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Potency of *Chenopodium ambrosioides* Powders and its Combinations with Wood Ash on *Sitophilus zeamais* in Stored Maize

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ABSTRACT

In an effort to develop easily available, affordable and biodegradable reduced-risk insecticides as alternatives to conventional synthetic post-harvest protectants against storage weevils, powders of *Chenopodium ambrosioides* were tested singly and in combination with the often used wood ash under ambient laboratory conditions on *Sitophilus zeamais* infested maize. This is a major impediment to sustained food security in Africa and other developing countries. The powders were mixed at varied doses of *C. ambrosioides* (0.5-2 g) powder per 50 g of maize grains; there was also a positive control of Actellic® powder and an untreated negative control. Grains treated with 2 g of *C. ambrosioides* powder caused 100% adult weevil mortality within 3 days after infestation. At 2 g, *C. ambrosioides* powders were as good as Actellic® powder on adult weevil mortality, protection of grains against damage and reduction of number of weevil progeny produced within 1-2 months of storage. The grain weight loss decreased with increased concentration of plant powder used but increased with longer duration of storage. The persistency effects of *C. ambrosioides* powder decreased over time till the powder lost its potency on adults beyond three weeks of grain storage. A proportional combination of 50:50% of *C. ambrosioides* powder and wood ash killed *S. zeamais* adults at a faster rate than either powder singly. The results indicate that the use of mixtures of *C. ambrosioides* powder and wood ash by small scale farmers is potentially a cost-effective and sustainable alternative or supplement to synthetic chemical insecticides for large-scale maize storage.

Key words: Post-harvest, progeny, powder combinations, persistency, protection

INTRODUCTION

Maize, (*Zea mais*) is one of the most commonly used cereals both as human food and animal feed in Africa. It is eaten in different forms such as boiled or grilled cobs or as various products of maize flour (Iken and Amusa, 2004). Its grains, cobs, stalks, leaves, tassels and silks all have commercial values in most settings though that of the grains is greatest (Paliwal, 2000). In most of Africa, the greater proportion of maize is produced by resource-poor small-scale farmers in remote villages with poor road networks and post-harvest storage facilities which often make them incur high post-harvest losses (Ntonifor and Monah, 2001). Insects are the main storage pests of maize (Akob and Ewete, 2007) since they cause qualitative and quantitative losses such as reduction of nutritive and market values, promotion of mold development and weight loss.

Losses due to post-harvest insect pests are a major problem hampering food security in Africa (Ogendo *et al*., 2004; Abebe *et al.*, 2009).

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) is largely responsible for most maize post-harvest losses in Cameroon (Akob and Ewete, 2007). These insect pests continue to reap a heavy toll on stored grains at farmer level in Cameroon due to the high costs of synthetic insecticides, poor road network and hence erratic supply to resource-poor farmers in remote areas (Koona, 2004). Moreover, given that maize is used as human food and feed for livestock renders the use of potentially toxic synthetic chemicals unacceptable since this may lead to problems of toxic residues, health and environmental hazards (Aslam *et al*., 2002). Due to these associated high costs and negative consequences of synthetic insecticides, small-scale farmers are more inclined to use traditional methods and plant derived products to store their produce (Ntonifor and Monah, 2001; Kim *et al*., 2003; Ntonifor *et al*., 2010). The old-age practice of traditional tropical farmers to mix a local plant with their grains prior to storage may be an attractive alternative to synthetic insecticides (Ukeh *et al*., 2010) due to their low toxicities, cost and easy availability. Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the major bioactive components of these plants (Ntonifor, 2011) Indigenous farmers in Cameroon often mix their grains with highly aromatic local plants thought to have insecticidal properties prior to storage (Ntonifor *et al*., 2010). An example of such aromatic plants used for post-harvest grain protection is *Chenopodium ambrosioides* (Chenopodiaceae). Given the current renewed interest in reducing environmental hazards and global warming, there is need for the re-evaluation, modification and intensification of environmental friendly and cost-effective pest management technologies such as the traditional botanical pest control agents. Therefore, the objectives of this study were to evaluate the effects of powders derived from *C. ambrosioides* on *Sitophilus zaemais* and also test the potency of various combinations of its powders with the frequently used wood ash for potential large-scale exploitation as an easily available and affordable plant-based post-harvest protectant.

