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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

Combinations of botanicals could enhance biological activity against insects. This in turn, will reduce amount of botanical used in storage protection. In this issue, 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 regarding adult toxicity, progeny inhibition, and reduction of damage and germination ability. *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 (40 g/kg) of *H. acida* wood ash (94.66%) and 25PG75HA (94.59%) within 14 days of exposure. 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% of each of two powders had antagonistic effect in weevil mortality. The three combinations of *H. acida* and *P. glandulosus* significantly reduced the progeny production. In term of inhibition of F_1, the combination 25PG75HA revealed more effective than the two other. The grain damage and population increase were significantly reduced. In general, the non-infested maize grain had a good germination rate than the infested ones. The treatments did not have negative effect on seed germination. From These results, the two powders and their binary combinations could be used to reduce grain infestation by insect while taking in account the proportions of insecticidal powders implied in the combination.

Keywords: *Sitophilus zeamais*, *Hymenocardia acida*, *Plectranthus glandulosus*, wood ash, leaf powder, binary combinations

Introduction

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 others food products, and also less demanding in term of storage technology, which can be self-made. The conserved and protected seeds permit availability of grains throughout the year thereby contributing to food security. Maize remains the most cultivated and most consumed cereal in Africa. The production of maize is done in the short period of the year whereas its commercialisation and consumption establish along the year. 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 (Gwinner etal., 1996).

During storage, maize grain is highly devastated by several pests, especially insect pests that are at the origin of the majority of damage occurring in the 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 (Ngamo and Hance, 2007). Maize grain does not escape to insect attack during storage. Among 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 for food security. 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 (Subramanyam and Hagstrum, 1995; Park etal., 2003). Furthermore, most 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. *Plecthrantus glandulosus* Hook leaf (Ngamo etal., 2007a; Nukenine etal., 2007) and wood ash (Ntonifor etal., 2001, Mulungu etal., 2010; Moyin-Jesu, 2010; Singh, 2011.) 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 (Ngassoum etal., 2001). The insecticidal properties of products from *P. glandulosus* have shown good insecticidal properties against stored maize grain pests (Nukenine etal., 2007; 2010; Ngamo etal., 2007b; Goudoum etal., 2010). Many authors have reported the effectiveness of wood ash as a grain protectant (Golob etal., 1982; Firdissa and Abraham, 1999; Akob and Ewete, 2007; Oguntade and Adekunle, 2010; Gemu etal., 2013). 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 both the amount of 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. glanduosus* leaf powder and *H. acida* wood ash regarding adult toxicity, progeny production, population growth, grain damage and germination.

Materials and MethodsSource of maize grains

The variety of maize used during all experimentation was Shaba, this provided by IRAD Wakwa in the Adamaoua region of Cameroon. Before experimentation, broken grains, the pieces of stone, sand and others 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 and 14 days of acclimatisation, 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. The weevils were reared on disinfested maize in 900 ml glass jars and kept under fluctuating laboratory conditions of 23.08 ± 2.05° C and 74.67 ± 14.36%). The culture was maintained and used as source of *S. zeamais* for bioassays.

Plants

Stems and branches of *H. acida* were collected in Ngaoundéré, Adamaoua 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, 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.

Leaves of *P. glandulosus* were collected in July 2012 in Ngaoundere located inVina Division, 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 on number 7656/SRF. The leaves were dried at room temperature for seven days, and then crushed until the powder passed through a 0.20 mm sieve. Wood ash and leaf powder of the two plants 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: 75PG25H

Toxicity and F_1 progeny bioassays

The toxicity bioassay was carried out under ambient laboratoryconditions. Four concentrations for 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 and their binary combinations were separately added to 50 g of maize in glass jars to constitute, respectively the contents of 5; 10; 20 and 40 g/kg. Then, the insecticidal materials plus grain were thoroughly mixed by manual shaking. The controls consisted of substrate without insecticidal products. A set of 20 insects of mixed sexes and 7 to 14-days-old were added into the jars containing the treated or untreated grains. All treatments were replicated four times. 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 according toSun and Johnson (1960). After the 14 days 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 for 5 weeks (Nukenine etal., 2007).

Damage and germination tests

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 three months, the live weevils and dead ones were counted. Damage assessment was performed by counting and weighingthe number of damaged and undamaged grain using the method of Adams and Schulten (Adams and Schulten, 1987).

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. Also, the non infested treated seeds were used to assess the effect of binary mixtures on germination ability. The number of germinated seeds was recorded after 10 days (Rao etal., 2006).

