Secondly, CNFs provide an avenue to conserve this resource by reducing the rate of loss of azadirachtin in many folds [12]. Hence it is important to generate further information on the biochemical effect of azadirachtin and its commercial insecticides. The objective of this study was to evaluate the efficacy of NeemPro® against the maize weevil on three maize varieties in the laboratory. This formulation protected stored bambara groundnut against the infestation of *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae) in the Adamawa region of Cameroon [13]. However, this is the first report of the same product in the protection of stored grains of three maize varieties, the most cultivated ones in Cameroon, against the infestation of *S. zeamais*.

Methods

Maize varieties

Grains of three maize varieties presented in Table 1 were collected from the Institute of Agricultural Research for Development (IARD), Nkolbisson-Yaounde (Cameroon). The moisture content of the grains was 11.30, 11.50 and 13.20 for CLH103, CMS8501 and SHABA, respectively.

Insects rearing

Maize weevils were reared on whole clean, undamaged and disinfested maize grains SHABA, the composite mostly grown by Adamawa farmers under ambient laboratory conditions. Adult weevils were obtained from a colony kept since 2005 in the Laboratory of Entomology at the University of Ngaoundere. Maize grains were sterilised in cold chamber at −14 °C for 21 days to kill any incipient infestation. The sterilised grains were conditioned during 14 days prior to rearing or bioassay processes. Twenty adults were released into ten glass jars (900 ml capacity) containing 500 g of conditioned grains each. The adults were removed after 2 weeks and the grains were kept under ambient laboratory conditions [temperature (T) = 21.9–24.4 °C and relative humidity (RH) = 75.3–78 %] for the development of progenies. Adults aged 7–14 days and mixed sexes were used for all bioassays.

Table 1 Genetic nature, grain surface texture and grain colour of the maize varieties used for the experiment

Variety	Genetic nature	Grain surface texture	Grain colour
CLH103	Hybrid	Semi-dent	Yellow
CMS8501	Composite	Corned	White
SHABA	Composite	Dent	White

Commercial insecticides

A commercial neem product, NeemPro® concentrated powder containing 0.1 % NeemAzal (0.03 g/kg Azadirachtin A) and mineral clay (diatomaceous earth) was provided by Trifolio-M GmbH Company, Lahnau, Germany. Malagrain DP 5 (5 % Malathion) was purchased from an agric-products shop at Ngaoundere, Cameroon.

Toxicity tests and F_1 progeny production

The application rates of NeemPro® were 0.75, 1.5, 3 and 6 g/kg after preliminary studies. These rates were obtained by adding 0.0375, 0.075, 0.15 and 0.3 g of the insecticide powder to 50 g of maize grains of each variety in a glass jar and shaken well by hands during 4 min to get uniform coating. Twenty (7–14 days old) adult weevils of mixed sexes were introduced into each jar. Each treatment was repeated four times. Treated and untreated controls were included. In the treated control, 0.025 g of Malagrain was introduced in 50 g grains of each maize variety (0.5 g/kg, recommended dose). For untreated control, neither NeemPro® nor Malagrain was used. All treatments were maintained in the laboratory at T of 21.7–25.6 °C and RH of 76.1–79 %, registered by a thermo-hygrometer EL-USB-2 (RH/TEMP DATA LOGGER) (Chine). The T and RH data were registered each 2 h from the beginning of the weevils rearing to the end of the experiments. Mortality was recorded 1, 3, 7 and 14 days after the infestation [14]. The mortality was corrected for control mortality using Abbott's formula [15].

On the 14 days post-infestation, where treatments were kept undisturbed for oviposition since the first day of the infestation, all insects were removed and the different jars containing grains were kept under the same experimental conditions (T = 22.6–25.6 °C and RH = 72.5–80 %). The counting of F_1 adults was done once a week for 5 weeks to avoid overlapping between the first and the second generations commencing 5 weeks post-infestation. The percent reduction in adult emergence or reproduction inhibition rate (IR %) was computed according to Chebet et al. [16] as shown in the following equation:

$$\text{Reproduction inhibition rate (\%)} = \frac{(\text{CN} - \text{TN})}{\text{CN}} \times 100$$

where CN is the number of newly emerged adult insects in the untreated control and TN is the number of newly emerged adult insects in the treated grains.

