Wini Goudoungou *et al. Agric & Food Secur (2018) 7:26*
https://doi.org/10.1186/s40066-018-0179-z

Agriculture & Food Security

RESEARCH

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Effectiveness of binary combinations of *Plectranthus glandulosus* leaf powder and *Hymenocardia acida* wood ash against *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract

Background: Botanicals are generally assumed to be more biodegradable, leading to less environmental problems. Combination of botanicals could enhance biological activity against insect pests. Hence, the amount of botanical used for the control of stored grain pests may be minimised. In this study, the bioassay was carried out on *Sitophilus zeamais* to assess the effectiveness of binary combinations of *Hymenocardia acida* wood ash and *Plectranthus glandulosus* leaf powder. The quantities of mixed products were added to maize grains to constitute the contents of 5, 10, 20 and 40 g/kg. Then, the bioassays on toxicity within 1, 3, 7 and 14 days exposure, progeny production, population increase, grain damage and germination ability of protected grains were carried out.

Results: The major compounds (pinene, α-pinene, α-terpineol, thymol, β-myrcene and 3-carene) of *P. glandulosus* leaf powder were monoterpenes. The major non-monoterpenic constituent was an oxygenated sesquiterpene, β caryophyllene oxide. The chemical analysis of *H. acida* ash showed that calcium (5800 mg/kg) and phosphorus (2782 mg/kg) recorded higher content than the other minerals. *Plectranthus glandulosus* leaf powder, *H. acida* wood ash and their binary combinations significantly induced mortality of *S. zeamais* adult ($P < 0.0001$). The higher mortality rate was achieved by the highest content within 14 days of exposure. The combinations of *P. glandulosus* leaf powder with *H. acida* at different proportions produced different interactions. The mixture of 75% *P. glandulosus* and 25% *H. acida* produced synergistic effect, whereas the mixture of 50:50 had antagonistic effect in weevil mortality. The three combinations of *H. acida* and *P. glandulosus* significantly reduced the production of the progeny compared to the control. From the application of 5 g/kg (lowest content), the number of emerging adults was highly reduced. The combination 25PG75HA revealed to be more effective than the two other against F_1 production. The grain damage and population growth were significantly reduced. In general, the non-infested maize grain had a good germination rate than the infested ones. The treatment did not have negative effect on seed germination.

Conclusions: From our results, the two powders and their binary combinations could be used to reduce grain infestation by insect while taking into account the proportions of insecticidal powders implied in the combination.

Keywords: *Sitophilus zeamais*, *Hymenocardia acida*, *Plectranthus glandulosus*, Wood ash, Leaf powder, Binary combinations

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Background

Cereals constitute the group of most consumed grain in sub-Saharan African, especially in Sahelian zones. In these zones, cereals are very interesting according to its conservation ability. Cereals are easily conserved compared to the other food products and also less demanding in terms of storage technology, which can be self-made. The conserved and protected seeds permit availability of seeds (food) throughout the year, thereby contributing to food security. Cereals such as sorghum, millets, wheat, maize and rice are major staple foods of most population. Botanically, cereals are grasses and belong to the monocot family Poaceae [1]. Maize remains the most cultivated and most consumed cereal in Africa. This staple food crop is grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic backgrounds in sub-Saharan Africa. Maize also has many uses in food industry (biscuit factory, pastry making, brewery, distilling, sweetening substance, etc.), textile industry, utilisation in pharmaceutical industry, biodegradable plastics, biofuel, alcohol and cosmetics production [2]. The production of maize is done in the short period of the year, whereas its commercialisation and consumption are done all year round. This makes imperative the storage and the protection of this grain. The insufficiencies of different storage methods in developing countries have not stopped to cause grain losses and this in unacceptable proportions [3].

During storage, maize grain is highly devastated by several pests, especially insect pest. Insects are at the origin of the majority of the damage occurring in the reserves of stored food products. Temperature and high humidity of the tropical climate favour proliferation of insects and micro-organisms which, in order to survive, devour the food products causing enormous damage [4]. Maize grain does not escape to insect attack during storage. Amongst the insect, maize grain pest, *Sitophilus zeamais* is the most detrimental. This pest causes quantitative and qualitative damage on stored maize. In this condition, the protection of this grain according to its multiple uses becomes a major necessity of food security. Food security could be enhanced by reducing stock losses. Damage caused by *S. zeamais* on maize could be reduced through chemical, biological, physical control and host plant resistance, which are important components of integrated pest management strategies. However, the use of synthetic residual chemicals dominates in Cameroon and other African countries. These chemicals, although effective, cause many environmental problems such as pollution, diseases and resistance in pests [5, 6]. Furthermore, the majority of farmers in Africa are resource-poor and have neither the means nor the skills to obtain and handle pesticides appropriately. Therefore, an environmentally safe and economically feasible pest control practice needs to be available.

Botanicals are products based on parts, powders, extracts or purified substances of plant origin. They are generally assumed to be more biodegradable, leading to less environmental problems. *Plectranthus glandulosus* Hook leaf [7–9] and wood ash [10–13] could stand out as good candidates for environmentally friendly control of storage beetle pests under Cameroonian conditions. *P. glandulosus* is an annual, glandular and strongly aromatic herb, used in folk medicine for the treatment of colds and sore throat in the Adamawa region of Cameroon [14]. The insecticidal properties of products from *P. glandulosus* have shown good insecticidal properties against stored maize grain pests [8, 9, 15–17]. The leaf powder of the plant, which is more accessible to rural farmers than synthetic chemicals, should be an alternative to control the different stages of insect pests. Many authors have reported the effectiveness of wood ash as a grain protectant [18–22]. The insecticidal efficacy of *Hymenocardia acida* wood ash needs to be determined since it is one of the plants which the wood is most used as firewood and charcoal in traditional kitchens in the northern part of Cameroon. Combinations of wood ash with *P. glandulosus* leaf powder could enhance biological activity against insects. This in turn will reduce the amount of both botanical and wood ash used in storage protection. Data concerning the effectiveness of the binary combinations between *H. acida* wood ash and *P. glandulosus* leaf powder are not available, although farmers mix dusts like wood ash with plant materials in stocks. As the stored grain in traditional facilities is used as seeds, the determination of the influence of grain protectant on seed germination is imperative.