Data analysis

Abbott's formula (Zar, 1999) 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 (SAS Institute, 2003; Finney, 1971). Tukey's test (P = 0.05) was applied for mean separation. Probit analysis (Finney, 1971; Abbott, 1925) 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}) reduction of F_1 progeny.

Results

Plectranthus glandulosus leaf powder, *H. acida* wood ash and their binary combinations significantly induced mortality of *S. zeamais* adult. This mortality increased with content and exposure time (Tab. 1). Low variation in term of efficacy was observed amongst the five tested powders. In general, this variation became highly significant (P< 0.0001) within 7 and 14 days. 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 14 days of exposure. *P. glandulosus* leaf powder induced low mortality compared to the other products at all exposure periods. The mortality rate of 57.24% was recorded with *P. glandulosus* leaf powder at its highest content (40 g/kg) within 14 days of exposure. 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 of exposure time. In term of induced mortality, the different products can be ranked as follows: *H. acida* wood ash > 25PG75HA > 50PG50HA > 75PG52HA > *P. glandulosus* leaf powder.

The lethal content of different powders and their combinations reduced; when the exposure period increased (Tab.2). 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 with 25% of *H. acida* wood ash produced synergistic effect whereas that made up by 50% of each of two powders had antagonistic effect.

Tab. 1 Cumulative mortality of *Sitophilus zeamais* adult induced by *Plectranthus glandulosus* leaf powder and *Hymenocardia acida* wood ash and their binary combinations (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	Products *P. glandulosus*	*H. acida*	25PG75HA	50PG50HA	75PG25HA	$F_{(4; 15)}$
1 day						
0	0.00 ± 0.0aA	0.00 ± 0.0cA	0.00 ± 0.00cA	0.00 ± 0.0cA	0.00 ± 0.0bA	-

5	3.82 ± 2.4aB	2.50 ± 1.4bcB	8.75 ± 3.1bcAB	11.25 ± 1.2bAB	21.25 ± 5.9abA	5.1*
10	6.25 ± 2.4aB	5.00 ± 8.7abB	22.50 ± 4.3abAB	20.00 ± 2.0bAB	28.75 ± 6.6aA	5.6*
20	7.50 ± 2.5aB	8.75 ± 1.2aB	25.00 ± 6.1abAB	20.00 ± 3.5bAB	36.25 ± 9.4aA	6.8*
40	16.25 ± 0.9aAB	12.50 ± 1.4aB	36.25 ± 5.5aAB	36.25 ± 5.5aAB	41.25 ± 8.5aA	5.0*
$\mathbf{F_{(4;15)}}$	2.48ns	19.66**	15.83***	40.80***	11.78**	
3 days						
0	0.00 ± 0.0cA	0.00 ± 0.0cA	0.00 ± 0.0cA	0.00 ± 0.0bA	0.00 ± 0.00bA	-
5	6.25 ± 3.1bcB	21.08 ± 3.7bAB	22.90 ± 7.5bAB	37.04 ± 4.4aA	25.06 ± 4.1aA	6.1*
10	11.38 ± 3.1abB	24.57 ± 2.0abAB	42.17 ± 7.2abA	40.92 ± 4.2aA	38.82 ± 8.0aA	6.2*
20	17.83 ± 5.0aB	33.66 ± 2.0aAB	44.42 ± 5.6abA	46.81 ± 7.3aA	40.07 ± 8.0aAB	3.9*
40	21.58 ± 3.9aB	37.41 ± 4.6aAB	57.57 ± 5.8aA	56.45 ± 6.2aA	52.83 ± 7.8aA	7.0*
$\mathbf{F_{(4;15)}}$	12.10***	66.26***	28.53***	40.77***	22.16***	
7 days						
0	0.00 ± 0.00cA	0.00 ± 0.00bA	0.00 ± 0.0bA	0.00 ± 0.00dA	0.00 ± 0.00bA	-
5	7.77 ± 2.6bB	63.55±12.8aA	36.55 ± 10.9aAB	43.71 ± 3.2cAB	36.92 ± 3.1aAB	5.9*
10	14.15 ± 3.2abB	72.69 ± 8.6aA	53.15 ± 9.6aA	58.92 ± 1.3bA	49.27 ± 5.4aA	10.6***
20	20.59 ± 3.7abB	77.96 ± 8.6aA	57.16 ± 9.1aA	69.72 ± 5.3aA	49.42 ± 7.0aA	11.6***
40	28.16 ± 4.7aC	87.11 ± 4.9aA	68.35 ± 4.4aAB	76.76 ± 1.0aA	56.14 ± 4.0aB	27.7***
$\mathbf{F_{(4;15)}}$	16.00***	18.27***	21.54***	202.13***	57.03***	
14 days						
0	0.00 ± 0.00dA	0.00 ± 0.00bA	0.00 ± 0.00cA	0.00 ± 0.00cA	0.00 ± 0.0cA	-
5	19.54 ± 2.6cB	69.08 ± 10.8aA	68.13 ± 10.3bA	63.16 ± 5.3bA	56.07 ± 3.9bA	7.9**
10	33.82 ± 3.5bB	82.60 ± 7.9aA	82.09 ± 3.6bA	80.92 ± 4.6aA	66.89 ± 5.8abA	15.3***
20	40.33 ± 4.1bC	90.64 ± 6.2aA	93.13 ± 1.41aA	82.17 ± 2.7aAB	72.44 ± 4.1abB	27.0***
40	57.24 ± 2.7aC	94.66 ± 3.7aA	94.59 ± 2.1aA	90.42 ± 1.3aAB	80.85 ± 1.4aA	47.3***
$\mathbf{F_{(4;15)}}$	106.53***	26.03***	65.27***	105.05***	122.33***	

