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Bioefficacy of Binary Combinations of *Plectranthus Glandulosus* Leaf Powder and *Acacia polyacantha* Wood Ash on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

Wini Goudoungou Jean
Department of Biological Sciences,
University of Bamenda, Bamenda, Cameroon &
University of Ngaoundéré, Ngaoundéré, Cameroon
Email: winigoudoungou@yahoo.fr

Ndjonka Dieudonné
Department of Biological Sciences,
University of Ngaoundéré, Ngaoundéré, Cameroon

Suh Christopher
Crop Protection Laboratory, IRAD, Bambui, Cameroon

Nukenine Elias Nchiwan
Department of Biological Sciences,
University of Ngaoundéré, Ngaoundéré, Cameroon

Abstract – **The maize weevil, *Sitophilus zeamais* is highly devastating and is regarded as the most cosmopolitan pest of stored maize grain across the tropics. Cheap, effective and environmentally friendly methods of reducing maize storage losses caused by this weevil are necessary to combat food insecurity, especially in sub-Saharan Africa. The insecticidal efficacy of binary combinations between wood ash from *Acacia polyacantha* and *Plectranthus glandulosus* leaf powder was assessed on *S. zeamais* regarding adult toxicity, progeny inhibition, suppression of population and reduction of damage. The masses of combined products were mixed with maize grain to constitute the contents of 5; 10; 20 and 40 g/kg to carry out the bioassays of toxicity within 1, 3, 7 and 14 days exposure, progeny production, population increase and grain damage. The binary mixtures of *P. glandulosus* leaf powder and *A. polyacantha* wood ash showed positive performances. The combination 50AP50PG was the most effective; it induced 100% mortality at its highest content (40 g/kg) within 14 days of exposure. The synergistic action was observed for combinations of *A. polyacantha* wood ash with *P. glandulosus* leaf powder for all the proportions. The combinations inhibited more than 80% of F_1 progeny relative to the control except combination 25AP75PG which reduced progeny by 78.82%. The combination 75AP25PG inhibited almost completely the production of progeny (> 95 %). The population of *S. zeamais* was considerably suppressed by all combinations thereby reducing maize damage and weight loss. Considering the results of this study the binary combinations of *A. polyacantha* wood ash and *P. glandulosus* leaf powder could be used as a suitable substitute of synthetic insecticides since it is economical and environmentally friendly.**

Keywords – ***Sitophilus zeamais*, Maize, Damage, Wood Ash, Leaf Powder, Binary Combinations, Synergistic Action.**

I. INTRODUCTION

The food situation has remained insecured and unpredictable in sub-Saharan Africa, where more than 50% of the population earn their livelihood from agriculture, leading to high levels of cyclic famine and poverty (Othira *et al.*, 2009). Therefore the fight against hunger and poverty needs to be intensified if this Millennium Development Goal is to be realized in sub-Saharan Africa. A major cause of food insecurity is grain loss during storage caused mainly by insect pests. Maize (*Zea mays*) is the leading cereal crop across Africa (FAO, 2009). The crop is grown in all the 10 regions of Cameroon and it is the most consumed cereal in the country. Unfortunately, maize is heavily devastated during storage by insect pests, especially its primary pest, maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). More so, storage of this crop is unavoidable in Cameroon and many African countries because production is done within a short period of the year while the crop is consumed and marketed all year round. Generally, over 70% of grains harvested in Africa are stored for human consumption or for marketing (Talabi, 1989).

Food security could be enhanced by reducing stock losses. Damages caused by *S. zeamais* on maize could be reduced through control measures (chemical , bio-control, physical 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 among others (Subramanyam and Hagstrum, 1995; Park *et al.*, 2003). 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 powders, extracts or purified substances of plant origin. They are generally assumed to be more biodegradable leading to less environmental problems. Powder and essential oils of *Plecthrantus glandulosus* Hook f. (Ngamo *et al.*, 2007; Nukenine *et al.*, 2007, 2010b) and wood ash (Mulungu *et al.*, 2010; Moyin-Jesu, 2010; Singh, 2011; Ntonifor *et al.*, 2011) stand out as good candidates for environmentally friendly control of storage pests under Cameroonian conditions.

The insecticidal properties of Products from *P. glandulosus* (Ngamo *et al.*, 2007b; Goudoum *et al.*, 2010;

Nukenine *et al.*, 2007, 2010a, 2010b) have shown good insecticidal properties against stored maize grain pests. The leaf powder of the plant, which is more accessible to rural farmers than the chemical, should be an alternative to manage the different stages of insect pests since it was tested and revealed effective to control adult population. The lowest effective dosages of botanicals need to be determined. Higher dosages of plant materials could increase their mammalian toxicity to dangerous levels. The combinations amongst different plant materials or with other insecticidal materials could enhance the effectiveness of products thereby reducing the quantity of used substances.

The use of chemically inert materials, such as wood ashes, diatomaceous earths, sand or other minerals, powders or plant products in large quantities to fill up the interstitial space in grain bulks and provide a barrier to insect movement, is quite widespread (FAO, 1999).

Many authors have reported the effectiveness of wood ash as a grain protectant (Golob *et al.*, 1982; Firdissa and Abraham, 1999). Golob *et al.* (1982) evaluated the effectiveness of admixing locally available powders at the rate of 1%, 5%, 15% and 30% (w/w) with maize and found that wood ash, among others, restricted infestation; wood ash at 30% being almost as effective as pirimiphos-methyl.

Acacia polyacantha, Mimosaceae of the genus *Acacia* is Widespread all over Africa in areas close to ground water but the tree can grow in poor dry savannah soil (Mulofwa *et al.*, 1994). *A. polyacantha* is further used for timber, building shelter, land improvement (nitrogen fixing, soil reclamation), used as fuel (firewood) and for ornamental purposes.

Combinations of inert dusts 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 related to the effectiveness of the binary combinations among inert dusts and *P. glandulosus* powder are not available, although farmers mix inert dusts like ash with plant materials in stocks.

The objective of this study was to evaluate the bioefficacy of binary combinations among *P. glandulosus* leaf powder and *A. polyacantha* wood ash on *S. zeamais* regarding adult toxicity, progeny production inhibition, suppression of population and reduction of damage.

II. MATERIALS AND METHODS

Insects rearing

Adults of *S. zeamais* were obtained from a colony maintained in the Applied Chemistry Laboratory of the University of Ngaoundere. The insect (weevil) culture was later transferred and kept in the Crop Protection laboratory of IRAD Bambui. The weevils were reared on disinfested maize in 900 ml glass jars and kept under ambient laboratory conditions (t = 23.08 ± 2.05° C, rh = 74.67 ± 14.36%). This culture was maintained and used as source of *S. zeamais*.