Population increase and grain damage

Four rates (0.75, 1.5, 3 and 6 g/kg of NeemPro® powder and 0.5 g/kg of Malagrain) for 200 g of each maize variety grain were admixed as described above. A lot of 30 adult insects of mixed sexes (7–14 days old) were introduced into each jar of each maize variety grain including treated and untreated controls. Each treatment with the same dosage for each variety was repeated four times. After 4 months, the numbers of alive and dead insects were removed and counted for each jar. Damage assessment was performed by measuring the weight of the sieved powder and that of the grains without powder (final weight). The amount of grain powder (frass plus faeces) was expressed as the total powder minus the weight of insecticide powder used. The percentage of grain weight loss was calculated by using the count-and-weigh method [17].

$$\text{Grain weight loss (\%)} = \left[\frac{(\text{Wu} \times \text{Nd}) - (\text{Wd} \times \text{Nu})}{\text{Wu}\,(\text{Nd} + \text{Nu})}\right] \times 100$$

where Wu is the weight of undamaged grains, Nd the number of damaged grains, Wd the weight of damaged grains and Nu the number of undamaged grains. All treatments were maintained in the laboratory conditions ($T = 24.1$–25.6 °C and RH = 70.7–74.5 %).

Seed germination

To assess the viability of seeds, seed germination test was similarly conducted according to the procedure described earlier by Demissie et al. [3] where 30 undamaged grains of each maize variety seed in each jar were randomly selected. The number of germinated seeds was recorded after 10 days.

Data analysis

The Statistical Analysis System [18] was used to analyse the data. Data on percentage of mortality, production of F_1 progeny, seed damage and seed germination were firstly arcsine-transformed [square root (x/100)]. The number of F_1 progeny produced was also log-transformed ($x + 1$). It is the transformed data that were subjected to the ANOVA procedure. The Tukey studentized test at $P = 0.05$ was used for mean separation. Finally, Probit analysis [19] was applied to determine lethal dosages causing 50 % (LC_{50}) and 95 % (LC_{95}) of *S. zeamais* mortality at 3, 7 and 14 days after treatment.

Results

Adult weevil mortality

The results of the phytotoxicity tests showed that NeemPro® caused significant mortality to *S. zeamais* in the three maize varieties (Table 2). Mortality increased with powder content and time post-exposure. At the highest dosage of 6 g/kg, 100, 95 and 89 % adult weevil mortality was achieved in CLH103, CMS8501 and SHABA, respectively, within 14 days of exposure. For the same time-point and in the same order, the lowest dosage of 0.75 g/kg caused 43, 18 and 10 % weevil mortality. The LC_{50} and LC_{95} values are presented in Figs. 1 and 2, respectively. After 14 days of exposure, CLH103 recorded better LC_{50} and LC_{95} values of 0.04 and 0.13 g/kg, respectively. Within 1 day of exposure, adult weevils exposed to Malagrain were dead and that was on the three maize varieties.

Emerging adult F_1 progeny

NeemPro® generally caused significant reduction in progeny production relative to the control, which was dose dependent (Table 3). Even the lowest dosage of 0.75 g/kg caused 84, 69 and 42 % suppression of F_1 progeny emergence in CLH103, CMS8501 and SHABA, respectively. Higher concentration levels roughly achieved complete suppression of progeny emergence in the three maize varieties. No progeny emergence was observed in Malagrain treatments.

Population increase, grain damage and germination

In general, the rate of increase of the population of *S. zeamais* was significantly reduced by NeemPro® (Table 4). From the dosages of 1.5 g/kg in CLH103 and 3 g/kg in CMS8501, the populations of the weevil were completely suppressed as in Malagrain. No alive insects were recorded after 4 months of maize storage, while with the highest content of 6 g/kg, 15 alive weevils were registered in SHABA variety. In addition, there were no significant differences between the main effects of the rate, NeemPro®, Malagrain and maize varieties in percentage grain damage, grain weight loss and germination (Table 4). However, there were slight differences of all the parameters in SHABA variety. Moreover, no undamaged grain was found in untreated tests and that was for the three maize varieties.