Therefore, the objective of this study was to assess the effectiveness of binary combinations between *P. glandulosus* leaf powder and *H. acida* wood ash regarding adult toxicity, progeny production, population growth, grain damage and germination.

Methods

Source of maize grains

The variety of maize used during all experimentation was Shaba provided by IRAD, Wakwa station in the Adamawa region of Cameroon. Before experimentation, broken grains, the pieces of stone, sand and other foreign materials were removed from the stock. Then, the maize was kept in the freezer at −20 °C for 14 days to allow its disinfestations. After disinfestations from all types of living organisms, the maize was kept in ambient conditions of laboratory for 14 days to allow its acclimatisation. After all these steps, the maize was ready for use as substrate for insect rearing and bioassays.

Insect rearing

Adults of *S. zeamais* were obtained from a colony maintained in rearing since 2005 in the Applied Chemistry Laboratory of the University of Ngaoundere. Then, the insect culture was transferred and kept in Crop Protection Laboratory of IRAD Bambui, North-West region of Cameroon. The weevils were reared on disinfested maize in 900-mL glass jars and kept under laboratory conditions (23.08 ± 2.05 °C and 74.67 ± 14.36% r.h.). The culture was maintained and used as source of *S. zeamais* for bioassays.

Preparation of *Hymenocardia acida* wood ash

Stems and branches of *H. acida* were collected in Ngaoundéré, Adamawa region of Cameroon (latitude 7°25′ North and longitude 13°35′ East, altitude of 1151 m above sea level). The identity of the plants was confirmed at the Cameroon National Herbarium in Yaounde, where voucher samples were deposited. *H. acida* is registered on number 50114/HNC. Woods were air-dried until moisture was completely lost and burnt separately in a traditional kitchen normally used in the region. The obtained ash was sieved and packaged in glass jars, labelled and kept in a freezer (at -4 °C) until subsequent use in the bioassays.

Preparation of *Plectranthus glandulosus* leaf powder

Leaves of *P. glandulosus* were collected in July 2012 in Ngaoundere, head quarter of the Adamawa region of Cameroon (latitude 7°25′ North and longitude 13°35′ East, altitude of 1151 m above sea level). The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde on number 7656/SRF. The leaves were dried at room temperature for 7 days and then crushed. The crushed leaves were ground until the powder passed through a 0.20-mm sieve. Then, a part of powder was stored in a freezer at -20 °C until needed for bioassays and the other part was used for essential oil extraction.

Preparation of binary combinations

The products were mixed in the following proportions to constitute the different binary combinations:

- 25% *P. glandulosus* leaf powder and 75% *H. acida* wood ash: 25PG75HA;
- 50% *P. glandulosus* leaf powder and 50% *H. acida* wood ash: 50PG50HA;
- 75% *P. glandulosus* leaf powder and 25% *H. acida* wood ash: 75PG25HA.

Analysis of volatile compounds of *Plectranthus glandulosus* leaf powder

The essential oil was extracted by hydrodistillation during 4 h using a Clevenger-type apparatus. The extracted oil was kept in a brown bottle at 4 °C to avoid degradation of chemical compounds by light until needed for gas chromatography–mass spectrometry (GC–MS) analysis.

Gas chromatography–mass spectrometry analysis was carried out with a chromatograph, model Agilent 7890A GC, equipped with an automatic injector and a column HP-1MS (15 m $\times$ 0.25 mm d.i; 0.25 µm film thickness) coupled to a mass detector Agilent 7890A MSD. The molecules were bombarded by an electronic beam of 70 eV. The gaze vector was helium (1 mL/min) with a pressure of 25 psi at the beginning of the column. The injector temperature was 250 °C. The programming of temperature consisted of a rise from 60 to 230 °C with the range of 2 °C/min and then 35 °C/min to reach 230 °C. The injection was done by split mode with the coefficient of 1/180. The injected quantity of essential oil of *P. glandulosus* was 0.2 µL. The detection was done by a quadripolar analyser constituted by an assembling of four parallel cylindrical electrodes. The bombing of essential oil by the electronic beam of 70 eV induced its ionisation and its fragmentation. Then, the positive ionic fragments formed the characteristic mass spectrum of compounds. The obtained spectra were compared with computerised database using NIST/EPA/NIH Mass Spectral Library, Wiley Register of Mass Spectral Data [23] and König et al. [24].

Determination of *Hymenocardia acida* wood ash mineral contents

The sample of ash was calcinated at 450 °C for 24 h using incinerator for a complete mineralisation [25]. Calcinated ash was dissolved in nitric acid (HNO_3) 1 M for digestion and then boiled. The solution was filtered after cooling. The filtrate obtained was used to proportion the following minerals: P, K, Ca, Mg, Na, Fe, Mn, Zn and Pb. Ca, K and Na were proportioned by flame photometry, while Mg, Fe, Mn, Zn and Pb were proportioned by atomic absorption spectrometry. The content of phosphate was measured by molecular absorption spectrophotometry.

Toxicity bioassay

The toxicity bioassay was carried out under ambient conditions of the laboratory. During experimentation, the temperature and relative humidity were recorded using a data logger (Data logger Model EL-USB-2, LASCAR, China). Four concentrations from each combination were considered. The masses of 0.25, 0.5, 1 and 2 g of *P. glandulosus* leaf powder and *H. acida* wood ash as well as their binary combinations were separately added to

50 g of maize in glass jars to constitute the contents of 5, 10, 20 and 40 g/kg, respectively. The insecticidal materials plus grain were thoroughly mixed by manual shaking. The controls consisted of substrate without insecticidal products. A group of 20 insects of mixed sexes and 7- to 14-days-old were added into each jar containing the treated or untreated grains. All treatments were replicated four times, and the experiment was arranged in a completely randomised design. Mortality was recorded 1, 3, 7 and 14 days post-infestation.