Source of maize grains

The variety of maize used during the experiment was Shaba. This variety was provided by IRAD Wakwa in the Adamawa region of Cameroon. Before experimentation, broken grains and impurities were removed from the stock. The maize was then kept in the freezer at – 4 °C for 14 days to allow for complete disinfestations, after disinfestations for all types of living organisms, the maize was kept in ambient laboratory conditions for 14 days to allow for acclimatisation. After all these steps, the maize was now ready for bioassays.

Preparation of Acacia polyacantha wood ash

Woods of *Acacia polyacantha* were collected respectively in Kousseri, Logone and Chari division, Far North region in Cameroon. The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde, where voucher samples were deposited. Woods were air-dried until complete moisture lost and burnt separately. For each plant material, 1kg g of ash was obtained. The ashes were packaged in glass jars, labelled and kept in a refrigerator (at – 4°C) until subsequent use in the bioassays.

Preparation of Plectranthus glandulosus leaf powder

The leaves of *P. glandulosus* were collected in July 2012 from Ngaoundere located in the Vina Division of the Adamawa region of Cameroon. The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde. The leaves were dried at room temperature for seven days, and then crushed. The crushed leaves were ground until the powder could pass through a 0.20 mm sieve. Part of the powder was stored in a freezer at –20°C for the bioassays and the other part was used for essential oil extraction.

Analysis of volatile compounds for Plectranthus glandulosus leaf powder

The essential oil was extracted by hydro distillation for 4hours using a Clevenger type apparatus. The extracted oil was kept in a dark bottle at 4° C until its use for GC-MS analysis.

The GC-MS analysis were carried out using a chromatograph model Agilent 7890A GC equipped with an automatic injector and a column HP-1MS (15 m × 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 gas 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 temperature programming was done by increasing the temperature from 60 to 230° C at an interval of 2° C/min then 35° C/min until 230° C was reached. 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 temperature of source was 150° C. The bombardment of essential oil with the electronic beam of 70 eV induced its ionisation and its fragmentation. Then the positive ionic fragments formed the characteristic mass spectrum of the compound. The obtained spectra were compared with computerized data base using NIST/EPA/NIH Mass Spectral Library (NIST, 1999), WileyRegister of Mass Spectral Data (Mc Lafferty and Stauffer 1994) and König *et al.*, (2001).

Determination of chemical composition of wood ashes

Mineral content

Ash was subjected to mineralisation in order to determine the mineral contents (Pauwels *et al.*, 1992). The ash sample was burnt at 450°C for a complete mineralisation. Burnt ash was dissolved in nitric acid (HNO_3) 1M for digestion then carried to boiling. The solution was filtered after cooling. The filtrate obtained was used to determine the following cations: P, K, Ca, Mg, Na, Fe, Mn, Zn and Pb. Ca, K, Na were determined by flame photometry using the extract obtained by mineralisation. Mg, Fe, Mn, Zn and Pb were proportioned by atomic absorption spectrometry. Concerning phosphate, its content was measured by molecular absorption spectrophotometry.

Determination of Acacia polyacantha wood ash pH

The ph of *A. polyacantha* wood ash was determined by using pH-meter CG818 (Schott-Geräte Gmbh, Germany). 2g of ash were dissolved in 10 ml of sterilised distilled water, and then the electrode of pH-meter was introduced into the solution. The pH values were directly read on pH-meter screen.

Preparation of different combinations

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

- 25% *P. glandulosus* leaf powder and 75% *A. polyacantha* wood ash to constitute product 75AP25PG;
- 50% *P. glandulosus* leaf powder and 50% *A. polyacantha* wood ash to constitute product 50AP50PG;
- 75% *P. glandulosus* leaf powder and 25% *A. polyacantha* wood ash to constitute product 25AP75PG.

These combinations were used to carry out the bioassays concerning toxicity, progeny production, population increase and grain damage.

Toxicity bioassay

The toxicity bioassays were carried out in ambient laboratory conditions . During experimentation the temperatures and the relative humidity were recorded using a data logger. Four concentrations for each product were considered. The masses of 0.25; 0.5; 1 and 2 for *P. glandulosus* leaf powder and *A. polyacantha* wood ash and their binary combinations were added each to 50g of maize (Shaba) to constitute respectively concentrations of 5; 10; 20; 40 g/kg. The controls consisted of substrate without insecticidal products. All treatments were replicated four times and the experiment was arranged in a completely randomized design. Mortality was recorded 1, 3, 7 and 14 days after application.

The co-toxicity coefficient per *P. glandulosus* leaf powder and wood ash mixture was determined. A co-toxicity coefficient of less than 80 is considered as antagonistic, between 80 and 120 as additive and higher than 120 as synergistic (Sun & Johnson, 1960). If mixture (M) compounds of two parts (A and B), and both components have appreciable LC_{50} values, 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 *A. polyacantha*):

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

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

$$\text{Actual TI of M} = \frac{\text{LC}_{50}\text{ of A}}{\text{LC}_{50}\text{ of M}} \times 100$$

$$\text{Theoretical TI of M} =$$

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

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

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:

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

This did not apply to this experiment because all the mixture combinations induced more than 20% of mortality.

F_1 progeny bioassay

After the 14 days mortality recordings, the 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 commencing 6 weeks after infestation. The emergence started only after the 5th week after infestation. After each counting session, the insects were removed from the jars. (Nukenine *et al.*, 2007).

Damage Bioassay

Four rates of the combined materials (5, 10, 20 and 40 g/kg), for 150 g seed were prepared as described above. Fifty unsexed weevils (7-14 days old) were introduced into each jar. Each treatment had four replications. After three months, the number of live and dead insects was counted. Damage assessment was performed by measuring and counting the number of damaged and undamaged grain using the method of Adams & Schulten (1978):

$$\text{Weight Loss(\%)} = \frac{(\text{Wu} \times \text{Nd}) - (\text{Wd} \times \text{Nu})}{\text{Wu}(\text{Nd} + \text{Nu})} \times 100$$

Where Wu is the weight of undamaged grain, Nd the number of damaged grain, Wd the weight of damaged grain and Nu the number of undamaged grain.