Discussion

Results of the present study show that the active ingredient of NeemPro®, azadirachtin, caused high mortality of *S. zeamais* on the one hand and completely hindered or significantly reduced progeny emergence on the other hand, indicating its potential use in the management of maize weevil. Earlier, the same NeemPro® was tested

Table 2 Corrected mortality of *Sitophilus zeamais* exposed to NeemPro® after 1, 3, 7 and 14 days on three maize varieties under laboratory conditions

Maize variety/content (g/kg)	% Mortality (mean ± SE)—exposure period (days)			
	1	3	7	14
CLH103				
0	0 ± 0	0 ± 0^{c}	0 ± 0^{b}	0 ± 0^{c}
0.75	0 ± 0	5 ± 2^{bc}	32.5 ± 13.92^{ab}	42.5 ± 9.24^{b}
1.5	0 ± 0	30 ± 13.69^{abc}	52.5 ± 22.59^{ab}	92.5 ± 4.33^{a}
3	0 ± 0	30 ± 3.54^{ab}	71.25 ± 15.73^{a}	92.5 ± 4.33^{a}
6	2.5 ± 1.44	43.75 ± 11.43^{a}	76.25 ± 12.81^{a}	100 ± 0^{a}
F value	3 ns	4.83*	4.36*	75.94***
CMS8501				
0	0 ± 0	0 ± 0^{b}	0 ± 0^{d}	0 ± 0^{c}
0.75	0 ± 0	3.75 ± 2.39^{b}	19.73 ± 4.11^{c}	17.73 ± 6.48^{bc}
1.5	0 ± 0	7.5 ± 3.23^{ab}	56.78 ± 8.04^{b}	57.95 ± 20.73^{ab}
3	0 ± 0	17.5 ± 5.95^{ab}	77.80 ± 12.37^{ab}	87.65 ± 6.15^{a}
6	1.25 ± 1.25	38.75 ± 11.43^{a}	93.1 ± 4.28^{a}	94.55 ± 2.42^{a}
F value	1 ns	6.63**	32.99***	16.28***
SHABA				
0	0 ± 0	0 ± 0^{c}	0 ± 0^{b}	0 ± 0^{c}
0.75	0 ± 0	3.75 ± 2.39^{bc}	10 ± 4.56^{b}	10.45 ± 3.56^{bc}
1.5	0 ± 0	8.75 ± 1.25^{ab}	24.25 ± 13.75^{ab}	26.20 ± 5.18^{b}
3	0 ± 0	12.5 ± 3.23^{ab}	55 ± 9.35^{a}	67.58 ± 11.92^{a}
6	0 ± 0	25 ± 7.36^{a}	63.75 ± 12.64^{a}	89.13 ± 6.32^{a}
F value	–	6.47**	8.4***	33.18***

Means in the same column for the same maize variety, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test); Number of replicates: 4; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *F* value: a ratio of two variances—the "between group" variance and the "within-group" variance

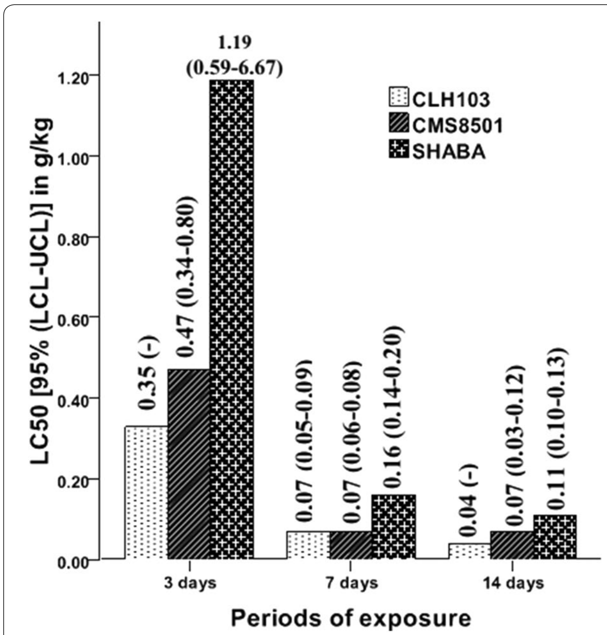

Fig. 1 LC_{50} of NeemPro® at 3, 7 and 14 days after treatment against adult *Sitophilus zeamais* on grains of three maize varieties under laboratory conditions