MATERIALS AND METHODS

Sources of insects and maize: A culture of *S. zeamais* was established to supply insects of similar ages for the experiments. A known weevil susceptible maize cultivar (KASAI) was procured and seeds with visible weevil emergent windows and other damage symptoms removed. The healthy seeds were then kept in the refrigerator at about -5°C for one month to eliminate hidden immature stages. *S. zeamais* adults were collected from infested maize grains of unknown cultivar obtained from local farmers in Cameroon in 2006. The weevils were identified with the aid of taxonomic keys and by comparing with laboratory voucher specimens at the Department of Plant and Animal Sciences, University of Buea, Cameroon, where the studies were conducted. The insects were sexed based on the methods of Halstead (1963). Stock cultures of the weevils were then maintained by transferring about 50 weevils per 500 g of the known weevil susceptible maize variety in each of ten 500 mL capacity glass jars. Each jar was covered with nylon mesh held in place with a rubber band to allow aeration and also prevent escape of weevils. The set-ups were then kept in the laboratory at ambient conditions of 25±5°C and 70±10% R.H (relative humidity).

Plant powders: Fresh samples of a mixture of leaves, inflorescence and stems of *Chenopodium ambrosioides* were harvested at the flowering stage, thoroughly washed with clean tap water then dried in an oven at 40°C for 5 days and then ground in a blender into a fine powder. The powdered sample was preserved in sealed containers for subsequent use. Wood ash, a frequently used traditional post-harvest protectant in Cameroon was collected from local residents. Pirimiphos-methyl (Actellic® dust) a standard synthetic post-harvest insecticide in Cameroon bought from a commercial store was used as the positive control.

Assessment of adult mortality: Wholesome untreated known weevil susceptible white-grained maize variety was used in all the experiments. Doses of 0.5, 1, 1.5 and 2 g of the plant powder, 0.025 g of Actellic® powder (positive control) and an untreated negative control were used. Each dose was added to a 500 mL capacity glass jar containing 50 g (ca. 150 grains) of the disinfested white grain maize variety. Twenty unsexed adult *S. zeamais* of one to five-day old were selected from the stock cultures and added to each jar and then covered with a nylon mesh fixed firmly with a rubber band. The jars were kept at ambient laboratory conditions of 25±5°C and 70±10% relative humidity in a completely randomized experimental design. Each treatment and the controls were replicated four times. Subsequently, each jar was opened daily and the number of dead weevils therein removed and counted; this continued till when all the insects in the jar died. An insect was considered dead if it did not respond to up to a third touch with a blunt object.

Assessment of grain damage: Doses of 0.5, 1, 1.5 and 2 g of the plant powder, 0.025 g of Actellic® powder and an untreated negative control as in the previous experiment were used in this study. Each dose was added to a 500 mL capacity glass jar-containing 50 g that is about 150 wholesome disinfested white grain maize variety. Twenty unsexed *S. zeamais* adults of one to five-day old were added to each jar and then covered with a nylon mesh held firmly with a rubber band as described earlier. The jars were kept at similar ambient conditions as in the previous experiment in a completely randomized experimental design. Each treatment and the controls were replicated four times. Subsequently, each jar was opened at 45 Days After Infestation (DAI) and then at 90 DAI to assess and calculate grain damage. The percentage weight loss (percent of holed seeds) of the maize grain was calculated using the count and weight method of Dobie *et al.* (1991).