Data analysis

Data on % cumulative corrected mortality, % reduction in F_1 progeny, % damage and % weight loss were arcsine-transformed [(square root(x/100)] and the number of F_1 progeny was log-transformed (x + 1). The transformed data was subjected to the ANOVA procedure using the Statistical Analysis System (Zar, 1999; SAS Institute, 2003). Tukey's test (P = 0.05) was applied for mean separation. Probit analysis (Finney, 1971; SAS institute, 2003) 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 of F_1 progeny. Abbott's formula (Abbott, 1925) was used to correct for control mortality before probit analysis and ANOVA.

III. RESULTS

Chemical composition of essential oil for P. glandulosus leaf powder

The volatile constituents of the essential oils from the powder of *P. glandulosus* 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 dominated chemical constituents were terpenic compounds. The major compounds were Thymol (10.1%), α-Terpineol (10.8%), α-Pinene (11.5%), 3-Carene (8.7%), β-Myrcene (9.7%), Pinene (11.5%), α-Pinene (11.2%) and L-β-Pinene (11.5%) which are the monoterpernes. The only major constituents, which were not monoterpenes, were β-Caryophyllene oxide (9.4%) which is oxygenated sesquiterpene and Acetophenone (5.5%) which is an aromatic compound.

Chemical composition of Acacia polyacantha wood ash

The value of *A. polyacantha* wood ash pH was 10.48. Some minerals were determined (Table 2). The highest mineral content was represented by magnesium (3742 mg/kg). The second most important mineral content was observed for calcium (2640 mg/kg) followed by phosphorus (1828 mg/kg) then potassium (1118 mg/kg). Manganese had the lowest content (0.011 g/kg) compared to the other minerals except lead (Pb) that was found in trace quantity (0.0019 mg/kg).

Mortality of adult induced by the insecticidal material alone and their binary combinations

The two products and their combinations induced significant mortality of *S. zeamais* adult (Table 3). This mortality varied significantly according to the products ($F_{(4;\ 300)}$ = 121.97; P<0.0001) and the concentrations ($F_{(4;\ 300)}$ = 374.38; P<0.0001). It was also period dependent ($F_{(3;\ 300)}$ = 381.19; P<0.0001).

Effect of the combination of Plectranthus glandulosus leaf powder and Acacia polyacantha wood ash on the bioefficacy

The LC values were generally higher with powders used alone than when combined i.e. *P. glandulosus* leaf powder with *A. polyacantha* (Table 4). The binary combinations of *P. glandulosus* leaf powder with *A. polyacantha* showed different toxicity parameters. The different values of lethal content (LC) decreased when the exposure period increased. values recorded were : 32.31; 26.59; and 42.13 g/kg respectively for 25AP75, 50AP50PG and 75AP25PG. But within 14 days of exposure the LC_{50} values were 1.09, 3.02 and 1.92 g/kg respectively for the same powders. The highest value of LC_{95} (144.61 g/kg) was observed with 25AP75PG whereas the lowest value (90.05 g/kg) was observed with the application of 50AP50PG. Different tendencies were observed for the three combinations according to the values of coefficient of co-toxicity (CTC). After 7 days of exposure, the combinations of *P. glandulosus* leaf powder and *A. polyacantha* wood ash revealed synergistic action and the values of coefficient of co-toxicity were greater than 120. The same tendency was observed after 14 days of exposure except for combination 50AP50PG, where an antagonistic action was observed.

Reduction of F1 progeny by the combined products and efficacy content (EC)

The three binary mixtures of *P. glandulosus* leaf powder with *A. polyacantha* wood ash induced significant reduction of F_1 progeny, which considerably increased by increasing the products concentration (Table 5). All the three combinations achieved significant reduction in adult emergence relative to the control at all content levels. Few insects emerged at 40 g/kg for all the mixtures. The combinations 75AP25PG inhibited almost completely (> 95 %) the production of F_1 progeny at 40 g/kg. Even at +he lowest (5 g/kg), the combinations considerably reduced the production of F_1 progeny compared to the control. At this content level the following combinations: 75AP25PG, 50AP50PG and 25AP75PG recorded respectively 27.25, 20.00 and 26.00 insects, whereas at content level 0 g/kg (control) 42.50 insects emerged from the maize. The values of EC_{50} and EC_{95} were different according to the combinations (Table 6). The lowest value of EC_{50} and EC_{95} were obtained respectively with 50AP50PG and 75AP25PG, which were 5.11 g/kg and 44.00 g/kg respectively. The highest values of EC_{50} (8.44 g/kg) and EC_{95} (165.65 g/kg) were recorded by 25AP75PG.

Suppression of population growth and reduction of grain damage

The combinations of *P. glandulosus* leaf powder with *A. polyacantha* wood ash significantly suppressed the population increase of *S. zeamais* after three months of storage (Figure 1). The combination 50AP50PG achieved the best performance compared to the other combinations (75AP25PG; 25AP75PG) at all the content levels. At 40 g/kg it was observed from grains treated with 50AP50PG lower number of life insects than dead insects. For this combination at 5 g/kg, the suppression of insect population was comparatively different from the control. The combinations 50AP50PG and 75AP50PG had the similar ability to suppress the population of *S. zeamais* at their highest content (40 g/kg), but at 5, 10 and 20 g/kg the difference was observed. The combination 50AP50PG was more effective than 75AP25PG. In terms of suppression of *S. zeamais* population increase, 25AP75PG was found to be less effective compared to the others combinations.

The percentages of perforated grain and weight loss were significantly reduced by the application of combinations of *P. glandulosus* leaf powder with *A. polyacantha* wood ash (Figure 2). These reductions of damage parameters were influenced by content levels and then the percentage of perforated grain and weight loss decreased when the products contents increased. The untreated maize (control) recorded 50 % of damaged grain with 13 % of weight loss. Even at the lowest concentration (5 g/kg) the damage reduction was considerate, the maize grain treated with combined products recorded less than 6 % of weight loss compared to the control where the maize grain lost almost 13 % of its weight. The percentage of weight loss (Figure 3) was related to that of perforated grain as, the higher the perforated grain,the higher the weight loss. The grains treated with 75AP25PG at 40 g/kg recorded the lowest damage (<15 % perforated grain, <2 % weight losses). At the same concentration, the maize grains treated with the combinations 50AP50PG and 25AP75PG recorded the lowest damage.