The co-toxicity coefficient per *P. glandulosus* leaf powder–*H. acida* wood ash mixture was calculated: A co-toxicity coefficient of less than 80 is considered as antagonistic, between 80 and 120 as additive and higher than 120 as synergistic [26]. When mixture (*M*) compounds of two parts (*A* and *B*) and both components have LC_{50}, then the following formulae are used (*A* serving as standard, it is represented in this study by *P. glandulosus* leaf powder, *B* represents wood ash for *H. acida*):

$$\text{Toxicity index (TI) of } A = 100,$$

$$\text{Toxicity index (TI) of } B = \frac{LC_{50} \text{ of } A}{LC_{50} \text{ of } B} \times 100,$$

$$\text{Actual TI of } M = \frac{LC_{50} \text{ of } A}{LC_{50} \text{ of } M} \times 100,$$

$$\text{Theoretical TI of } M = \text{TI of } A \times \% \text{ of } A \text{ in } M + TI \text{ of } B \times \% \text{ of } B \text{ in } M,$$

$$\text{Co-toxicity coefficient} = \frac{\text{Actual TI of } M}{\text{Theoretical TI of } M} \times 100.$$

If one component of the mixture alone (for example a wood ash) causes low mortality at all doses (< 20%), then the co-toxicity coefficient of the mixture should be calculated by the formula:

$$\text{Co-toxicity coefficient} = \frac{LC_{50} \text{ of } A \text{ alone}}{LC_{50} \text{ of } A \text{ in the mixture}} \times 100.$$

F_1 progeny bioassay

After the 14-day mortality recordings, all insects and products were discarded. The grains were left inside the bottles, and the counting of F_1 adults was carried out once a week during 5 weeks. The emergence started only from 5th week after infestation. After each counting session, the insects were removed from the jars [8].

Damage bioassay

Four rates of the binary combinations (5, 10, 20 and 40 g/kg) were mixed with 150 g of maize grain as described above. Fifty unidentified sex weevils (7–14 days old) were introduced into each jar. Each treatment had four replications. After 3 months, the live weevils and dead ones were counted. Damage assessment was performed by counting and weighing the number of damaged and undamaged grain using the method of Adams and Schulten [27].

$$\text{Weight loss (\%)} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u(N_d + N_u)} \times 100,$$

where W_u is the weight of undamaged grain, N_d the number of damaged grain, W_d the weight of damaged grain, and N_u the number of undamaged grain.

Test of germination

Seed germination was tested using 30 randomly picked grains from non-perforated grains after separation of the perforated from the non-perforated in each jar. In order to assess the effect of binary mixtures on germination ability, the seeds were treated with different contents and stored as previously described, but without insect. The seeds from the two lots (infested and non-infested) of stored maize grains were placed on moistened paper in 9-cm glass Petri dishes. The number of germinated seeds was recorded after 10 days [28].

Data analysis

Abbott's formula [29] was used to correct for control mortality before analysis of variance (ANOVA) and probit analysis. Data on cumulative corrected mortality, reduction in F_1 progeny, damage, weight loss and germination percentage were arcsine-transformed [(square root(x/100)], and the number of F_1 progeny was log-transformed ($x+1$). The transformed data were subjected to the ANOVA procedure using the statistical analysis system [30, 31]. Tukey's test ($P = 0.05$) was applied for mean separation. Probit analysis [31, 32] was conducted to determine lethal dosages causing 50% (LC_{50}) and 95% (LC_{95}) mortality of *S. zeamais* at 1, 3, 7 and 14 days after treatment application. The probit analysis was also used to determine the effective content causing 50% (EC_{50}) and 95% (EC_{95}) reduction in F_1 progeny.

Results

Chemical analysis

Plectranthus glandulosus leaf powder

The volatile constituents of the essential oils from *P. glandulosus* leaf powder were identified by their retention indices and mass spectra in comparison with those of standard synthetic compounds. The results of the chemical analysis are presented in Table 1. The dominant chemical constituents were terpenic compounds. The major compounds were β-pinene (11.5%), α-pinene (11.2%), α-terpineol (10.8%), thymol (10.1%), β-myrcene (9.7%) and 3-carene (8.7%), which are monoterpenes. The

Table 1 Chemical constituents of essential oil of *Plectranthus glandulosus* leaf

Compounds	% Composition
Hydrocarbonated monoterpenes	
1R-α-Pinene	11.2
β-Pinene	11.5
(+)-4-Carene	0.3
3-Carene	8.7
β-Phellandrene	Tr
Paracymene	7.5
Limonene	2.5
γ-Terpinene	0.1
β-Thujene	0.2
2-Methyladamantane	0.8
(E)-β-Ocimene	0.3
(E)-4,8-Dimethyl-1,3,7-nonatriene	Tr
α-Terpinene	1.3
β-Myrcene	9.7
Hydrocarbonated sesquiterpenes	
7-β-[H]-silphiperfol-5-ene	1.2
Silphin-1-ene	Tr
Silphiperfola-5,7(14)-diene	0.1
α-Copaene	2.7
Pethybrene	0.3
Modhephene	0.3
α-Isocomene	0.2
α-Curcumene	0.2
γ-Gurjumene	Tr
(Z,E)-α-Farnesene	2.5
β-Selinene	0.1
Oxygenated monoterpenes	
α-Terpineol	10.8
O-Acetylthymol	0.8
Thymol	10.1
(E,E)-2,4-Decadienal	0.1
8,9-Dihydrothymol	Tr
Bornyl acetate	Tr
Neryl acetate	0.3
α-Terpenyle	0.1
(E)-β-Damascenone	0.4
β-Cyclocitral	0.1
4′-Methoxyvalerophenone	Tr
p-Methoxycumene	Tr
Oxygenated sesquiterpenes	
Cubebol	Tr
β-Caryophyllene oxide	9.4
β-Oplopenone	Tr
Aromatic compounds	
3,4-Xylenol	Tr
2-(2-Butynyl)-cyclohexanone	0.1
Acetophenone	5.5
Aldehydes	
(E)-2-Nonanal	0.1
Undecanal	0.2
Decanal	0.1
Lauraldehyde	0.1
(E)-2-Decenal	0.1
Nonenal	0.3
Ketones	
Decan-2-one	Tr
Tridecan-2-one	Tr
Ester	
cis-hex-3-enyl-acetate	0.5

Tr in trace (< 0.1)

Table 2 Chemical composition of wood ash

Mineral	Content (mg/kg)
Calcium	5800
Magnesium	851
Potassium	997
Sodium	289
Iron	778
Zinc	786
Lead	0.0019
Manganese	0.011
Phosphor	2782

major non-monoterpenic compound was an oxygenated sesquiterpene, β caryophyllene oxide (9.4%).

