Residual efficacy of two synthetic insecticides in controlling the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: curculionidae) in storage

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Abstract

The use of insecticides is an integral component among the strategies for effective management of stored product insect pests. This study was conducted to assess the toxicity of two synthetic insecticides, viz; Dichlorvos and Profenofos+Cypermethrin on *Sitophilus zeamais* in storage. Adult weevils were exposed to grains treated with the insecticides and filter papers impregnated with concentrations of 1.250mg/ml, 1.000mg/ml, 0.500mg/ml, 0.250mg/ml, 0.125mg/ml and 0.000mg/ml Dichlorvos, and 2.750mg/ml, 2.200mg/ml, 1.100mg/ml, 0.550mg/ml, 0.275mg/ml and 0.000mg/ml of Profenofos+Cypermethrin. Mortality was recorded over a period of 3 to 24 hours for the filter paper bioassay while residual action of insecticide-treated grains was conducted 30 and 60 days after treatment. The filter paper bioassays revealed that mortalities of weevils exposed to the two insecticides ranged from 0.00-86.67 % and 13.33-100.00 % at 6 and 12 hours respectively. The LC_{50} values from impregnated filter paper at 6 hours were 0.672mg/ml and 1.074mg/ml while LC_{50} values at 12 hours were 0.291mg/ml and 0.027mg/ml for Dichlorvos and Profenofos+Cypermethrin, respectively. The LC_{50} toxicity factor for Profenofos+Cypermethrin and Dichlorvos at 12 hours of exposure to *S. zeamais* was in a ratio of 10.78:1. The 30-days post-treatment residual efficacy of both insecticides gave 100.00% mortality of insects in 6 hours of exposure whereas at 60 days post-treatment, 100.00% mortality was achieved in 6 hours and 12 hours of exposure to Profenofos+Cypermethrin and Dichlorvos respectively. A mean number of 128.00±1.15 adult weevils emerged when 40.00±0.00 adults were introduced to untreated grains resulting in 5.00% weight loss of grains after 30 days. There was a significant direct relationship (r=1.00; p<0.05) between weight loss in untreated grains, and adult emergence. This study has revealed the efficacy of Dichlorvos and Profenofos+Cypermethrin as candidate insecticides for controlling *S. zeamais* infestation on maize.

Keywords: Profenofos+Cypermethrin; dichlorvos; toxicity; residual action; maize; weevils.

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Introduction

Maize (*Zea mays*) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (Purseglove, 1992). Maize was introduced into Africa in the 1500s and has since become one of Africa's dominant food crops (FARA, 2009). As in many other regions, it is consumed as a vegetable although it is a grain crop. Worldwide production of maize is 785 million tons annually, with the largest producer, the United States, producing 42%. Africa produces 6.5% and the largest African producer is Nigeria with nearly 8 million tons (FARA, 2009). It is estimated that by 2050, the demand for maize in developing countries will double, and by 2025, maize will have become the crop with the greatest production globally (CIMMYT and IITA, 2010).

In Nigeria, maize is one of the important grains not only on the basis of the number of farmers that engage in its cultivation, but also in its economic value (Olaniyi and Adewale, 2010). The cultivation of maize was formerly for subsistence purposes, but it has gradually become an important commercial crop on which many agro-allied industries depend as the raw material (Kimeju and DeGroote, 2010). Maize is cultivated in the rainforest and the derived-savannah zones of the country and compared to other cereals, it is high-yielding, easy to process, and cheaper. The grains are rich in Vitamins A, C and E, carbohydrates, essential minerals, and contain 9% protein. They are also rich in dietary fiber and calories which are a good source of energy (Olaniyi and Adewale, 2010).

Storage is an important aspect of food security in developing countries. This is especially important since most cereals, including maize, are produced on a seasonal basis, and in many places, there is only one harvest a year, which itself may be subject to failure (Iken and Amusa, 2004). Such seasonal production usually leads to fluctuating supply at the international, regional, national or at household levels. Storage however helps to even out fluctuations in market supply, from one season to the next and from one year to the next, by taking produce off the market in surplus seasons, and releasing it back onto the market in lean periods (Isman, 2006).