IV. Discussion

The two insecticidal materials alone were found to be toxic against adult *S. zeamais*. This toxic action increased with increasing exposure period. Even with the lowest concentration, the mortality became higher with increasing period. This situation was well elucidated by decreasing of values of LC from 3rd day to the 14th day. The mortality rate also increased with increasing product concentration. It was noticed a difference amongst the four insecticidal materials in terms of toxicity that was confirmed by their lethal content, which were very low for *A. Polyacantha* ash. *P. glandulosus* leaf powder recorded the highest values of LC. The increase of adult mortality according to the exposure period and concentration was due to the increase of the quantity of active ingredients contained in the insecticidal materials. The *A. polyacantha* wood ash was highly toxic and this toxicity could be due to abrasive property of ash and may be the mineral content. Gwinner *et al.* (1996) reported that the effect of ash varies according to the type of ash, which is related to its mineral content. Baier and Webster (1992) found kitchen ash applied at the rate of 20% w/w controlled bruchid reproduction throughout a 39 weeks storage period. *P. glandulosus* leaf powder was the less effective in terms of toxicity compared to the other products, however it was significantly effective against weevil compared to the control. In the present study, *P. glandulosus* leaf powder caused at its highest concentration (40 g/kg) 57.24% mortality rate within 14 days. With similar concentration level and time post-exposure, Nukenine *et al.* (2011) recorded 74%. The difference in toxicity recorded by the different studies could be due to time of harvest, age of plant at harvest, environmental conditions, among others, which are known to greatly influence the insecticidal activity of botanicals (Kaushik *et al.,* 2007; Nukenine *et al.,* 2010a). Concerning the mode of action, powders have the tendency of blocking the spiracles of insects which impairs respiration and death ensues (Iloba & Ekrakene, 2006). *P. glandulosus* powder contains several monoterpenes (Ngassoum *et al.,* 2001; Nukenine *et al.,* 2007), which could be toxic to the weevil by reversible competitive inhibition of acetyl cholinesterase by occupation of the hydrophobic site of the enzyme's active centre (Ryan & Byrne, 1988). Further research is required to determine the exact mode of action.

The efficacy of insecticidal compounds is related to time of exposure, it decreases when the storage period increases. It is therefore important to determine the action duration of each insecticidal material used in grain storage and its persistence . In our experiment, the efficacy of insecticidal products slightly decreased when the storage period increased. The loss of efficacy was negligible in general. Ntonifor *et al.* (2011) reported that 1g of leaf powder of *Chenopodium ambrosioides*/50g of maize caused 100% mortality during the first week. But at the second week at the same concentration, the mortality rate decreased to 87.5%. Thus *C. ambrosioides* lost its potency and needed to be retreated. This report makes obligatory the monitoring of stored grains to avoid re-infestation by insect pests.

The combinations of *P. glandulosus* leaf powder with *A. polyacantha* wood ash at different proportions produced different effects such as synergism and antagonism. Generally the combinations were synergetic except for combination 50AP50PG where antagonism was observed. The combinations of insecticidal materials have the advantages to increase the efficacy by complementing the bio-efficacy of the individual products and simultaneously lowering their use on the one hand and broadening the spectrum of activity and overcoming pest resistance to individual pesticide on the other hand (Das, 2014). While all the combinations or mixtures produce beneficial effects, negative effects can also occur such as reduced pest control, phyto-toxicity and incompatibility problems between materials (Regupathy *et al.*, , 2004). The combinations of *P. glandulosus* leaf powder with *A. polyacantha* on mortality produced synergistic effect, whereas combinations of this leaf with *H. acida* wood ash induced antagonistic effect except for 75% of *H. acida* ash with 25% leaf powder after 14 days which produced a significant synergism. The same performance was observed for combination of 50% of *A. polyacantha* wood ash with *P. glandulosus* leaf powder. In general, the mixtures of leaf powder improved the efficacy unlike when these materials are used individually. The additive effect was also observed, the effect of two materials is equal to the sum of each component given alone (1+3=4). The synergism has been demonstrated in this experiment by the decreasing of LC_{50} values compared to those of single material. For example after 7 days of exposure, the LC_{50} for *A. polyacantha* and *P. glandulosus* were respectively 77.47 and 166.04 g/kg whereas the LC_{50} values of 25AP75PG, 50AP50PG and 75AP25PG were respectively 17.16, 15.27 and 15.75 g/kg. The proportions of two products used in combinations can produce different performances. The combinations of 25HA75PG and 50HA50PG produced respectively synergistic and antagonistic effects. The same tendency concerning the variations in efficacy for different proportions of products was observed by Ntonifor *et al.* (2010), they 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% and100% mortalities of *C. maculatus* respectively after 3 days of exposure. Shalan *et al.* (2005) found that mixtures of some botanical extracts were more effective and economical than phytochemicals alone in controlling certain insects. The different binary mixtures significantly inhibited the production of F_1 *S. zeamais*. In different combinations, there were ash and leaf of *P. glandulosus*. In addition to increase the mortality, the combinations of these products have effects on the development of *S. zeamais*. The presence of *P. glandulosus* leaf powder in combinations potentiates the effect of ash. There are physical and chemical action, which are the desiccation by ashes and intoxification by the chemical compounds contained in *P. glandulosus* leaf. The mixtures act on the fecundity by the impairement of gonotropic cycle of adults which might prevent the eggs from hatching. The mixtures can also disturb or delay the development of larvae in adults. Karso & Al Mallah (2014) found that the mixture of soybean oil and Acetamprid pesticide gave the highest average mortality of *Trogoderma granarium* larvae and which varied according to the proportions. The binary mixtures conserved their efficacy alone and the storage period of maize grain before the infestation by adult weevils. However, that efficacy slightly decreased. That situation can be explained by the fact that the ashes absorb the water contained in grains and then potentiate the effect of *P. glandulosus* leaf powder. But when the storage last, the benefit effect of *P. glandulosus* leaf was lost and the desiccant effect of ash was reduced by absorption of water contained in relative humidity since the work was done in ambient laboratory conditions. The mortality of *S. zeamais* induced by leaf powder of *Peumus boldus* was greatly influenced by powder storage time before insect infestation (Bustos-Figueroa *et al.*, 2009). These authors observed that the toxicity of this plant was high after 24 hours of storage before infestation, but significantly decreased over the time. However in the present study, the combinations permitted the insecticidal materials to become more persistent compared to these products used individually.