Means ± S.E. followed by the same capital letter in a line and the same lowercase 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.

The three combinations of *H. acida* and *P. glandulosus* significantly reduced the production of progeny compared to the control (Tab. 3). From the application of 5 g/kg (lowest content), the number of emerging adults was highly reduced. The highest inhibition of emerging insects was recorded at the highest content (40 g/kg) of the three combinations made up by *P. glandulosus* leaf powder and *H. acida* wood ash; 25PG75HA, 50PG50HA and 75PG25HA inhibited adult F_1 progeny production by 95.49, 83.39 and 80.92, respectively. Generally, 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} was achieved by 75PG25HA (10.69 g/kg).

Tab. 2 Lethal contents and co-toxicity coefficients of binary combinations (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Products	Slope	R^2	LC50(95% FL) (g/kg)	LC95(95% FL) (g/kg)	Co-toxicity coefficient (CTC)	Significance of CTC	χ2
3 days							
P. glandulosus	0.98 0±0.25	0.973	213.69 (87.60; 2902)β	—			1.50ns
H. acida	0.569±0.20	0.966	135.32 (50.48; 18401)β	—			0.292ns
75PG25HA	0.728 ± 0.19	0.923	34.119 (21.81; 99.05)	6180 (766.378; 4210605)β	547.111	synergistic	0.622ns
50PG50HA	0.525 ± 0.19	0.924	25.27 (14.68; 132.30)	—	21.414	antagonistic	0.349ns
25PG75HA	0.925 ± 0.19	0.904	24.774 (17.92; 42.31)	1485 (377.425; 38229)β	601.378	synergistic	1.342ns
7 days							
P. glandulosus	0.908 ± 0.23	0.999	166.041 (73.016; 1706)	—			0.121ns

H. acida	0.826 ± 0.21	0.988	1.946 (0.280; 3.910)	190.821 (73.336; 3007)β			0.323ns
75PG25HA	0.483 ± 0.19	0.848	18.166 (9.275; 69.952)	—	41.394	antagonistic	0.564ns
50PG50HA	0.946 ± 0.19	0.973	6.798 (3.711; 9.538)	372.549 (138.058; 3695)β	56.589	antagonistic	0.217ns
25PG75HA	0.851 ± 0.19	0.942	10.816 (6.810; 15.197)	928.122 (249.182; 26174)β	23.896	antagonistic	0.545ns
14 days							
P. glandulosus	1.088±0.19	0.976	28.645 (21.379; 46.464)	928.767 (306.688; 9366)β			0.826ns
H. acida	1.268±0.24	0.929	1.936 (0.618; 3.316)	38.341 (25.041; 91.006)			0.195ns
75PG25HA	0.769 ± 0.19	0.981	3.036 (0.605; 5.481)	416.348 (124.349; 15575)β	212.073	synergistic	0.085ns
50PG50HA	1.00 ± 0.22	0.861	1.967 (0.459; 3.634)	86.344 (44.754; 415.547)β	3.937	antagonistic	0.986ns
25PG75HA	1.381 ± 0.25	0.896	2.175 (0.837; 3.509)	33.773 (23.036; 70.093)	116.067	additive	0.583ns

ns: P > 0.05; *: P <0.05, β : the LC values were obtained by extrapolation, # : the Fudicial limit values for LC could not be computed due to very low variations in mortality among the different contents of insecticidal material.