One of the main challenges during this storage process is insect pests' infestation. Grain loss in Africa due to insect pests' damage in storage systems is estimated at 20 to 30 % (Isman, 2006). The maize weevil, *Sitophilus zeamais* (Motschulsky) is a cosmopolitan pest of stored cereals. It is one of the most damaging pests of stored grains, causing a significant decrease in the nutritional quality of the grains and major losses in economic trade. Worldwide, *S. zeamais* causes more than 20% grain loss for untreated maize (Nukenine *et al* 2002). The foraging capacity of this pest is reported to be due to its highly efficient digestive system (De Sousa and Conte, 2013). Infestations initiated in the field may further develop in storage as the grain dries whether stored as cobs or bulk grain. Apart from the indirect effects, arising from the production of heat by the insects, the major effect of infestation by the maize weevil is the damage to grain by feeding activities of the adults and the development of immature stages within the grain. This not only reduces the grain quality but also produces a considerable amount of grain dust mixed with frass, thereby necessitating control measures (Shelton, 2009). In recent years, post-harvest losses to storage insect pests such as the maize weevil, have been recognized as an increasingly important problem in Africa (Shelton, 2009). Cheap and effective methods for reducing *S. zeamais* damage are needed in African countries including Nigeria.

Insecticides, especially gaseous fumigants and residual insecticides, still remain a very important component among strategies for effective management of stored product insect pests. Methyl bromide and phosphine fumigants were applied on broad scale against stored grain insects but increasing resistance problems limit their use. Chaudhry (2000) listed over 11 species of stored grain insects that developed resistance to phosphine. Due to increasing problems with fumigants, residual insecticides have become an important tool for the control of stored grain pests. Residual pesticides stay longer in food commodities and are less expensive than fumigants. Their application is easy and safe as compared to fumigants (Farkhanda and Abida, 2013).

Organophosphates and pyrethroids are two commonly used groups of residual insecticides that are effective against a wide range of stored food pests (Athanassiou *et al* 2004), and their synergistic effect on pests have been reported (Vasquez-Castro *et al* 2012). However, there is need for periodic evaluation of these groups of insecticides to update their current efficacy status, as insects evolve over time and there could be potential development of resistance (Don-Pedro, 2009). It is therefore against this backdrop, that two synthetic insecticides, an organophosphate (Dichlorvos), and a combination of an organophosphate and a pyrethroid (Profenofos+Cypermethrin), were assessed for their toxicity and residual efficacy in protecting maize grains in storage against the maize weevil, *S. zeamais*.

Materials and methods

Source of maize grains and insecticides

Maize grains were obtained from Agege local market, Lagos, Nigeria, and disinfested in a dry oven for 24 hours at a temperature of 40^{o}C in order to kill any adult insect, larvae or eggs present in the cowpea. The local marketers had assured the authors that the maize grains had not been treated with pesticides before purchase. The disinfested maize grains were left under ambient laboratory conditions at temperature 28^o±2^{o}C and relative humidity 72%±5% for 24 hours for the moisture contents to stabilize before being used for the experiments. The insecticides, Nopest® (Dichlorvos – 100%, EC) and Sharp shooter® (Profenofos 40% + Cypermethrin 4%) were procured from the pesticide market at Idumagbo, Lagos.

Insect culture

A culture of *Sitophilus zeamais* was established from untreated and infested cowpea seeds which were obtained from the market. The infested grains were kept in kilner jars covered with muslin cloth to allow aeration and prevent insects from escaping. Adult weevils were removed and used to set up another culture on disinfested grains, and as a result a steady monoculture stock was maintainedin the laboratory under ambient conditions.