Effect on hidden eggs and immature stages: Batches of 50 g disinfested white maize grains were infested with 20 one to five-day old unsexed adults of *S. zeamais* weevils in 500 mL glass jars then covered with a nylon mesh fixed firmly with a rubber band. The jars were kept at similar ambient conditions as in the previous experiments for 10 days to allow the weevils mate and lay. Thereafter, the adults were removed and the grains treated with 0.5, 1, 1.5 or 2 g of *C. ambrosioides* powder and 0.025 g of Actellic® powder (positive control) while nothing was added to the negative control. The jars were re-covered as earlier described and kept at ambient conditions as stated above. After every two days, each jar was opened and the number of adult weevils that emerged counted; this continued for a period of 90 days following the removal of adults. Thereafter, the number of adults that emerged from each treatment after 30, 60 and 90 days was calculated. Each treatment and the controls were replicated four times.

Evaluation of persistency of powders: The persistency of *C. ambrosioides* was also assessed by treating 50 g of disinfested white maize grains with, 0.5, 1, 1.5 or 2 g of the powder and the positive and negative controls with 0.025 g of Actellic® powder and nothing, respectively. The set up was kept at laboratory conditions as described above. Once each week, 20 newly emerged adults from the stock culture were then added to each jar and subsequently the numbers that died within five days were counted and removed. The experiment was terminated when subsequent insects added did not die.

Bioassay with mixtures of plant powder and wood ash: Given that wood ash is often used by traditional farmers to protect their grains against stored insect pests and also that previous bioassays with 2 g of *C. ambrosioides* powders caused 100% of weevil mortality within three days post infestation, various proportional mixtures of these protectants were then tested. Proportional mixtures of 0:2, 0.5:1.5, 1:1, 1.5:0.5, or 2:0 g of *C. ambrosioides* powders and wood ash were tested; 0.025 g of Actelhic® powder was used as the positive control and nothing was added to the untreated control. Each of these treatments was applied to 50 g of disinfested white grained maize seeds in the test jars and then 10 pairs of weevils also added. Each treatment and the controls were replicated 4 times. The experiment was then kept under similar conditions as in the previous experiments. Daily, each jar was opened and the number of dead insects therein counted; this continued till after 5 days when the experiment was terminated. Subsequently, the percent weevil mortality per day was calculated.

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Scientists (SPSS). Treatment means were compared using the LSD test set at p = 0.05 significant level.

RESULTS

Assessment of adult mortality: Adult weevil mortality on grains treated with *C. ambrosioides* powders increased with the concentration of powder used and duration of exposure. Grains treated with 0.5 g of the powder caused <50% of *S. zeamais* adult mortality at three Days After Infestation (DAI) while 2 g of it caused 100% mortality within the same period (Table 1). However, by the seventh day after exposure, all the weevils in grains treated with any concentration of the plant powder died. The untreated grains (negative control) and those treated with 0.025 g of Actellic®

Table 1: Mortality of *Sitophilus zeamais* adults caused by treating 50 g of stored maize grains with varied concentrations of *Chenopodium ambrosioides* or 0.025 g Actellic® powder

Days exposed	Concentration (g) 0	0.5	1	1.5	2	0.025 Actellic®	LSD (p = 0.05)
1	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	35.6±2.5bA	49.0±2.5cA	100±0.0dA	3.5
2	0.0±0.0aA	31.5±4.5bB	69.0±2.5bB	81.5±2.5bB	81.5±2.5bB	100±0.0cA	15.0
3	0.0±0.0aA	49.0±4.0bC	86.5±4.0cC	95.0±4.0cC	100±0.0cdC	100±0.0cdA	10.5
4	0.0±0.0aA	66.5±3.5bD	96.5±2.5cC	97.5±2.5cC	100±0.0cC	100±0.0cA	6.5
5	0.0±0.0aA	75.0±2.5bDE	99.0±2.0cC	99.0±1.0cC	100±0.0cC	100±0.0cA	3.5
6	0.0±0.0aA	84.0±2.5bE	100±0.0cC	99.0±2.0cC	100±0.0cC	100±0.0cA	3.5
7	0.0±0.0aA	100±0.0bE	100±0.0bC	100±0.0bC	100±0.0bC	100±0.0bA	0.0
LSD (p = 0.05)	0.0	13.0	10.5	4.5	6.0	0.0	

Values are cumulative Mean±SD. Means with the saine upper case and lower case letters in a column and row, respectively are not significantly different (p = 0.05).

powder (positive control) had zero and 100% weevil mortality at seven and one day after infestation, respectively.