Hymenocardia acida wood ash

Different contents of ions were recorded concerning the mineral determination (Table 2). Calcium (5800 mg/kg) and phosphorus (2782 mg/kg) recorded the higher content than the other minerals. Iron, zinc and magnesium had almost the same content. The lower content was recorded for manganese (0.011 mg/kg) and lead (0.0019 mg/kg).

Adult mortality

Plectranthus glandulosus leaf powder, *H. acida* wood ash and their binary combinations induced significant mortality of *S. zeamais* adult (Table 3). This mortality increased with content and exposure time for each product (Table 4). The insecticidal efficacy varied slightly amongst the tested products. The higher mortality rate was achieved by the highest content (40 g/kg) of *H. acida* wood ash (94.66%) and 25PG75HA (94.59%) within

Table 3 Test of between-subjects effects concerning mortality

Source	Sum of squares	df	Mean square	F	Signification
Product	37794.240	4	9448.560	97.007	< 0.0001
Days after infestation	97,723.529	3	32,574.510	334.436	< 0.0001
Content	145,458.329	4	36,364.582	373.348	< 0.0001
Product × days after infestation	18,876.462	12	1573.039	16.150	< 0.0001
Product × content	10,291.142	16	643.196	6.604	< 0.0001
Days after infestation × content	25,227.056	12	2102.255	21.583	< 0.0001
Product × days after infestation × content	6185.378	48	128.862	1.323	0.086
Error	29,220.380	300	97.401		
Total	857,756.358	400			

df degree of freedom

Table 4 Cumulative mortality of *Sitophilus zeamais* adult induced by *Plectranthus glandulosus* leaf powder, *Hymenocardia acida* wood ash and their binary combinations under fluctuating laboratory conditions (temp. = 22.76 ± 2.02 °C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	Products					$F_{(4; 15)}$
	P. glandulosus	*H. acida*	25PG75HA	50PG50HA	75PG25HA	
1 day						
0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}	–
5	3.82 ± 2.41^{aB}	2.50 ± 1.44^{bcB}	8.75 ± 3.15^{bcAB}	11.25 ± 1.25^{bAB}	21.25 ± 5.91^{abA}	5.14*
10	6.25 ± 2.39^{aB}	5.00 ± 8.75^{abB}	22.50 ± 4.33^{abAB}	20.00 ± 2.04^{bAB}	28.75 ± 6.57^{aA}	5.64*
20	7.50 ± 2.50^{aB}	8.75 ± 1.25^{aB}	25.00 ± 6.12^{abAB}	20.00 ± 3.54^{bAB}	36.25 ± 9.44^{aA}	6.82*
40	16.25 ± 5.91^{aAB}	12.50 ± 1.44^{aB}	36.25 ± 5.54^{aAB}	36.25 ± 5.54^{aAB}	41.25 ± 8.51^{aA}	5.04*
$F_{(4; 15)}$	2.48 ns	19.66**	15.83***	40.80***	11.68**	
3 days						
0	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	–
5	6.25 ± 3.15^{bcB}	21.08 ± 3.73^{bAB}	22.90 ± 7.37^{bAB}	37.04 ± 4.39^{aA}	25.06 ± 4.10^{aA}	6.09*
10	11.38 ± 3.13^{abB}	24.57 ± 1.99^{abAB}	42.17 ± 7.18^{abA}	40.92 ± 4.17^{aA}	38.82 ± 7.98^{aA}	6.24*
20	17.83 ± 4.97^{aB}	33.66 ± 2.01^{aAB}	44.42 ± 5.58^{abA}	46.81 ± 7.31^{aA}	40.07 ± 7.87^{aAB}	3.95*
40	21.58 ± 3.87^{aB}	37.41 ± 4.63^{aAB}	57.57 ± 5.84^{aA}	56.45 ± 6.18^{aA}	52.83 ± 7.85^{aA}	7.02*
$F_{(4; 15)}$	12.10***	66.26***	28.53***	40.77***	22.16***	
7 days						
0	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{bA}	–
5	7.77 ± 2.59^{bB}	63.55 ± 12.84^{aA}	36.55 ± 10.94^{aAB}	43.71 ± 3.25^{cAB}	36.92 ± 3.14^{aAB}	5.89*
10	14.15 ± 3.21^{abB}	72.69 ± 8.58^{aA}	53.15 ± 9.47^{aA}	58.92 ± 1.35^{bA}	49.27 ± 5.36^{aA}	10.59***
20	20.59 ± 3.70^{abB}	77.96 ± 8.65^{aA}	57.16 ± 9.11^{aA}	69.72 ± 5.31^{aA}	49.42 ± 6.99^{aA}	11.63***
40	28.16 ± 4.73^{aC}	87.11 ± 4.91^{aA}	68.35 ± 4.40^{aAB}	76.76 ± 1.02^{aA}	56.14 ± 3.97^{aB}	27.67***
$F_{(4; 15)}$	16.00***	18.27***	21.54***	202.13***	57.03***	
14 days						
0	0.00 ± 0.00^{dA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	–
5	19.54 ± 2.63^{cB}	69.08 ± 10.78^{aA}	68.13 ± 10.30^{bA}	63.16 ± 5.27^{bA}	56.07 ± 3.93^{bA}	7.87**
10	33.82 ± 3.52^{bB}	82.60 ± 7.91^{aA}	82.09 ± 3.59^{abA}	80.92 ± 4.61^{aA}	66.89 ± 5.78^{abA}	15.27***
20	40.33 ± 4.12^{bC}	90.64 ± 6.23^{aA}	93.13 ± 1.41^{aA}	82.17 ± 2.71^{aAB}	72.44 ± 4.13^{abB}	27.04***
40	57.24 ± 2.71^{aC}	94.66 ± 3.72^{aA}	94.59 ± 2.15^{aA}	90.42 ± 1.35^{aAB}	80.85 ± 1.45^{aA}	47.34***
$F_{(4; 15)}$	106.53***	26.03***	65.27***	105.05***	122.33***	

