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Toxicity of fine powders, filter cake and Triplex against *Sitophilus zeamais* adults

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Abstract

Filter cake and Triplex are powdered by-products of aluminum sulfate and soap factories, respectively. There is limited data about the use of these powders as grain protectants. This study was aimed at determining contact toxicity of both powders against *Sitophilus zeamais*, a common pest of stored grains. Lethal concentration of both powders to *S. zeamais* was determined by exposing 10 adults for 12 h in 9 cm diameter concrete arenas inside Petri dishes dusted with filter cake (0 - 8 g/m^2) or Triplex (0 - 9 g/m^2). Lethal time was determined by exposing adults to 3 g/m^2 filter cake and 9 g/m^2 Triplex for 1 to 24 h. Each treatment was replicated 3 times. At the intended exposure time, adults were transferred to 150-ml round plastic containers with 30 g of wheat and held at 28 degree Celsius and 65% r.h. for 14 d to determine mortality. Adult progeny production was determined after 42 d. A 50% mortality of adults was obtained at 0.61 g/m^2 of filter cake and 1.61 g/m^2 of Triplex concentrations with a 12 h exposure. The corresponding effective concentrations for 50% reduction of progeny production were 0.18 g/m^2 of filter cake and 2.66 g/m^2 of Triplex. Lethal times for 50% mortality of adults after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 4.42 and 4.29 h, respectively. The corresponding effective times for 50% reduction of progeny production after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 1.74 and 2.34 h respectively. The overall result indicated that filter cake was highly toxic to *S. zeamais* than Triplex. Therefore, filter cake is a potential powder to be included in the integrated pest management practice in small holder farmers' storage structures after tested under real field conditions.

Keywords: Filter cake; Triplex; *Sitophilus zeamais*; Toxicity

1. Introduction

Grain losses due to insect pests in sub-Saharan Africa are very high, and the magnitudes of losses vary from country to country and from region to region (Abate et al., 2015). In countries like Ethiopia, about 80% of all grain produced is estimated to be stored at the farm or village level (Tadesse and Eticha, 2000). Grain storage losses in Ethiopia due to insect pests were estimated to be in the range of 10 to 21% (Abraham et al., 2008). A number of chemical insecticides used by Ethiopian small holder farmers to protect their grain in storage have been reported by several researchers (Abraham et al., 2008; Girma et al., 2008a,b; Hengsdijk and De Boer, 2017; Mengistie et al, 2017; Dessalegn et al., 2017). Recently, chemical pesticides, regardless of their inherent hazards, are used extensively in the fast changing agricultural sector of Ethiopia (Nigatu et al., 2016). However, Ethiopia is confronted with a number of problems related to unsafe handling of pesticide distribution and use, such as use of unsafe storage facilities, improper training on safe use of pesticides, and inadequate infrastructure to regulate safe use of pesticides (Mengistie et al., 2017). A survey done by Nigatu et al. (2016) on knowledge, attitude, and practices of farmers and farm workers in Ethiopia reported that 85% of farm workers (pesticide mixers/loaders, sprayers, and application supervisors) (n = 601) and 100% of female re-entry farm workers (harvesters, pesticide assessors, irrigation workers, irrigation supervisors, packing and sorting workers, transport/push car workers), (n = 275) did not receive pesticide-related training. In addition, 62% of farm workers did not shower after pesticide application, and none of the small-scale farm workers (n = 258) used personal protective equipment. A considerable increment in chemical pesticide usage intensity, illegitimate usage of DDT and Endosulfan on food crops, and direct import of pesticides without the formal Ethiopian registration process were also reported by Nigatu et al. (2016).

Therefore, there is a need to explore products that are safe and effective in controlling insects in smallholder farmers' traditional storages in Ethiopia. Two such products are filter cake and Triplex (Girma et al., 2008a,b; Tadesse and Subramanyam, 2018). Filter cake is a by-product of aluminum sulfate factory (Awash Melkassa Aluminium Sulphate & Sulfuric Acid Share Company, Melkassa Awash, Ethiopia (AMASSASC)). Triplex is a by-product of Mohammed International Development Research and Organization Companies (MIDROC) soap factory (Star Soap and Detergent Industries (SSDI Private Limited Company), Addis Ababa, Ethiopia). A study on elemental composition of both powders using energy-dispersive X-ray spectroscopy indicated that silicon and oxygen were dominant elements (Tadesse and Subramanyam, 2018). The same study showed 100% mortality when adults of *Sitophilus zeamais* Motschulsky were exposed to 7.5 g/m^2 of filter cake and 10 g/m^2 of both powders for 24 h on treated concrete arenas. Girma et al. (2008a) reported 92% mortality 3 d after *S. zeamais* were exposed to three genotypes of maize treated with 1, 2.5, and 5% (w/w) of filter cake. Similarly, a 0.25% (w/w) Triplex treated maize showed no significant mean percentage mortality of *S. zeamais* (93%) when compared with that of a synthetic insecticide, pirimiphos-methyl (100%), 7 months after treatment (Girma et al., 2008b). However, the toxicity of both products on multiple range of concentration and time was not known. Toxicity study is the investigation of either short or long-term toxic effects of a drug or chemical on animals (Saganuwan, 2017). Short-term toxic effect is determined using median lethal dose (LD_{50}), the dose required to kill half the members of a tested population after a specified test duration which was first introduced by Trevan in 1927 (Trevan, 1927) and revised many times (Saganuwan, 2017). It is frequently used as a general indicator of a substance's acute toxicity and lower LC_{50} is indicative of higher toxicity (Criswell and Campbell, 2013). Therefore, knowing the toxicity of filter cake and Triplex will help to use both products at the safer and effective level. This study was designed to determine the LC_{50}, lethal time (LT_{50}), effective concentration (EC_{50}) and effective time (ET_{50}) of filter cake and Triplex against *S. zeamais* economically, an important stored grain pest in Ethiopia (Abraham et al., 2008; Girma et al., 2008a,b; Tilahun and Hussen, 2014).