Bioassays

Stock solutions of 1.25 ml/L of Dichlorvos and 6.25 ml/L of Profenofos+Cypermethrin were prepared separately using acetone as solvent. The stock concentrations (100%) of Dichlorvos and Profenofos+Cypermethrin were determined by using half and quarter, respectively, of the recommended dosage by the manufacturer after preliminary mortality tests were conducted. A serial dilution of 80%, 40%, 20% and 10% of the stocks were prepared which corresponded to 1.000 mg/ml, 0.500 mg/ml, 0.250 mg/ml and 0.125 mg/ml for Dichlorvos and 2.200 mg/ml, 1.100 mg/ml, 0.550 mg/ml and 0.275 mg/ml for Profenofos+Cypermethrin. Acetone alone was used as control. Filter paper was impregnated with 10mls of specified concentration of the insecticides and left to dry for 12 hours. Ten unsexed adult *S. zeamais* were then introduced into each petri dish lined with treated filter paper, with three replicates per treatment. For control experiments, insects were introduced to filter paper impregnated with only acetone in three replicates per setup. Lethal toxicity was determined after 3, 6, 12 and 24 hours of the weevil exposure to the impregnated filter papers. Mortality was confirmed if the insect remained still when probed with a blunt pin.

Residual action of insecticides on *S. zemais* was assessed at 30 and 60 days after treatment. One hundred grams (100 g) of disinfested whole maize grains was immersed separately in 100% (stock solution concentration), 80% and 0% (control) dilutions of Dichlorvos and Profenofos+Cypermethrin, in petri dishes. Three replicates of each treatment and untreated maize grains were set up. The petri dishes were left uncovered for 12 hours for the maize grains to dry. Each of the 100 g treated maize grains was transferred from petri dishes into separate 1lb kilner jars and covered with muslin cloth held in place with a rubber band for aeration. The set up was left in the laboratory at ambient temperature of 28±2^{0}C and relative humidity of 72%±5%. After 30 days of treatment, 40 un-sexed adults *S. zeamais* were introduced into each jar, and mortalities were recorded at 6 and 12 hours of exposure of weevils to the treated maize grains, after which all insects were removed. At 60 days post treatment, 40 un-sexed adults *S. zeamais* were again introduced into each jar and the mortalities were recorded after 6 and 12 hours of exposure of the weevils to the maize grains.

Data on percentage mortality of weevils on treated filter papers and maize grains was corrected using Abbott's (1925) formula:

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100$$

Where Pt = Corrected mortality, Po = Observed mortality on treatment, and Pc = Control mortality.

Weight loss of grains due to weevil infestation

One hundred grammes each of untreated oven-sterilised maize grains were placed in three 2lb kilner jars and separately infested with 40 un-sexed *S. zeamais* adults. The jars were left under ambient laboratory conditions for two weeks to allow for oviposition of the weevils, after which the weevils were completely removed. The kilner jars were left for another 30 days and the number of emerged adult weevils were recorded as well as the weight of the maize grains. The following parameters were recorded viz; the mean number of adult weevils that emerged from the maize grains; mean weight loss in maize grain due to infestation by *S. zeamais* (Odeyemi and Daramola, 2002); and the relationship between maize grain weight loss and adult emergence weredetermined. Percentage weight loss was determined as follows:

$$\%\text{Weight loss} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Data analysis

Mortality data were subjected to Probit analysis to determine LC_{50} (Lethal Concentration 50) values. The relationship between maize grain weight loss and adult emergence was tested using Pearson correlation (*r*). Statistics were performed using the SPSS programme version 20.

Results

Determination of toxicity (LC_{50}) of dichlorvos and profenofos+cypermethrin on Sitophilus zeamais