Mean ± S.E. followed by the same capital letter in a line and the same lower letter in a column do not differ significantly at $P < 0.05$ (Tukey's test)

Each datum represents the mean of four replicates of 20 insects each

ns $P > 0.05$; *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$

14 days of exposure. *Plectranthus glandulosus* leaf powder induced low mortality (only 57.24% after 14 days at 40 g/kg) compared to the other products at all exposure periods. Low mortality rate was recorded at 5 g/kg for the different powders. However, this lowest content (5 g/kg) induced significant mortality with increasing exposure time. The combinations 25PG75HA, 50PG50HA and 75PG25HA at 5 g/kg induced 8.75, 11.25 and 21.25%, respectively, within 1-day exposure. However, the same combinations in the same order, at the same content level within 14-day exposure, provoked 68.13, 63.16 and 56.07% mortality of *S. zeamais*. According to the mortality rate, the different products can be ranked as follows: *H. acida* wood ash > 25PG75HA > 50PG50HA > 75PG52HA > *Plectranthus glandulosus* leaf powder.

Toxicity and effect of the binary combinations

The lethal content of different powders and their combinations reduced, when the exposure period increased (Table 5). The LC_{50} of *P. glandulosus* leaf powder was 213.64 g/kg within 3 days, but it reduced to 28.65 g/kg within 14 days. The LC_{50} of *H. acida* was 135.23 and 1.94 g/kg, respectively, within 3 days and 14 days. The same tendency was observed with the LC_{95}, *H. acida* wood ash recorded 190.82 and 38.34 g/kg LC_{95} values, respectively, within 7-day and 14-day exposure period. Between the 7th and 14th day after exposure, LC_{50} and LC_{95} values of the different combinations reduced with increasing exposure periods, respectively, from 18.17 to 3.04 and 458.17 to 416.35 g/kg for 75PG25HA, 6.80 to 1.97 and 372.55 to 86.34 g/kg for 50PG50HA and 10.82 to 2.18 and 928.12 to 33.77 g/kg for 25PG75HA.

The combinations of *P. glandulosus* leaf powder with *H. acida* at different proportions produced different interactions. The combination made up by 75% of *P. glandulosus* leaf powder with 25% of *H. acida* wood ash produced synergistic effect, whereas that made up by 50% each of two powders had antagonistic effect. The additive effect was observed at 14th day of exposure with 25PG75HA mixture.

Reduction of progeny production

The three combinations of *H. acida* and *P. glandulosus* significantly reduced the production of progeny compared to the control (Table 6). From the application of 5 g/kg (lowest content), the number of emerging adults was highly reduced in treated samples (14.25 emerged adults) than in untreated ones (42.50 emerged adults).

Table 5 Lethal contents and co-toxicity coefficients of binary combinations of *Plectranthus glandulosus* leaf powder with *Hymenocardia acida* wood ash on *Sitophilus zeamais* under fluctuating laboratory conditions (temp. = 22.76 ± 2.02 °C; r.h. = 69.87 ± 9.93%)

Products	Slope	R^2	LC_{50}(95% FL) (g/kg)	LC_{95}(95% FL) (g/kg)	Co-toxicity coefficient (CTC)	Significance of CTC	χ^2
3 days							
P. glandulosus	0.980 ± 0.253	0.973	213.694 (87.601; 2902)[a]	–			1.523[ns]
H. acida	0.569 ± 0.199	0.966	135.326 (50.483; 18,401)[a]	–			0.289[ns]
75PG25HA	0.728 ± 0.193	0.923	34.119 (21.812; 99.052)	6180 (766.378; 4,210,605)[a]	547.111	Synergistic	0.622[ns]
50PG50HA	0.525 ± 0.188	0.924	25.278 (14.683; 132.309)	34,126 (1340; 3.453E[12])[a]	21.414	Antagonistic	0.349[ns]
25PG75HA	0.925 ± 0.194	0.904	24.774 (17.920; 42.318)	1485 (377.425; 38,229)[a]	601.378	Synergistic	1.342[ns]
7 days							
P. glandulosus	0.908 ± 0.231	0.999	166.041 (73.016; 1706)	–			0.121[ns]
H. acida	0.826 ± 0.210	0.988	1.946 (0.280; 3.910)	190.821 (73.336; 3007)[a]			0.323[ns]
75PG25HA	0.483 ± 0.188	0.848	18.166 (9.275; 69.952)	45,817 (1355; 7.715E[15])[a]	41.394	Antagonistic	0.564[ns]
50PG50HA	0.946 ± 0.195	0.973	6.798 (3.711; 9.538)	372.549 (138.058; 3695)[a]	56.589	Antagonistic	0.217[ns]
25PG75HA	0.851 ± 0.191	0.942	10.816 (6.810; 15.197)	928.122 (249.182; 26,174)[a]	23.896	Antagonistic	0.545[ns]
14 days							
P. glandulosus	1.088 ± 0.198	0.976	28.645 (21.379; 46.464)	928.767 (306.688; 9366)[a]			0.8263[ns]
H. acida	1.268 ± 0.249	0.929	1.936 (0.618; 3.316)	38.341 (25.041; 91.006)			0.195[ns]
75PG25HA	0.769 ± 0.199	0.981	3.036 (0.605; 5.481)	416.348 (124.349; 15,575)[a]	212.073	Synergistic	0.085[ns]
50PG50HA	1.00 ± 0.222	0.861	1.967 (0.459; 3.634)	86.344 (44.754; 415.547)[a]	3.937	Antagonistic	0.986[ns]
25PG75HA	1.381 ± 0.255	0.896	2.175 (0.837; 3.509)	33.773 (23.036; 70.093)	116.067	Additive	0.583[ns]