All the treatments significantly reduced grain damage and population increase, compared to the control (Tab. 4). And, the reductions of grain damage and population growth were dose-dependent. The number of insects, grain damage and weight loss decreased when the concentration of powders increased. Concerning the different parameters, a difference was observed in term of effectiveness according to the combination. The number of insects was also considerably reduced. Even at the lowest content (5 g/kg), the three combinations revealed 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 more effective compared to the other combinations.

The germination rate varied with treatment. In general, the non-infested maize grain had a good germination rate than the infested ones (Tab. 5). In non-infested grain, the germination percentage was almost the same; it varied according neither to the combination nor to the content. But, with maize grain infested by *S. zeamais*, the germination rate increased with ascending the dosage. The germination percentage of infested maize grain was highest at the highest content (40 g/kg). Without insect, the germination was significantly higher even without insecticidal powder (94.33 %) whereas with insect the germination rate was the lowest one (21.67 %).

Tab. 3 Progeny production of *Sitophilus zeamais* in maize treated with binary mixtures (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Content	25PG75HA	50PG50HA	75PG25HA	$F_{(2;9)}$
	Mean number of F1 adult progeny			
0	42.50 ± 1.71aA	42.50 ± 1.17aA	42.20 ± 1.17aA	—
5	14.25 ± 0.85bB	23.00 ± 2.86bAB	28.50 ± 4.65abA	5.05*
10	9.25 ± 1.65bcB	15.75 ± 1.65bcAB	23.75 ± 3.79bA	7.97*
20	4.75 ± 1.44cdB	8.25 ± 0.63cdAB	14.50 ± 3.80bcA	4.34*
40	2.00 ± 0.82dB	7.00 ± 0.82dA	8.00 ± 1.08cA	12.40*
$F_{(4;15)}$	145.94***	70.41***	16.23***	
	Inhibition of adult emergence relative to control (%)			
0	0.00 ± 0.00dA	0.00 ± 0.00dA	0.00 ± 0.00dA	—
5	66.51 ± 1.23cA	45.92 ± 6.42cAB	33.27 ± 10.56cB	5.47*

10	78.32 ± 3.87bA	62.88 ± 3.97bAB	44.53 ± 8.27bcB	8.66*
20	88.91 ± 3.47abA	80.42 ± 2.05aAB	66.60 ± 7.45aB	5.31*
40	95.49 ± 1.74aA	83.39 ± 2.32aB	80.92 ± 3.17aB	9.88*
$F_{(4; 15)}$	233.90***	86.76***	19.96***	
EC_{50} (95%FL) g/kg	2.55(1.29; 3.76)	5.57(3.54; 7.39)	10.69(6.74; 15.09)	

Means ± S.E. followed by the same capital letter in a line and the same lowercase letter in a column do not differ significantly at P< 0.05 (Tukey's test). *: P < 0.05, ***: P< 0.0001.

Tab. 4 Population increase of *Sitophilus zeamais* and grain damage recorded in stored maize treated with the binary combinations (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	**Products**					
	25PG75HA	**50PG50HA**	**75PG25HA**	**25PG75HA**	**50PG50HA**	**75PG25HA**
	Number of live insects			Number of dead insects		
0	240.00 ± 12.2a	240.00 ± 12.2a	240.00 ± 12.2a	50.50 ± 8.5a	50.50 ± 8.5a	50.50 ± 8.5a
5	54.75 ± 25.1b	118.50 ± 12.5b	170.00 ± 7.07	69.02 ± 7.5a	58.00 ± 8.9a	63.50 ± 8.4a
10	36.50 ± 4.6b	102.25 ± 2.2bc	128.25 ± 13.4b	69.00 ± 1.3a	62.02 ± 2.7a	64.75 ± 2.6a
20	38.75 ± 7.1b	89.25 ± 5.7bc	72.75 ± 2.5c	59.12 ± 1.4a	75.50 ± 1.5a	82.00 ± 12.7a
40	26.50 ± 7.27b	68.00 ± 10.0c	44.75 ± 11.6c	58.50 ± 0.9a	71.50 ± 2.5a	53.75 ± 5.5a
$F_{(4; 15)}$	45.08***	51.25***	58.05***	2.32ns	3.02ns	2.22ns
	Grain damage			Weight loss (%)		
0	49.61 ± 4.54a	49.61 ± 4.54a	49.61 ± 4.54a	12.81 ± 1.49a	12.81 ± 149a	12.81 ± 1.49a
5	21.92 ± 3.59b	28.02 ± 1.55b	36.81 ± 1.97b	3.22 ± 0.82b	5.07 ± 1.10b	5.87 ± 1.16b
10	18.20 ± 2.54b	25.13 ± 1.43b	30.41 ± 0.72b	3.02 ±1.36b	4.15 ± 0.91b	3.85 ± 1.14b
20	17.25 ± 2.60b	20.12 ± 3.01b	27.95 ± 0.68bc	2.94 ± 1.29b	3.92 ± 1.00b	3.43 ± 0.23b
40	13.56 ± 1.17b	17.20 ± 3.2b	17.60 ± 2.0c	1.54 ± 1.1b	3.09 ± 0.7b	2.79 ± 0.6b
$F_{(4; 15)}$	22.25***	18.21***	23.68***	13.83***	13.86***	15.93***