The results obtained from bioassay using impregnated filter paper revealed that the percentage mean mortality of the insects exposed to different concentrations of both insecticides was dose/time dependent. The percentage mean mortality of weevils exposed to Dichlorvos ranged from 0.00-53.33 %, 0.00-86.67 %, 13.33-100.00 % and 66.67-100.00 % at 3, 6, 12 and 24 hours, respectively (Figure 1a). A similar trend of increasing mortality with time and dose was observed for weevils exposed to Profenofos+Cypermethrin. However, there was a more rapid increase in mortality of weevils exposed to Profenofos+Cypermethrin than Dichlorvos in this experiment, with higher mortality achieved in all concentrations at 6 hours (Figure1b). The control experiments showed no mortality of the weevils after 24 hours. The probit analysis showed that the 3, 6, 12 and 24 hours LC_{50} values for Dichlorvos were 1.214 mg/ml, 0.672 mg/ml, 0.291 mg/ml and 0.084 mg/ml respectively, whereas for Profenofos+ Cypermethrin, LC_{50} values were 3.728 mg/ml, 1.075 mg/ml, 0.027 mg/ml and indeterminate respectively.

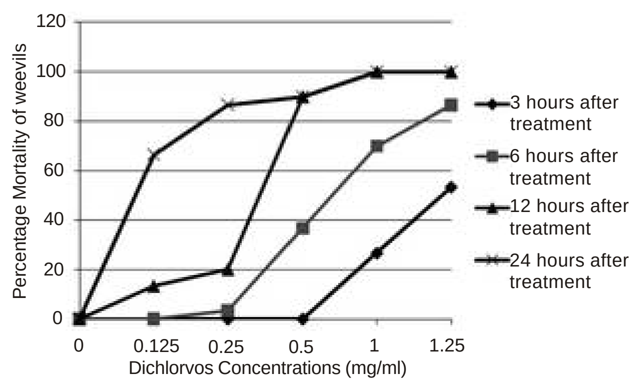

Figure 1a. Toxicity of Dichlorvos to *Sitophilus zeamais* at 3, 6, 12 and 24 hours after treatment.

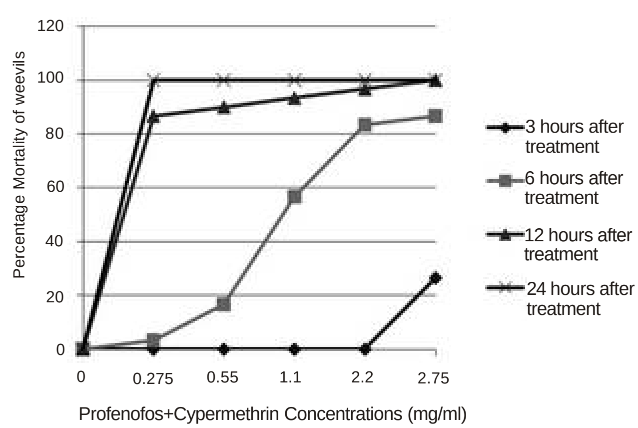

Figure 1b. Toxicity of Profenofos+Cypermethrin to *Sitophilus zeamais* at 3, 6, 12 and 24 hours after treatment.

Profenofos+Cypermethrin LC_{50} at 24 hours could not be calculated since weevils exposed to all concentrations of the chemical had attained 100% mortality at that time. The computed toxicity factor revealed that Dichlorvos was initially 3.07 times more toxic than Profenofos+ Cypermethrin at 3 hours but later on after 12 hours, Profenofos+Cypermethrin became 10.78 times more toxic to weevils than Dichlorvos.

Residual toxicity of Dichlorvos and Profenofos+ Cypermethrin on maize grains 30 and 60 days after treatment against Sitophilus zeamais

The residual effects for determined doses of Dichlorvos and Profenofos+Cypermethrin on maize grains treated showed that both insecticides induced 100% mortality at 6 hours of exposure to *S. zeamais* after 30 days of treatment (Figure 2a). However, after 60 days of treatment of maize grains, Profenofos+Cypermethrin gave 100.00% mortality after 6 hours of exposure to the weevils (Figure 2b) while 100.00% mortality of weevils was achieved for Dichlorvos after 12 hours).