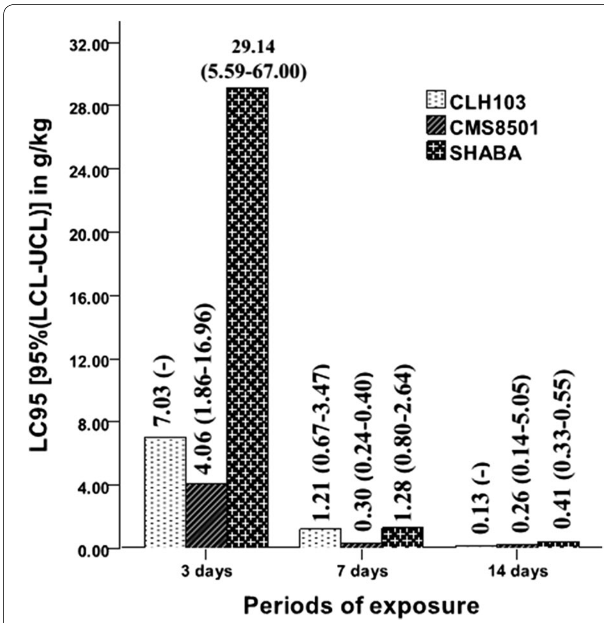

Fig. 2 LC_{95} of NeemPro® at 3, 7 and 14 days after treatment against adult *Sitophilus zeamais* on grains of three maize varieties under laboratory conditions

Table 3 Progeny production of *Sitophilus zeamais* on grains of three maize varieties treated with NeemPro® under ambient laboratory conditions

Maize variety/content (g/kg)	Mean number of F_1 adult progeny	% reduction in adult emergence relative to control
CLH103		
Malagrain	0 ± 0^{b}	100 ± 0^{a}
0	85.75 ± 17.31^{a}	0 ± 0^{c}
0.75	14.25 ± 3.82^{b}	84.25 ± 3.52^{b}
1.5	4.5 ± 1.66^{b}	93.95 ± 2.57^{ab}
3	2.25 ± 0.75^{b}	96.75 ± 1.51^{a}
6	1.25 ± 1.25^{b}	98.48 ± 1.52^{a}
F-value	20.56***	375.89***
CMS8501		
Malagrain	0 ± 0^{d}	100 ± 0^{a}
0	125.25 ± 4.59^{a}	0 ± 0^{c}
0.75	38.25 ± 10.84^{b}	68.58 ± 9.65^{b}
1.5	17.75 ± 4.44^{bc}	85.58 ± 4.03^{ab}
3	6.25 ± 2.75^{cd}	$94.83 \pm 2.35a^{b}$
6	2.75 ± 1.8^{d}	97.7 ± 1.5^{a}
F-value	75.92***	69.62***
SHABA		
Malagrain	0 ± 0^{c}	100 ± 0^{a}
0	79.25 ± 4.94^{a}	0 ± 0^{c}
0.75	44.75 ± 8.17^{ab}	42.33 ± 11.99^{b}
1.5	32 ± 7.87^{bc}	58.28 ± 1.28^{ab}
3	20.75 ± 8.25^{bc}	72.5 ± 11.21^{ab}
6	9.75 ± 4.23^{c}	87.03 ± 5.67^{a}
F value	15.04***	13.07***

Means in the same column for the same maize variety, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test); Number of replicates: 4; *** $P < 0.001$; *F* value: a ratio of two variances—the "between group" variance and the "within-group" variance

for its ability to protect Bambara groundnut against the infestation of *Callosobruchus maculatus*, regarding adult mortality as well as F_1 progeny and larval inhibition. The insecticide was admixed with Bambara groundnut seeds. The product caused 68.75 and 98.75 % adult mortality respectively within 1 and 6 days with 6 days-LC_{50} of 0.001 g/kg. The product completely inhibited F_1 progeny production at 3 g/kg [13]. In the same vein, Nukenine et al. [20] evaluated the effectiveness of NeemAzal PC KG 0.1 (0.1 % azadirachtin A) against *S. zeamais* in maize grains and found that within 14 days of exposure, maximum mortality of 99 and 100 % reduction in F_1 progeny were achieved at 12 g/kg. All tested concentrations completely suppressed the population increase of the weevil, had no damaged grains and recorded no weight loss.

In addition, the inhibition of *S. zeamais* progeny emergence and maize grain damage as a result of treatment with NeemPro® was probably due to the huge array of azadirachtin activities on the insect's hormone system. It has been proved that azadirachtin disrupts or inhibits development of insect eggs, larvae or pupae, preventing the moulting of larvae or nymphs, disrupting mating and sexual communication, deterring females from laying eggs, sterilising adults, poisoning larvae, thus preventing adult maturation by inhibiting the formation of chitin, the essential substance for the insect to form an exoskeleton [6, 10, 21, 22].