The combinations of insecticidal materials improved 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. The combinations of *A. polyacantha* and *H. acida* wood ashes with *P. glandulosus* leaf at different proportions reduced considerably the damage by lowering the number of perforated grain and weight loss and at the same time by inhibiting the population increase. But, the control due to the combinations was less effective compared to the ashes used individually as done in the previous experiment. That could be justified by the variations of environmental conditions, which were different in the two experiments. In this experiment, temperature and relative humidity were 22.76 ± 2.02°C and 69.87 ± 9.93% respectively, whereas in the experiment with single product they were 23.51±1.96°C and 55.79 ± 9.00% r.h. In the present study, the relative humidity was higher and the temperature was lower compared respectively to those of the other experiment. All these conditions reduced the efficacy of combined products. As previously explained moisture reduces the efficacy of inert material. Hill (1990) reported that wood ash was useful as a physical barrier on grains but it can also possess various chemical properties according to its botanical source. Chatterjee (1984) revealed that the ashes and sand which were widely used acted as hygroscopic substances and reduced the moisture content of the commodities to some extent with which they were mixed and indirectly affected the insect multiplication. The *P. glandulosus* leaf thanks to its chemical composition controlled the proliferation of the insects, which explains the efficacy of combinations in short storage period. When the storage period increased, the efficacy decreased by loss of their volatiles compounds which confer its toxicity against insects. A similar observation was done by Mwangangi & Mutsiya (2013), who showed that the efficacy of *Ocimum basilicum* powder deteriorated the fastest leading to 80, 77, 44, 20 and 15 % mortality over 0, 7, 14, 21 and 28 days of storage. To improve the combinations of ashes with plant powders effectiveness and allow the components to ensure a good preservation of grains, it is important to avoid moisture increase.

V. CONCLUSION

In conclusion the combinations of *A. polyacantha* wood ash with *P. glandulosus* leaf powder increased the insecticidal efficacy of both products. But this synergistic action was not observed in all proportions, because the antagonistic action was recorded for the combination of 50% of *A. polyacantha* wood ash with 50% of *P. glandulosus* leaf powder (50AP50PG) after 14 days of exposure. Thus the proportions of each product involved in the mixture and their chemical composition influences the bioactivity on insect pests.

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Table 1: Chemical constituents of essential oil from *Plectranthus glandulosus* leaf powder

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
Bornyle acetate	tr
Neryle 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: < 0.1

Table 2: Chemical composition of Acacia polyacantha wood ash

Minerals	**Content (mg/kg)**
Calcium (Ca)	2640
Magnesium (Mg)	3742
Potassium (K)	1118
Sodium (Na)	289
Iron (Fe)	608
Zinc (Zn)	786
Lead (Pb)	0.0019
Manganese (Mn)	0.011
Phosphor (P)	1828

Table 3: Corrected cumulative mortality of *Sitophilus zeamais* adult induced by *Acacia polyacantha* wood ash, *Plectranthus glandulosus* leaf powder and theirs binary combinations under ambient conditions of laboratory (t = 22.76 ± 2.02° C; hr = 69.87 ± 9.93%)

Content (g/kg)	**Products**					**$F_{(4 ; 15)}$**
	A. polyacantha	***P. glandulosus***	**75AP25PG**	**50AP50PG**	**25AP25PG**	
			1 day			
0	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}	
5	1.25 ± 1.25^{Aa}	3.82 ± 2.41^{Aa}	10.00 ± 5.00^{ABa}	16.25 ± 5.15^{Aa}	15.00 ± 4.08^{Aa}	2.42^{ns}
10	5.00 ± 2.04^{Ab}	6.25 ± 2.39^{Ab}	13.75 ± 7.47^{ABab}	23.75 ± 5.54^{Aa}	23.75 ± 5.15^{Aa}	4.23*
20	6.25 ± 2.39^{Ab}	7.50 ± 2.50^{Ab}	16.25 ± 5.15^{Aab}	25.00 ± 3.45^{Aa}	25.00 ± 6.12^{Aa}	3.64*
40	7.50 ± 2.50^{Ab}	16.25 ± 5.91^{Aab}	26.25 ± 7.18^{Aab}	31.25 ± 4.73^{Aa}	28.75 ± 5.15^{Aab}	3.46*
$F_{(4 ; 15)}$	2.84^{ns}	2.48^{ns}	5.46*	20.47***	15.17***	
			3 days			
0	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Da}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	
5	15.26 ± 5.49^{Abc}	6.25 ± 3.15^{BCc}	19.15 ± 2.19^{Cab}	31.98 ± 2.89^{Ba}	26.91 ± 3.08^{Bab}	10.13***

10	16.65 ± 5.41^{Abc}	11.38 ± 3.13^{ABc}	32.89 ± 0.44^{Bab}	34.48 ± 5.29^{Bab}	39.41 ± 7.29^{ABa}	9.05**
20	16.65 ± 5.41^{Ab}	17.83 ± 4.97^{Ab}	39.81 ± 1.68^{ABa}	42.31 ± 3.06^{Ba}	46.32 ± 7.13^{ABa}	8.54**
40	27.06 ± 6.41^{Abc}	21.58 ± 3.87^{Ac}	47.44 ± 3.06^{Aab}	58.95 ± 4.13^{Aa}	49.94 ± 7.30^{Aa}	9.34**
$F_{(4\,;\,15)}$	6.40*	12.10***	112.21***	82.01***	40.86***	
			7 days			
0	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{C}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	
5	20.73 ± 5.38^{Aab}	7.77 ± 2.59^{Bb}	34.81 ± 4.34^{Bb}	35.31 ± 7.53^{Ba}	32.68 ± 4.90^{Ba}	5.78*
10	25.94 ± 5.90^{Aab}	14.15 ± 3.21^{ABb}	46.49 ± 2.05^{ABa}	40.79 ± 7.46^{ABa}	41.06 ± 2.13^{ABa}	5.45*
20	35.03 ± 8.47^{Aab}	20.59 ± 3.70^{ABb}	49.05 ± 6.97^{ABab}	56.76 ± 6.41^{ABa}	51.75 ± 8.30^{ABa}	4.43*
40	41.82 ± 5.64^{Aab}	28.16 ± 4.73^{Ab}	65.43 ± 8.12^{Aa}	62.87 ± 3.84^{Aa}	64.32 ± 6.69^{Aa}	7.80**
$F_{(4\,;\,15)}$	16.76**	16.00***	45.78***	37.36***	31.64***	
			14 days			
0	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Da}	0.00 ± 0.00^{Ca}	
5	19.96 ± 9.37^{Bb}	19.54 ± 2.63^{Bb}	69.88 ± 1.40^{Ba}	68.42 ± 4.30^{Ca}	69.96 ± 4.54^{Ba}	27.27***
10	50.00 ± 3.94^{Ab}	33.82 ± 3.52^{ABc}	71.98 ± 1.42^{Ba}	73.91 ± 2.82^{BCa}	76.75 ± 4.06^{ABa}	31.15***
20	67.40 ± 6.19^{Aa}	40.33 ± 4.12^{ABb}	84.80 ± 3.56^{ABa}	83.41 ± 5.13^{Ba}	83.48 ± 2.06^{ABa}	18.11**
40	75.51 ± 5.85^{Ab}	57.24 ± 2.71^{Ac}	91.67 ± 2.78^{Aab}	100.00 ± 0.00^{Aa}	88.96 ± 5.58^{Aab}	17.39***
$F_{(4\,;\,15)}$	40.00***	106.53***	124.96***	195.91***	106.30***	