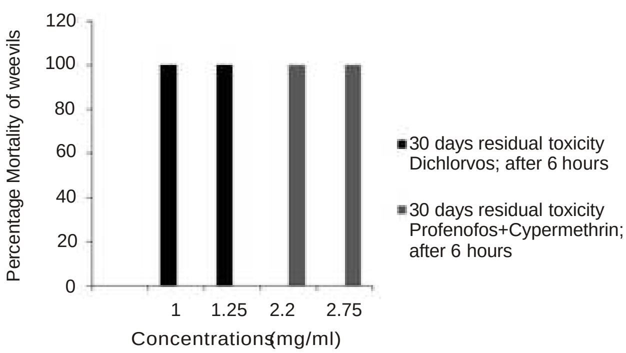

Figure 2a. Residual toxicity of Dichlorvos and Profenofos+Cypermethrin on *Sitophilus zeamais*in stored maize grains at 30 days post-treatment.

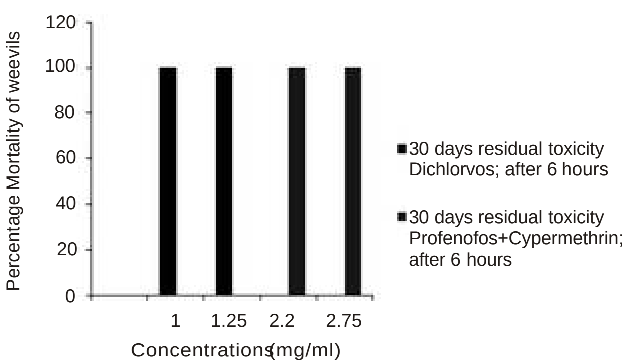

Figure 2b. Residual action of Dichlorvos and Profenofos+Cypermethrin on *Sitophilus zeamais* in stored maize grains at 60 days post-treatment.

Maize weight loss and adult emergence

The mean number of emerged adult *S. zeamais* from untreated maize after 30 days storage was 128.00±1.15 as against 40.00±0.00 from the initial culture, implying a reproductive fitness factor of 1:3 (Table 1). The mean weight loss of maize grain (which was equivalent to the percentage mean weight loss because 100 g initial weight was used) was 5.00±0.17 and this was achieved within 1 reproductive cycle of 6 weeks (2 weeks of oviposition and 4 weeks for development to adult). This implies that 128.00±1.15 emerged weevils brought about 5.0% decrease in weight in the grains. There was a significant positive correlation between the weight loss of the maize and the number of emerged *S. zeamais* over the study period (r=1.000; p<0.01).

Table 1. Mean values (X±SE) The relationship between maize grain weight loss and adult emergence after 30 days.

Maize/Insect	A (g)	B (g)	C (g)	Mean±SE weight(g)/ Weevils	Ratio of introduced insects to emerged insects
Initial Weight (g)	100.00	100.00	100.00	100.00±0.00	
Final Weight (g)	94.70	95.30	95.00	95.00±0.17	
Weight loss (g)	5.30	4.70	5.00	5.00±0.17	
Number of insects at set up	40.00	40.00	40.00	40.00±0.00	1.00
Emerged Insects	130.00	126.00	128.00	128.00±1.15	3.20

Legend: A, B and C = Replicates; SE=Standard Error of mean.

Discussion

This study shows that insecticides, Dichlorvos and Profenofos+Cypermethrin had high toxicity against the maize weevil, *S. zeamais*. The mortality recorded for impregnated filter paper bioassay at 6 and 12 hours ranged from 0.00-86.67% and 86.67-100.00% respectively for Dichlorvos while the same trend was observed for Profenofos+Cypermethrin. In both cases, insect mortality was dose/time dependent. This result is in consonance with the work of Kljajic *et al* (2006) in which all the insecticides investigated in their study were found to increase in toxicity to adults of all three *Sitophilus* species with duration of exposure. Farkhanda and Abida observed an increasing trend in percentage mortality with increasing insecticide concentration and at 48 hours of insecticide treatment, 100% mortality was recorded but that of cypermethrin was quicker in action on filter papers (Farkhanda and Abida, 2013). This same trend was observed in the present study as Profenofos+Cypermethrin gave a faster knockdown of the weevil within 6 hours of exposure whereas it took 12 hours for Dichlorvos to effect 100% mortality. The reason for differential toxicity lies with the mechanism of action of both insecticides. The Profenofos+ Cypermethrin is a combination of organophosphate and pyrethroid which acts on the synapses as well as the

Na-K channels while Dichlorvos, an organophosphate, inhibits the action of Acetylcholinesterase enzyme (AChE) at the synaptic junctions (Eric *et al* 2007). The dual nature of the Profenofos+Cypermethrin implies that its higher toxicity has a primarily mechanistic basis.