Assessment of grain damage: Grains treated with *C. ambrosioides* powders suffered a significantly lower grain damage ($p<0.05$) compared to the untreated grains (Table 2). The grain weight loss decreased with increased concentration of plant powder used but increased over a longer duration of storage. Grains treated with 1 g of *C. ambrosioides* powder did not suffer from any grain weight loss at 45 and 90 days after storage. Grain treated with 0.5 g of *C. ambrosioides* powder and stored for 90 days suffered about double the grain weight loss at 45 days of storage. There were no remarkable differences in grain weight loss between grains treated with Actellic® powder and those treated with the various concentrations of *C. ambrosioides* powders.

Effect on hidden eggs and immature stages: Adult counts at 30, 60 and 90 days after treatment (DAT) showed that all concentrations of *C. Ambrosioides* powders variedly affected the development of *S. zeamais* immature stages hidden inside the maize grains. At 30 DAT, grains treated with each of 0.5-2 g of *C. Ambrosioides* powder caused a significant ($p<0.05$) delay in adult emergence. Two gram was not significantly ($p>0.05$) different from Actellic® powder, the standard grain protectant in Cameroon. At 60 and 90 DAT, relatively higher numbers of adults emerged from all treatments but again significantly ($p<0.05$) fewer emerged from grains that were treated with plant powders or Actellic® powder compared to the untreated (negative) control (Table 3). The highest mean number of adults of 48.8 emerged from the untreated control at 90 DAT but this was very similar to the 48.5 that emerged at 60 DAT from the same treatment.

Evaluation of persistency of powders: The persistency effects of *C. Ambrosioides* powder decreased over time. *S. zaemais* adults introduced on grains treated with 1 g of *C. Ambrosioides* powder had 100% mortality during the first week of treatment application. However, adult weevils introduced the second week had 87.5, 98.5 and 99% mortality on grains treated with 1, 1.5 and 2 g of *C. ambrosioides* powder, respectively. These mortalities even reduced further by the third week after treatment application (Table 4). Beyond this period, *C. ambrosioides* powder lost its potency and needed to be re-treated. In contrast, grains treated with Actellic® powder still caused 100% mortality of adults by the fourth week after treatment application.

Table 2: Maize grain weight loss due to *Sitophilus zeamais* damage at 45 and 90 days after treatment with various doses of *Chenopodium ambrosioides* powder or 0.025 g Actellic® powder

	45 days of exposure		90 days of exposure	
Dosage (g /50 g)	*Chenopodium ambrosioides*	0.05 Actellic®	*Chenopodium ambrosioides*	0.025 Actellic®
0	1.2±0.6[a]	NA	8.0±1.3[a]	NA
0.05	NA	0.0±0.0	NA	0.0±0.0
0.5	0.2±0.1[b]	NA	1.4±0.6[b]	NA
1.0	0.0±0.0[c]	NA	0.0±0.0[c]	NA
1.5	0.0±0.0[c]	NA	0.0±0.0[c]	NA
2.0	0.0±0.0[c]	NA	0.0±0.0[c]	NA
LSD (p = 0.05)	0.8	NA	4.4	NA

Values are Mean±SD. Means with the same letters in a column are not significantly different (p = 0.05). NA = Not applicable

Table 3: Number of adult *Sitophilus zeamais* that emerged at 30, 60 and 90 Days After Treatment (DAT) of 50 g of stored maize grains with different concentrations of *Chenopodium ambrosioides* or 0.025 g Actellic® powder