Means ± S.E. followed by the same lowercase 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.

Tab. 5 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* in laboratory conditions (t = 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 ± 1.3a	94.33 ± 1.3a	94.33 ± 1.3a	21.67 ± 2.1d	21.67 ± 2.1d	21.67 ± 2.1c
5	91.08 ± 0.2a	90.83 ± 1.5a	94.83 ± 1.9a	84.67 ± 0.8c	81.67 ± 3.1c	78.33 ± 0.9b
10	92.00 ± 0.8a	94.50 ± 1.8a	92.83 ± 0.8a	88.33 ± 1.7bc	84.17 ± 2.5bc	83.33 ± 1.3ab
20	92.17 ± 0.7a	94.17 ± 1.5a	93.42 ± 1.6a	92.33 ± 0.8ab	93.33 ± 1.3a	86.67 ± 2.4ab
40	91.17 ± 0.3a	92.08 ± 0.7a	92.20 ± 1.7a	97.50 ± 1.6a	95.00 ± 1.7a	90.00 ± 2.3a
$F_{(4; 15)}$	2.72ns	1.3ns	0.41ns	430.01***	180.43***	218.78***

Means ± S.E. followed by the same lowercase letter in a column do not differ significantly at P< 0.05 (Tukey's test); ns: P > 0.05; ***: P< 0.0001.

Discussion

The binary mixtures of *P. glandulosus* leaf powder and *H. acida* wood ash provoked significant mortality of *S. zeamais*. 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, broadening the spectrum of activity and reducing the chance of resistance development, on the other hand (Das, 2014). However, with mixtures, negative effects can also occur such as reduction of efficacy, phyto-toxicity and incompatibility problems between materials (Regupathy, 2004). The combinations of 75% of *P. glandulosus* leaf powder with 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, it produced a significant synergism. In general, the mixtures composed by different 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 proportions of two products used in combinations can 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 same products was observed by Ntonifor etal. (2010); these authors found that the combinations of *Syzygium aromaticum* (L.) (Myrtaceae) and *Cyperus aequelis* (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.

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 *S. zeamais* development. The presence of *P. glandulosus* leaf powder in combinations may potentiate the effect of ash. There are physical and chemical action, 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 (2014) found that the mixture of soya oil and Acetamprid pesticide gave the highest average mortality of *Trogoderma granarium* larvae and 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 of damage and the suppression of *S. zeamais* population growth were positively correlated. Combinations of *H. acida* wood ash with *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 (1990) 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. *P. glandulosus* leaf, thanks to its chemical compounds controlled the proliferation of insect, which explain 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 (2013), 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.

In many African countries, stored grains provide not only grains for food but also seeds for planting. The untreated maize in presence of insects recorded the week germination rate. In this case the seed loss 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 (2011) found that seed germination was significantly reduced when untreated maize seeds were exposed to *S. oryzae* and *T. castaneum*. 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.

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 (Chung and Miller, 1995; Bustos-Figueroa etal., 2009). 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 etal. (2009) observed that the leaf powder of *Peumus 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 25% of *P. glandulosus* leaf powder with 75% of wood ash could be due to the higher content of ash in the combination. According to Philogène (1972), 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, Fe, which are important for plant growth. Parimelazhagan and Francis (1999) 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 (Hedimbi *et al.*, 2012). 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.

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 permit to the maize grains to conserve their viability without affecting negatively germination rate. The beneficial effect of the combinations could be enhanced by using the appropriated proportions. Then, other proportions in combination of the two powders need to be tested in order to find out the most 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.

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