The probit analysis for impregnated filter paper experiment revealed that the 6 hours LC_{50} values for Profenofos+Cypermethrin and Dichlorvos were 1.074mg/ml and 0.672mg/ml while at 12 hours, LC_{50} values were 0.027mg/ml and 0.291mg/ml, respectively. At 12 hours, Profenofos+Cypermethrin was about 11 times more toxic than Dichlorvos. This is in agreement with Farkhanda and Abida (2013) who conducted a comparative study on the toxicity of Cypermethrin and Dichlorvos against lesser grain borer, *Rhyzopertha dominica* (Fabricius) on impregnated filter paper and their result confirmed that Cypermethrin were more toxic than Dichlorvos with a toxicity factor of about 5.44 to 1.

The result of our study is also in agreement with that of Rajput (2010) who worked on Cypermethrin and Dichlorvos on rice weevil, *Sitophilus oryzae* and found that Cypermethrin alone was four times more toxic than Dichlorvos. Obviously, in this study, the results lend credence to the fact that there could be synergistic action between Cypermethrin and Profenofos over Dichlorvos alone. This hypothesis agrees with Vasquez-Castro *et al* (2012) who recorded higher mortality of *Sitophilus* spp. on wheat grains treated with a mixture of organophosphate fenitrothion+pyrethroid esfenvalerate than esfenvalerate alone.

The residual action of both insecticides 60 days post-treatment of maize grains against *S. zeamais* gave mortalities of 88.33% (Dichlorvos) and 100% (Profenofos+Cypermethrin) within 6 hours while 100% mortality was achieved after 12 hours for Dichlorvos. Thus, indicating that Profenofos+Cypermethrin was faster-acting than Dichlorvos but both insecticides were effective at controlling the insect pest. This result is in agreement with the findings of earlier authors such as Farkhanda and Abida (2013). It has been reported that the consumption of food produce already treated with Dichlorvos does not raise concerns since the levels of dietary exposure to man (from food residues monitoring data) are considered minimal (HSE, 2014). This is possibly because dichlorvos is known to break down by hydrolysis into less toxic substances during processing of grains in mills, thereby making flour products safer for human consumption (Don-Pedro 2009).

Weevils damage grains, reducing output and quality of product. In this study, the mean weight loss of maize grain and the mean emerged adults of *S. zeamais* was 5.00±0.17 and 128.00±1.15 respectively within 1 reproductive cycle of 6 weeks. There was a perfect direct correlation between the weight of the maize and the number of *S. zeamais* emerging over the study-period.

According to FAO, losses as a result of insect infestation during the short period of storage from harvest to sale at the farm-gate range between 10% and 20% (FAO, 1991). Apart from weight loss, weevil activities in stored grains may also cause reduction in grain quality and promotion of fungal growth. Thus, in order to increase maize yield and minimize weevil infestation, insecticide application at the correct dosage is inevitable.

Conclusion

The protection of maize grains from the ravaging effects of the maize weevil, *S. zeamais*, in storage is imperative as the study shows that there is a significant direct relationship between maize grain weight loss and insect emergence. The two insecticides tested, Dichlorvos and Profenophos+Cypermethrin, gave good residual contact toxicity to *S. zeamais*; 30 and 60 days post-treatment. It can therefore be concluded from this study, that Dichlorvos and Profenophos+Cypermethrin are candidate-insecticides for the management of *S. zeamais* infestation on stored maize grains.

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