[ns] $P > 0.05$; *: $P < 0.05$

[a] The LC values were obtained by extrapolation

The progeny production inhibition ability might depend on the proportion of each botanical involved in the mixture, and thus, 2, 7 and 8 adults emerged, respectively, in grain treated with 25PG75HA, 50PG50HA and 75PG25HA at the content of 40 g/kg. At 5 g/kg, 25PG75HA inhibited the F_1 progeny production by more than 60%, whereas 50PG50HA and 75PG25HA caused a reduction of less than 50%. The higher inhibition rate of emerging insects was recorded at the highest content (40 g/kg) of the three combinations, with 95.49, 83.39 and 80.92 reduction, recorded, respectively, for 25PG75HA, 50PG50HA and 75PG25HA. Overall, the combination 25PG75HA was revealed more effective than the two other (50PG50HA and 75PG25HA). This combination recorded the lowest EC_{50} (2.55 g/kg), whereas the highest EC_{50} (10.69 g/kg) was recorded for 75PG25HA (Tables 7 and 8).

Suppression of population increase and reduction in grain damage

All the treatments significantly reduced grain damage and population increase, compared to the control. The number of insects, grain damage and weight loss decreased when the concentration of powders increased. Concerning the different parameters, the difference was observed in terms of effectiveness according to the combination.

The number of insects was also considerably suppressed. Even at the lowest content (5 g/kg), the three combinations revealed to be very effective; the grain treated with 25P75HA recorded 21.92 damaged grain and 3.22% weight loss, whereas the non-treated grain recorded 49.61 damaged grain and 12.81% weight loss. At their highest content level (40 g/kg), the damage was almost completely suppressed. 25PG75HA revealed to be more effective compared to the other combinations; the grain treated with 25PG75HA recorded fewer insects than those treated with the two other combinations (50PG50HA, 75PG25HA). At 40 g/kg, 26.50 lived insects and 58.50 dead insects were counted from 25PG75HA treatment, whereas from 50PG50HA and 75PG25HA, 68 and 44.75 lived insects and 71.50 and 53.75 dead insects were recorded, respectively.

Germination rate after 3 months of storage

The germination rate varied with treatment. In general, the non-infested maize grain had a good germination rate than the infested ones. The germination rates from the non-infested treated grains did not varied amongst the treatments. However, these rates increased with ascending dosage with respect to treated infested grains. At 40 g/kg, 97.50, 95 and 90% germination rates were recorded from the treatments 25PG75HA, 50PG50HA and 75PG25HA, respectively. Without insect, the germination was significantly higher even without insecticidal

Table 6 Progeny production of *Sitophilus zeamais* in maize treated with binary mixtures from *Plectranthus glandulosus* leaf powder with *Hymenocardia acida* wood ash under fluctuating laboratory conditions (temp. = 22.76 ± 2.02 °C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	25PG75HA	50PG50HA	75PG25HA	$F_{(2; 9)}$
Mean number of F_1 adult progeny				
0	42.50 ± 1.71^{aA}	42.50 ± 1.17^{aA}	42.20 ± 1.17^{aA}	–
5	14.25 ± 0.85^{bB}	23.00 ± 2.86^{bAB}	28.50 ± 4.65^{abA}	5.05*
10	9.25 ± 1.65^{bcB}	15.75 ± 1.65^{bcAB}	23.75 ± 3.79^{bA}	7.97*
20	4.75 ± 1.44^{cdB}	8.25 ± 0.63^{cdAB}	14.50 ± 3.80^{bcA}	4.34*
40	2.00 ± 0.82^{dB}	7.00 ± 0.82^{dAB}	8.00 ± 1.08^{cA}	12.40*
$F_{(4; 15)}$	145.94***	70.41***	16.23***	
Inhibition of adult emergence relative to control (%)				
0	0.00 ± 0.00^{dA}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{dA}	–
5	66.51 ± 1.23^{cA}	45.92 ± 6.42^{cAB}	33.27 ± 10.56^{cB}	5.47*
10	78.32 ± 3.87^{bA}	62.88 ± 3.97^{bAB}	44.53 ± 8.27^{bcB}	8.66*
20	88.91 ± 3.47^{abA}	80.42 ± 2.05^{aAB}	66.60 ± 7.45^{aB}	5.31*
40	95.49 ± 1.74^{aA}	83.39 ± 2.32^{aB}	80.92 ± 3.17^{aB}	9.88*
$F_{(4; 15)}$	233.90***	86.76***	19.96***	
EC_{50} (95%FL) g/kg	2.553(1.291; 3.764)	5.567(3.540; 7.390)	10.694(6.741; 15.088)	

Mean ± S.E. followed by the same capital letter in a line and the same lower case letter in a column do not differ significantly at $P < 0.05$ (Tukey's test)