Moreover, NeemPro® protected the three maize varieties. These results corroborate earlier findings of Demissie et al. [3] who reported that Silicosec, filter cake and wood ash protected grains of three maize genotypes against *S. zeamais*. The rate of seed germination was not affected by NeemPro® as observed in Malagrain. Nukenine et al. [20] reported similar findings where NeemAzal did not have negative effects on maize seed germination (germination rates of 92.23 % at 3 g/kg to 97.77 % at 12 g/kg were recorded).

Besides, Neempro® was used to protect the tree maize varieties in this study. The reason of using three varieties was that a product may protect a variety more than others. Someone may conclude that a product has protected a variety from weevils' attack whereas the variety itself was resistant against the insect. This is due to the level of different physical and biochemical parameters such as grain hardness, kernel weight, protein content,

Table 4 Population increase (mean number of progeny for 4 jars ± SE) and damage parameters of *Sitophilus zeamais* on grains of three maize varieties admixed with NeemPro® and stored for 4 months under laboratory conditions and percentage of seed germination

Maize variety/content (g/kg)	Number of insects alive	Grain damage (%)	Weight loss (%)	Germination (%)
CLH103				
Malagrain	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	84.13 ± 1.24^{a}
0	669 ± 0^{a}	113 ± 0^{a}	99.99 ± 0.01^{a}	0 ± 0^{b}
0.75	5 ± 5^{b}	1 ± 1^{b}	0.13 ± 0.13^{b}	85.56 ± 2.94^{a}
1.5	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	86.67 ± 1.93^{a}
3	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	76.67 ± 1.93^{a}
6	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	82.22 ± 2.94^{a}
F value	86.63***	8607.81***	999.99***	218.06***
CMS8501				
Malagrain	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}	86.41 ± 1.02^{ab}
0	748 ± 28^{a}	106 ± 0^{a}	99.94 ± 0.01^{a}	0 ± 0^{c}
0.75	40 ± 20^{b}	5 ± 2^{b}	1.13 ± 0.44^{b}	85.55 ± 2.22^{ab}
1.5	6 ± 6^{bc}	1 ± 1^{bc}	0.61 ± 0.61^{b}	82.22 ± 2.94^{ab}
3	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}	78.89 ± 5.56^{ab}
6	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}	72.22 ± 294^{b}
F value	86.63***	970.42***	17674.85***	110.92***
SHABA				
Malagrain	0 ± 0^{d}	0 ± 0^{b}	0 ± 0^{b}	57.73 ± 2.05^{c}
0	762 ± 66^{a}	98 ± 0^{a}	99.98 ± 0.01^{a}	0 ± 0^{b}
0.75	476 ± 60^{a}	81 ± 12^{a}	58.31 ± 24.36^{a}	26.67 ± 13.47^{ab}
1.5	135 ± 48^{b}	28 ± 5^{b}	5.85 ± 1.12^{b}	37.78 ± 2.94^{a}
3	54 ± 21^{bc}	12 ± 2^{b}	1.54 ± 0^{b}	33.33 ± 1.93^{a}
6	15 ± 11^{c}	7 ± 3^{b}	0.69 ± 0.29^{b}	30 ± 3.33^{a}
F value	47.11***	44.29***	16.5***	6.27***

Means in the same column for the same maize variety, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test); number of replicates: 4; *** $P < 0.001$; *F* value: a ratio of two variances—the "between group" variance and the "within-group" variance

pericarp thickness, moisture content showed by different varieties [23]. In the present study, the product seems to have protected CLH103 and CMS8501 varieties more than SHABA in population increase, grain damage and germination. This may be due to the moisture content of SHABA which was 13.20 against 11.30 and 11.50 for CLH103, CMS8501, respectively.

Conclusions and recommendations

The study shows that NeemPro® is very effective against *S. zeamais* on the grains of three maize varieties (CLH103, CMS8501 and SHABA). This insecticide, not only kills the adult weevils, but also affects their progeny production. Additionally, it protects stored grains of the three varieties for 4 months without affecting the seed germination power. However, the product seems to have protected CLH103 and CMS8501 varieties more than SHABA. Therefore, NeemPro® can be used as post-harvest grain protectant against the infestation of the noxious *S. zeamais*. With this in mind, further research is needed in the future to investigate the effect of this botanical insecticide on other stored products pests and to determine the biochemical parameters of the three maize varieties.

Abbreviations

CLH: cameroon lowland hybrid; CMS: cameroon maize selection; LCL: lower confidence limit; UCL: upper confidence limit; LC: lethal concentration.

Authors' contributions

CA and ENN conceived the idea, designed the experiments and analysed the data. SPYD and GTF conducted the experiments. SPYD wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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