Days after Treatment	Concentration (g) 0	0.5	1	1.5	2	0.025 Actellic®	LSD (p = 0.05)
30	26.0±1.6[a]	5.0±1.0[b]	5.0±0.5[b]	1.8±0.6[c]	0.6±0.3[c]	0.3±0.2[c]	3.1
60	48.5±2.4[a]	26.3±1.4[b]	23.0±1.4[b]	18.5±0.7[b]	5.0±1.3[c]	8.0±0.0[c]	10.2
90	48.8±2.4[a]	26.7±1.2[b]	25.0±1.8[b]	19.5±0.7[b]	5.0±1.3[c]	8.0±0.0[c]	9.5

Values are Mean±SD. Means with the same letters in a row are not significantly different (p = 0.05)

Table 4: Mortality of *Sitophilus zeamais* adults at various weeks of infestations of 50 g of stored maize grains treated with varied concentrations of *Chenopodium ambrosioides powder* or 0.025 g Actellic® powder

Weeks of infestation	Concentration (g/ 50 g of grains) 0	0.5	1.0	1.5	2	0.5 Actellic®	LSD (p = 0.05)
1	0.0±0.0[a]	99.0±2.0[b]	100[b]	100[b]	100[b]	100[b]	1.5
2	0.0±0.0[a]	54.0±3.0[b]	87.5±3.5[c]	96.5±2.5[cd]	99.0±2.0[d]	100[d]	10.5
3	0.0±0.0[a]	24.0±4.0[b]	34.0±4.5[b]	74.0±5.5[c]	96.0±4.5[cd]	100[d]	23.5
4	0.0±0.0[a]	5.0±1.5[a]	8.0±2.5[ab]	15.5±2.0[b]	25.0±2.5[c]	100[d]	9.5
5	0.0±0.0[a]	1.5±0.5[a]	0[a]	1.5±1.0[a]	5.5±1.5[a]	100[b]	19.5

Values are Mean±SD. Means with the same letters in a row are not significantly different (p = 0.05)

Table 5: Mortality of *Sitophilus zeamais* adults caused by treating 50 g of stored maize grains with different proportional combinations of *Chenopodium ambrosioides* powder and wood ash or 0.025 g Actellic® powder sole

Days exposed	Proportional combinations (g:g) 0:0	0.5:1.5	1:1	1.5:0.5	2.0:0	0:2.0	0.025 Actellic®	LSD (p = 0.05)
1	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	100±0.0aA	3.5
2	0.0±0.0aA	35.0±1.5bB	70.0±4.5cB	80.0±2.5cB	90.0±4.0cdB	0.0±0.0aA	100±0.0dA	15.0
3	0.0±0.0aA	51.0±4.0cB	85.0±3.0dC	92.5±3.5deC	100±0.0eC	34.0±2.0bB	100±0.0eA	10.5
4	0.0±0.0aA	75.0±5.0bC	96.5±5.0cCD	97.5±3.5cC	100±0.0cC	75.0±5.5bC	100±0.0cA	6.5
5	0.0±0.0aA	96.0±2.0bC	100±0.0bD	100±0.0bC	100±0.0bC	96.5±3.0bD	100±0.0bA	3.5
LSD (p = 0.05)		16.0	13.0	10.5	7.5	8.0	0.0	

Values are cumulative Mean±SD. Means with the same upper case and lower case letters in a column and row, respectively are not significantly different (p = 0.05).

Bioassay with mixtures of *C. ambrosioides* powder and wood ash: Proportional combinations 1:1 g of *C. ambrosioides* powder and wood ash killed 85% of *S. zeamais* adults at 3 days after infestation (DAI) and by 5 DAI, there was 100% weevil mortality. An increased concentration of *C. ambrosioides* in the mixture to 1.5:0.5 g of *C. ambrosioides* and wood ash killed 92.5% of the weevils at 3 DAI and again caused 100% mortality at 5 DAI compared to the untreated (negative) control that had zero percent weevil mortality (Table 5).