*$P < 0.05$; ***$P < 0.0001$

Table 7 Population increase in *Sitophilus zeamais* and grain damage recorded in stored maize treated with the binary combinations of wood ash of *Hymenocardia acida* with leaf powder of *Plectranthus glandulosus* under fluctuating laboratory conditions (temp. = 22.76 ± 2.02 °C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	25PG75HA	50PG50HA	75PG25HA	25PG75HA	50PG50HA	75PG25HA
	Number of lived insects			Number of dead insects		
0	240.00 ± 12.25^{a}	240.00 ± 12.25^{a}	240.00 ± 12.25^{a}	50.50 ± 8.53^{a}	50.50 ± 8.53^{a}	50.50 ± 8.53^{a}
5	54.75 ± 25.15^{b}	118.50 ± 12.58^{b}	170.00 ± 7.07^{b}	69.02 ± 7.52^{a}	58.00 ± 8.98^{a}	63.50 ± 8.41^{a}
10	36.50 ± 4.56^{b}	102.25 ± 2.25^{bc}	128.25 ± 13.44^{b}	69.00 ± 1.35^{a}	62.02 ± 2.71^{a}	64.75 ± 2.66^{a}
20	38.75 ± 7.18^{b}	89.25 ± 5.75^{bc}	72.75 ± 2.53^{c}	59.12 ± 1.37^{a}	75.50 ± 1.50^{a}	82.00 ± 12.67^{a}
40	26.50 ± 7.17^{b}	68.00 ± 10.00^{c}	44.75 ± 11.62^{c}	58.50 ± 0.96^{a}	71.50 ± 2.50^{a}	53.75 ± 5.54^{a}
$F_{(4;\ 15)}$	45.08***	51.25***	58.05***	2.32^{ns}	3.02^{ns}	2.22^{ns}
	Grain damage			Weight loss (%)		
0	49.61 ± 4.54^{a}	49.61 ± 4.54^{a}	49.61 ± 4.54^{a}	12.81 ± 1.49^{a}	12.81 ± 149^{a}	12.81 ± 1.49^{a}
5	21.92 ± 3.59^{b}	28.02 ± 1.55^{b}	36.81 ± 1.97^{b}	3.22 ± 0.82^{b}	5.07 ± 1.10^{b}	5.87 ± 1.16^{b}
10	18.20 ± 2.54^{b}	25.13 ± 1.43^{b}	30.41 ± 0.72^{b}	3.02 ± 1.36^{b}	4.15 ± 0.91^{b}	3.85 ± 1.14^{b}
20	17.25 ± 2.60^{b}	20.12 ± 3.01^{b}	27.95 ± 0.68^{bc}	2.94 ± 1.29^{b}	3.92 ± 1.00^{b}	3.43 ± 0.23^{b}
40	13.56 ± 1.17^{b}	17.20 ± 3.27^{b}	17.60 ± 2.00^{c}	1.54 ± 1.08^{b}	3.09 ± 0.67^{b}	2.79 ± 0.62^{b}
$F_{(4;\ 15)}$	22.25***	18.21***	23.68***	13.83***	13.86***	15.93***

Mean ± S.E. followed by the same capital letter in a line and the same lower case letter in a column do not differ significantly at $P < 0.05$ (Tukey's test)

^{ns} $P > 0.05$; *$P < 0.05$; ***$P < 0.0001$

Table 8 Germination of stored grains treated with binary combinations of *Hymenocardia acida* wood ash with *Plectranthus glandulosus* leaf powder and infested, and non-infested by *Sitophilus zeamais* under laboratory conditions (temp.: 22.76 ± 2.02 °C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	Products					
	25PG75HA	50PG50HA	75PG25HA	25PG75HA	50PG50HA	75PG25HA
	Non-infested			Infested		
0	94.33 ± 1.36^{a}	94.33 ± 1.36^{a}	94.33 ± 1.36^{a}	21.67 ± 2.15^{d}	21.67 ± 2.15^{d}	21.67 ± 2.15^{c}
5	91.08 ± 0.21^{a}	90.83 ± 1.50^{a}	94.83 ± 1.90^{a}	84.67 ± 0.83^{c}	81.67 ± 3.19^{c}	78.33 ± 0.96^{b}
10	92.00 ± 0.79^{a}	94.50 ± 1.83^{a}	92.83 ± 0.88^{a}	88.33 ± 1.67^{bc}	84.17 ± 2.50^{bc}	83.33 ± 1.36^{ab}
20	92.17 ± 0.74^{a}	94.17 ± 1.50^{a}	93.42 ± 1.64^{a}	92.33 ± 0.83^{ab}	93.33 ± 1.36^{a}	86.67 ± 2.36^{ab}
40	91.17 ± 0.32^{a}	92.08 ± 0.79^{a}	92.20 ± 1.73^{a}	97.50 ± 1.60^{a}	95.00 ± 1.67^{a}	90.00 ± 2.36^{a}
$F_{(4;\ 15)}$	2.72^{ns}	1.30^{ns}	0.41^{ns}	430.01***	180.43***	218.78***

Mean ± S.E. followed by the same capital letter in a line and the same lower case letter in a column do not differ significantly at $P < 0.05$ (Tukey's test)

powder (94.33%), whereas with insect the germination rate was very low (21.67%).

Discussion

The binary mixtures of *P. glandulosus* leaf powder and *H. acida* wood ash provoked significant mortality of *S. zeamais*. The different binary combinations of these two substances produced different effects such as synergism, antagonism and additivity. The combination of insecticidal materials has the advantages to increase efficacy by complementing the bio-efficacy of the individual products and simultaneously lowering their doses on the one hand, and broadening the spectrum of activity and reducing the chance of resistance development on the other hand [33]. However, with mixtures, negative effects can also occur such as reduced efficacy, phyto-toxicity and incompatibility problems between materials [34]. The combinations of 75% of *P. glandulosus* leaf powder and 25% of *H. acida* on *S. zeamais* mortality produced synergistic effect, whereas combination made up by 50% of *P. glandulosus* leaf and 50% *H. acida* wood ash induced antagonistic effect within 14 days, which produced a significant synergism. In general, the mixtures composed by the individual insecticidal materials improved in efficacy. The additive effect was also observed; the effect of two materials is equal to the sum of each component given alone (1 + 3 = 4), which was observed in the present study by the combination 25PG75HA (25% *P. glandulosus* leaf powder and 75% *H. acida* wood ash) within 14 days of exposure.

The synergism has been demonstrated in this experiment by the decreasing LC_{50} values compared to those of single material. The proportions of two products used in combinations may produce different performances according to the involved proportions. The combinations of 75PG25HA and 50PG50HA produced, respectively, synergistic and antagonistic effect. The same tendency concerning the variations in efficacy for different proportions of products was observed by Ntonifor et al. [35]. They found that the combinations of *Syzygium aromaticum* (L.) (Myrtaceae) and *Cyperus aequalis* (Vahl) (Cyperaceae) at the proportions of 0:2, 0.5:1.5, 1:1, 2:0 (g:g) induced 36.3, 93.8, 98.8, 100% mortality of *C. maculatus*, respectively, within 3 days of exposure. Shaalan et al. [36] found that mixtures of *Khaya senegalensis* A. Juss (Meliaceae) and *Daucus carota* Linnaeus (Apiaceae) seed extracts were more effective and economical than phytochemicals alone in controlling *Aedes aegypti* Linnaeus (Diptera: Culicidae) and *Culex annulirostris* Skuse (Diptera: Culicidae) mosquitoes.