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

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

^{ns} $P > 0.05$, * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

Table 4: Lethal contents and co-toxicity coefficients of different combinations of *Plectranthus glandulosus* leaf powder with *Acacia polyacantha* wood ash on *Sitophilus zeamais* under fluctuating laboratory conditions (t = 22.76 ± 2.02° C; hr = 69.87 ± 9.93%)

Products	**Slope**	***R^2***	**LC_{50}(95% FL) (g/kg)**	**LC_{95}(95% FL) (g/kg)**	**Co-toxicity coefficient**	**Signification of CTC**	**χ^2**
				3 days			
A. polyacantha	0.43 ± 0.22	0.71	—$^{\beta}$	—			1.46^{ns}
P. glandulosus	0.98 ± 0.25	0.973	213.69 (87.60; 2902)$^{\beta}$	—			1.52^{ns}
25AP75PG	0.65 ± 0.19	0.89	32.31 (19.97; 117.09)	—			0.48^{ns}
50AP50PG	0.77 ± 0.19	0.89	26.59 (17.99; 57.54)	—			0.96^{ns}
75AP25PG	0.83 ± 0.20	0.93	42.13 (26.95; 116.59)$^{\beta}$	—			0.83^{ns}
				7 days			
A. polyacantha	0.69 ± 0.20	0.99	77.47 (39.07; 688.41)$^{\beta}$	—			0.08^{ns}
P. glandulosus	0.91 ± 0.23	0.99	166.04 (73.02; 1706)$^{\beta}$	—			0.12^{ns}
25AP75PG	0.92 ± 0.19	0.98	17.16 (12.47; 25.39)	—	752.69	synergistic	0.17^{ns}
50AP50PG	0.84 ± 0.19	0.96	15.27 (10.59; 22.82)	—	691.95	synergistic	0.40^{ns}
75AP25PG	0.85 ± 0.19	0.94	15.75 (11.06; 23.55)	—	496.61	synergistic	0.70^{ns}
				14 days			
A. polyacantha	1.67 ± 0.33	0.92	12.51 (2.66; 33.06)	121.24 (40.21; 3443.32)$^{\beta}$			5.20^{ns}
P. glandulosus	1.07 ± 0.19	0.97	28.64 (21.37; 46.46)	928.77 (306.69; 9366)$^{\beta}$			0.83^{ns}
25AP75PG	0.77 ± 0.22	0.99	1.09 (0.05; 2.77)	144.61(56.51; 3274)$^{\beta}$	1987.15	synergistic	0.01^{ns}
50AP50PG	1.51 ± 0.59	0.95	3.02$^{\#}$	37.29$^{\#}$	64.49	antagonism	5.35*
75AP25PG	0.98 ± 0.22	0.90	1.92 (0.41; 3.61)	90.05 (45.60; 480.64)$^{\beta}$	758.30	synergistic	1.50^{ns}

^{ns}: $P > 0.05$; *: $P < 0.05$; CTC: Cotoxicity coefficient, —: the LC values are too large or estimation impossible to inadequate mortality, $^{\beta}$: the LC values are obtained by extrapolation, $^{\#}$: the values of Fudicial limit for these LC values could not be computed by the software.

Table 5: F_1 progeny production of *Sitophilus zeamais* in maize grains treated with binary mixtures of *Plectranthus glandulosus* leaf powder and *Acacia polyacantha* wood ash under fluctuating conditions of laboratory (t = 22.76 ± 2.02° C; hr = 69.87 ± 9.93%)

Content (g/kg)	**Mean number of F1 adult progeny**	**% reduction in adult emergence relative to control**
75AP25PG		
0	42.50 ± 1.71^{A}	0.00 ± 0.00^{D}
5	27.25 ± 4.03^{B}	34.55 ± 11.45^{C}
10	18.00 ± 3.19B	56.59 ± 8.84^{BC}
20	8.25 ± 1.65^{CD}	80.04 ± 4.60^{AB}
40	2.00 ± 1.41^{D}	95.18 ± 3.45^{A}
$F_{(4; 15)}$	37.60***	29.25***
50AP50PG		
0	42.50 ± 1.71^{A}	0.00 ± 0.00^{C}
5	20.00 ± 3.53^{B}	53.42 ± 7.58^{B}
10	17.00 ± 3.08^{B}	60.14 ± 7.26^{B}
20	13.25 ± 3.12^{BC}	69.06 ± 7.35^{AB}
40	4.75 ± 1.03^{C}	88.56 ± 2.67^{A}
$F_{(4; 15)}$	27.73***	31.92***
25AP75PG		
0	42.50 ± 1.71^{A}	0.00 ± 0.00^{D}
5	26.00 ± 2.68^{B}	38.10 ± 7.78^{C}
10	19.75 ± 2.06^{BC}	$52.97 \pm 5.96\mathrm{B}^{C}$
20	12.60 ± 0.82^{CD}	71.41 ± 3.00^{AB}
40	9.00 ± 1.22^{D}	78.82 ± 2.93^{A}
$F_{(4; 15)}$	53.87***	43.16***

Means ± S.E. followed by the same letter in column do not differ significantly at $P < 0.05$ (Tukey's test).
ns P > 0.05, * P < 0.05, ** P < 0.001, *** P < 0.0001.

Table 6: Efficacy content of binary combinations of *Plectranthus glandulosus* leaf powder with *Acacia polyacantha* wood ash inducing 50 and 95% reduction in F_1 progeny in ambient conditions (t = 22.76 ± 2.02° C; hr = 69.87 ± 9.93%)

Products	**Slope + SE**	**R^2**	**EC$_{50}$ (95%FL) g/kg**	**EC$_{95}$(95%FL) g/kg**	**χ^2**
75AP25PG	2.22 ± 0.12	0.65	7.96(5.39; 10.40)	44.00(28.44; 107.33)$^{£}$	1.50^{ns}
50AP50PG	1.12 ± 0.11	0.56	5.11(1.42; 8.24)	148.83(58.10; 3376.75)$^{£}$	3.50^{ns}
25AP75PG	1.27 ± 0.10	0.62	8.44(5.63; 11.11)	165.65(82.31; 686.61)$^{£}$	3.25^{ns}

^{ns} P > 0.05, EC: Efficacy Content, FL: Fudicial Limit, $^{£}$: the values of EC are obtained by extrapolation.