DISCUSSION

High *S. zeamais* adult mortalities were recorded in grains treated with *Chenopodium ambrosioides* powders compared to the untreated control. This could be attributed to the chemical composition of the plant since *C. ambrosioides* is known to contain insecticidal compounds such as ascaridole, cymol and α-terpine (Quarles, 1992). This is consistent with Delobel and Malonga

(1987) who reported that *C. ambrosioides* extracts caused mortality of *Corryedon serratus*, a stored product insect pest of groundnuts. Similarly, Tapondjou *et al.* (2002) reported that leaf powders of *C. ambrosioides* were potent against six stored product beetles. It is therefore likely that *C. ambrosioides* has several insecticidal constituents that are active against all Coleopterans.

The level of damage by *S. zeamais* on maize was significantly higher in the untreated grains compared to those treated with various concentrations of *C. ambrosioides* powder or Actellic® powder. This shows that *C. ambrosioides* powder offers good grain protective activity similar to that of Actelhc® powder within 1-2 months of grain storage. The lower grain damage suffered by grains treated with *C. ambrosioides* powders may be attributed to the early adult mortality which prevented the insects from damaging the grains and also hindered them from starting a new cycle of oviposition. The insecticidal activity of derivatives of the highly aromatic *C. ambrosioides* has been attributed to the presence of several active ingredients that operate via different modes of action (Chiasson *et al.*, 2004).

Significantly fewer adults emerged from grain treated with *C. ambrosioides* powder or Actellic® powder as compared to the untreated grains. This shows that each of these powders prevented egg hatching and/or the development of the immature stages of the weevils to adulthood in contrast to what prevailed in the untreated grains. Presumably, the *C. ambrosioides* powder has larvicidal and/or ovicidal effects in addition to its adulticidal activities. Obviously, the highly fragrant *C. ambrosioides* powders contain fairly complex phytochemicals with synergistic and/or potentiating interactions that are lethal both to the adult and juvenile stages of *S. zeamais*.

The residual effect of *C. ambrosioides* lasted for three weeks since re-infestation with adult weevils beyond this period did not lead to significant mortalities. This was probably due to the evaporation of the volatiles since the containers were constantly opened weekly to count dead weevils. It might have also been due to early biodegradability of the active constituents in the powder. Therefore, if grains are stored in open bins, they will need to be re-treated at about 3-4 weeks intervals. This corroborates earlier observations that the inability powders to offer a longer duration of protection could be as a result of the powder settling at the bottom of the containers (Niber, 1994).

The proportional combination of 50:50% of *C. ambrosioides* powder and wood ash led to higher mortalities of the weevils. It is possible that such combinations led to additive and/or synergistic effects of the plant powder and wood ash. Such improved grain protection from combination "therapy" in storage protection with plant derived powders had earlier been reported by Ntonifor *et al.* (2010). Using such proportional combinations is advantageous in that the powders likely have different modes of actions and chemical compositions and this will slow down the development of resistance. The proportional combination of plant powders and wood ash offers a greater potential for large-scale traditional grain storage given that such combinations are cheaper since the wood ash in the combination is easier to obtain and costs nothing. Such proportional combinations can easily be vulgarized to tropical farmers since they are more receptive to post-harvest protection methods that are within their technical and financial means (Ntonifor *et al.*, 2010). Therefore, these combinations can serve as easily affordable, available and biodegradable alternatives or supplements to the costly and less environmentally friendly synthetic post-harvest protectants.

CONCLUSION

Present results demonstrated the potent lethal activities of *C. ambrosioides* powder and its 50:50% proportional combinations with wood ash against *S. zeamais* adults. *C. ambrosioides* powder also caused a reduction in the number of weevil progeny produced. The fact that at 2 g, *C. ambrosioides* powders as well as its 50:50% mixture with wood ash were as good as Actellic® powder may enable farmers or small scale enterprises develop their own plant-based, cost-effective, eco-friendly and sustainable post-harvest pesticide formulations as alternatives or supplements to the conventional synthetics. Obviously, efficacy and safety tests for such novel post-harvest formulations will be required prior to their vulgarization for large-scale use. However, given that *C. ambrosioies* is often used as a medicinal herb, it is likely that it will not cause a safety problem.

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