The three binary combinations of *P. glandulosus* leaf powder and *H. acida* wood ash considerably inhibited the production of *S. zeamais* progeny. In addition to increasing mortality, the combinations of these products have an effect on the development of *S. zeamais*. The presence of *P. glandulosus* leaf powder in combinations may potentiate the effect of ash. There are physical and chemical actions, which are the desiccation by ash and poisoning by the chemical compounds contained in *P. glandulosus* leaf. Mixtures can disturb or delay the development of larvae in adults. Karso and Al Mallah [37] found that the mixture of soya oil and Acetamiprid pesticide gave the highest average mortality of *Trogoderma granarium* larvae, which varied according to the proportions.

The combinations of insecticidal materials improve the protection of stored grain by reducing the qualitative and quantitative losses. The reduction in damage and the suppression of *S. zeamais* population growth were positively correlated. Combinations of *H. acida* wood ash and *P. glandulosus* leaf powder at different proportions considerably reduced damage, by lowering the number of perforated grains and weight loss, and at the same time by inhibiting the population increase. Hill [38] reported that wood ash was useful as a physical barrier on the grain. However, it can also possess various chemical properties according to its botanical source. *Plectranthus glandulosus* leaf, thanks to its chemical compounds, controlled the proliferation of insect, which explains the efficacy of combinations in short storage period. When the storage period increased, the efficacy decreased by loss of their volatile compounds which confer its toxicity against insects. Similar findings were reported by Mwangangi and Mutsiya [39], who showed that the efficacy of *Ocimum basilicum* Linnaeus (Lamiaceae) powder deteriorated the fastest leading to 80, 77, 44, 20 and 15% mortality over 0, 7, 14, 21 and 28 days of storage. Then, to improve effectiveness of ash plant powders combinations and allow components to ensure a good preservation of grains, it is important to avoid the increase in moisture.

In many African countries, stored grains provide not only grains for food, but also seeds for planting. Thus, the conservation of seed viability after the application of protectant is necessary. During the experiment, in the presence of weevils, germination rate increased with increase in product contents. The untreated maize in the presence of insects recorded the week germination rate. In this case, the seed loses their germination ability due to the high *S. zeamais* infestation that lays its eggs on grain. The larvae develop and feed inside the grain by consuming the germ of the seed, thus diminishing the viability of the seeds. Usha Rani and Devanand [40] found that seed germination was significantly reduced when untreated maize seeds were exposed to *S. oryzae* and *T. castaneum*. But when the maize seeds were stored with combinations of *P. glandulosus* leaf powder and wood ash without weevils, no difference was observed amongst treatments and content levels and they conserved significant germination rate. Higher levels of the products improved their ability to protect grain, leading to a greater germination capacity. The different powders did not present any adverse effect on maize seed germination. It has been shown that the storage of maize seeds for prolonged periods after treatment with various botanical concentrations does not have any adverse effect on the seed viability.

In the present study, no adverse effect was observed on germination ability. But, some findings reported the inhibiting effect of some plant extracts on seed germination [41, 42]. The application of lower concentrations of *Murraya koenigii* Linnaeus (Rutaceae) and *Capsicum annuum* Linnaeus (Solanaceae) extracts caused a normal germination, but the same plants at higher concentrations caused 30–35% inhibition of seed germination. Bustos-Figueroa et al. [42] observed that the leaf powder of *P. boldus* used alone or mixed with lime did not affect the percentage of maize seed germination. This corroborates our findings about the germination rate recorded with the treatments. Higher germination rate recorded by the combinations of 25% of *P. glandulosus* leaf powder and 75% of wood ash could be due to the higher content of ash in the combination. According to Philogène [43], the ash does not affect germination but could enhance growth because of the cations that it contains. *H. acida* wood ash contains high quantity of Ca, K, P, Na and Fe, which are important for plant growth. Parimelazhagan and Francis [44] found that leaf extracts of *Cerastium viscosum* Linnaeus (Caryophyllaceae) increased seed germination and improved seedling development of rice seeds. In general, grains in storage facilities lost their viability and germination chances as the post-harvest storage period increases [45]. That could explain the loss of viability partly even when the seeds do not have damage. The combinations protected the maize grains against the destruction of their germination capacity by weevils, and they did not influence negatively seed germination.

Conclusions

The binary combinations of *P. glandulosus* leaf powder and *H. acida* wood ash at different proportions effectively protect maize grain against infestation by *S. zeamais* in storage. The binary combinations did not alter the viability of maize grains, and thus, the germination rate. The beneficial effect of the combinations could be enhanced by using the appropriated proportions. Other proportions which may be involved in the mixture of these botanicals may be tested in order to the find out the most appropriate efficient combination. Further studies need to be carried out concerning mammalian toxicity that could be attributed to the use of these products in grain storage. Also, the investigations need to be undertaken in order to assess the effect of these powders on the organoleptic and technological properties of treated grains.

Abbreviations

25PG75HA: 25% *Plectranthus glandulosus* leaf powder + 75% *Hymenocardia acida* wood ash; 50PG50HA: 50% *Plectranthus glandulosus* leaf powder + 50% *Hymenocardia acida* wood ash; 75PG25HA: 75% *Plectranthus glandulosus* leaf powder + 25% *Hymenocardia acida* wood ash; temp.: temperature; r.h.: relative humidity; LC: lethal content; EC: efficacy content; *df*: degree of freedom; FL: fudicial limit.

Authors' contributions

JWG, ENN and DN conceived the idea, designed the experiments and analysed the data. JWG, CS and TG carried out the experiments. JWG wrote the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

The authors are thankful to the laboratory of soil analysis and environmental chemistry of the faculty of Agronomic Sciences of the University of Dschang (Cameroon) for the analysis of wood ash mineral content and the Research Institute on Medicinal Plants (IMPM) of Yaoundé for the extraction of essential oils and the determination of its volatile constituents using GC–MS. They also express their gratitude to IRAD (Institute of Agricultural Research for Development) Bambui (Cameroon) for providing facilities to carry out this research work.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data sets used and/or analysed during this study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

Not applicable.

Publisher's Note

Received: 5 September 2017 Accepted: 21 March 2018
Published online: 03 April 2018

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