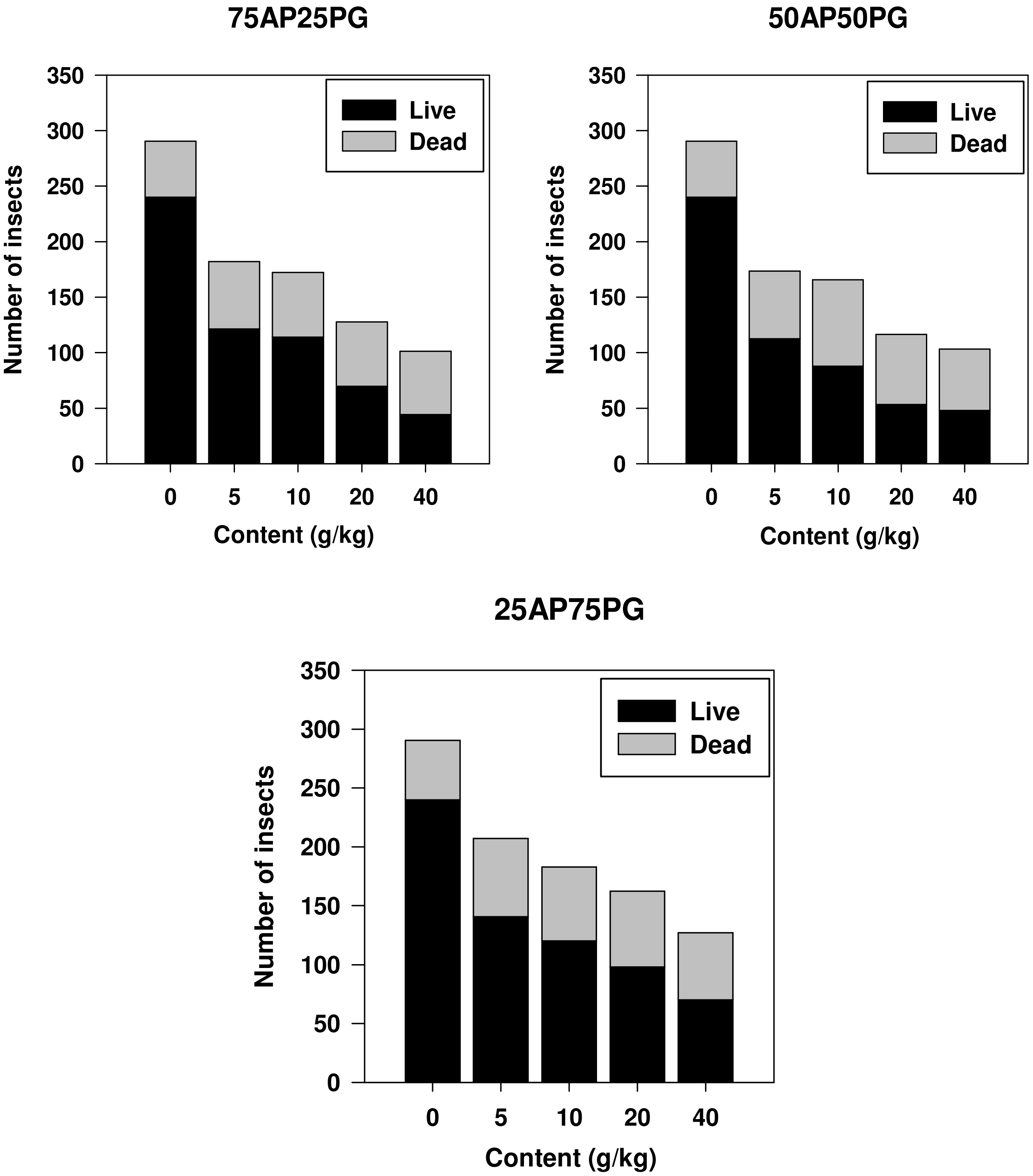

Fig.1. Population increase in stored maize treated with binary combinations of *Acacia polyacantha* wood ash and *Plectranthus glandulosus* leaf powder under fluctuating laboratory conditions (t = 22.76 ± 2.02° C; hr = 69.87 ± 9.93%)

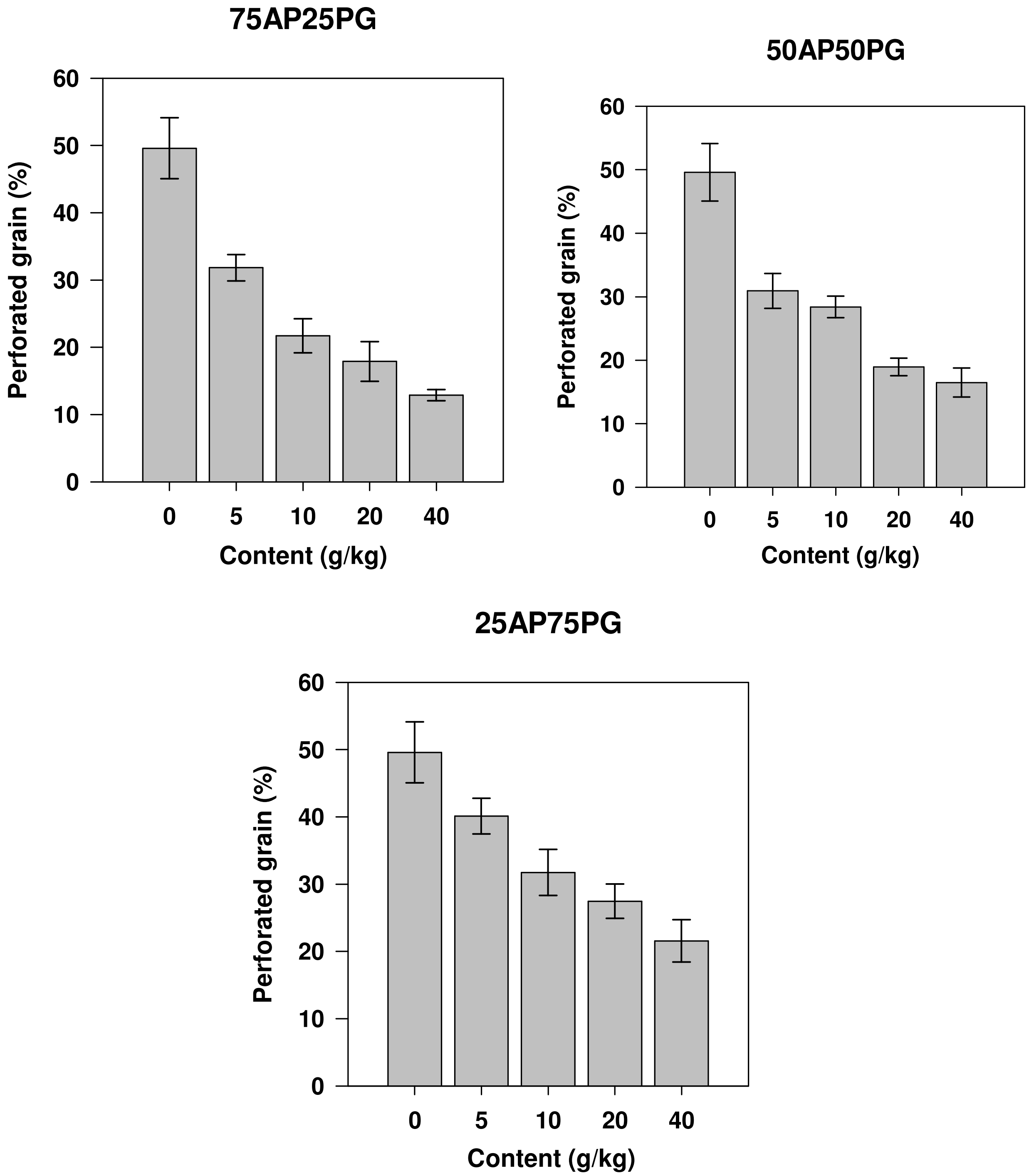

Fig.2. Perforated grain recorded in stored maize treated with binary combinations of *Acacia polyacantha* wood ash and *Plectranthus glandulosus* leaf powder under fluctuating laboratory conditions (t = 22.76 ± 2.02° C; hr = 69.87 ± 9.93%)

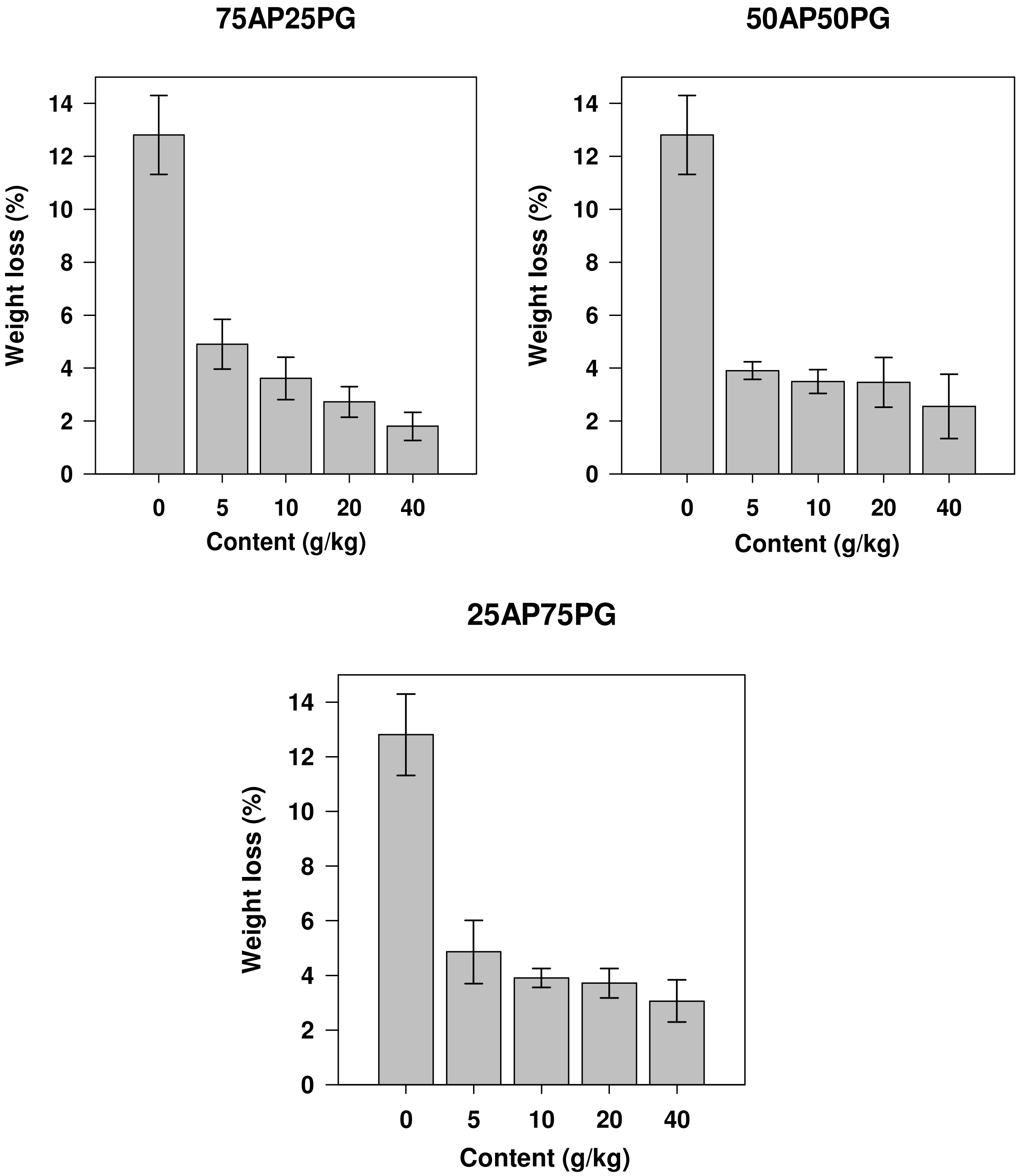

Fig.3. Weight losses recorded in stored maize treated with binary combinations of *Acacia polyacantha* wood ash and *Plectranthus glandulosus* leaf powder under fluctuating laboratory conditions (t = 22.76 ± 2.02° C; hr = 69.87 ± 9.93%)