2. Materials and methods

2.1. Concrete-poured Petri dishes

Rockite®, a ready-to-mix concrete product (Hartline Products Co., Inc., Cleveland, Ohio, USA), was mixed with tap water in 2:1 ratio (grams to milliliter) to make a slurry. The slurry was poured into 9 cm diameter and 1.5 cm high plastic Petri dishes (Fisher Scientific, Denver, Colorado, USA). Slurry was poured to cover one half of the Petri dish's height. Slurry filled Petri dishes were allowed to dry on a laboratory bench for 24 h. Polytetrafluoroethylene (Insecta-a-Slip, Bio Quip Products, Inc., Rancho Dominguez, California, USA) was used to coat the inside walls of Petri dishes to prevent insects from crawling on sides of dishes.

2.2. Application of powders to concrete arenas and insect exposure

Brass frame, stainless steel wire cloth sieve with #80 mesh size (Seedburo Equipment Company, Des Plaines, Illinois, USA) were used to sift filter cake and Triplex powders. Concrete arenas of dishes were treated with the fines (particle size < 177 microns) at the following concentrations: 0 (untreated control), 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 g/m^2 of filter cake or 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 g/m^2 of Triplex. A laboratory strain of *S. zeamais* was reared at 28 °C and 65% r.h. on organic hard red winter wheat (Heartland Mills, Marienthal, Kansas, USA) of 12.5% moisture content (wet basis) at the department of Grain Science and Industry, Kansas State University. Adults of *S. zeamais* were separated from wheat using a 2.38 mm diameter round-holed aluminum sieve (Seedburo Equipment Company, Des Plaines, Illinois, USA). Ten unsexed newly emerged two weeks old adults were exposed to untreated concrete arenas (control) and arenas receiving each of the 9 concentrations of filter cake or Triplex for 12 and 24 h in an environmental chamber with 28 °C and 65% r.h. A separate set of experiment, 10 adults from the same culture were exposed to 3 g/m^2 of filter cake or 9 g/m^2 of Triplex separately for 1,2,4,6, 8, 10,12, 14, 16, 18, 20, 22, 24 h to determine the lethal times of both powders. Each powder-concentration-time combination was replicated three times. After the intended exposure time period insects were transferred carefully with a camel's hair brush to 150 ml round plastic containers holding 30 g of cleaned, organic hard red winter wheat (Heartland Mills) of ~12.5% moisture content (wet basis). The plastic containers had perforated lids with wire-mesh screens to facilitate air diffusion. Containers were incubated on the same chamber at 28 °C and 65% r.h. After 14 d, wheat from each container was sifted using a 2.38 mm circular round-holed aluminum sieve to separate insects from wheat. Insects that did not respond when gently prodded by a camel's hair brush were considered dead. A third set of experiment with same concentrations and exposure times was set to determine adult progeny production 42 d, after exposure to filter cake or Triplex. Adult progeny produced was counted from each container and the 10 starting adults were subtracted.

2.5. Data analysis

The mean ± SE mortality of *S. zeamais* on untreated (control) (n = 3) arenas at all exposure times ranged from 0.0 to 0.33 ± 0.33 and 0.0 to 0.67 ± 0.33 for filter cake and Triplex, respectively. Therefore, mortality data of *S. zeamais* exposed to filter cake or Triplex were corrected for responses in the control treatment (Abbott, 1925). Probit analysis was used to generate probit regression estimates and lethal concentration (LC) or lethal time (LT) producing 50 and 99% mortality. Similarly, probit analysis was used to generate effective concentrations (EC) and effective times (ET) for the 50 and 99% reduction of progeny production. The LC_{50}, LT_{50}, EC_{50} and ET_{50} values of filter cake were compared to the corresponding values of Triplex using ratio tests (Robertson et al., 2007). All the data were analyzed using SAS software (SAS Institute, 2012). Differences between any two LC_{50} or LT_{50}, EC_{50} or ET_{50} values were considered to be significantly different ($P < 0.05$) if the 95% CL for the ratio did not include 1 (Robertson et al., 2007).

3. Results

3.1. Concentration and corrected mortality responses of S. zeamais

Mean ± SE percentage corrected mortality of *S. zeamais* adults exposed to 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 g/m^2 concentrations of filter cake at 12 h ranged from 41.58 ± 6.87 to 100 ± 0.00%. For 1, 2, 3, 4, 5, 6, 7, 8 and 9 g/m^2 concentrations of Triplex, the mortalities ranged from 20.96 ± 9.09 to 96.56 ± 3.44%. The corresponding mortalities of *S. zeamais* adults after exposure to 3 g/m^2 of filter cake or 9 g/m^2 of Triplex for 1,2,4,6, 8, 10,12, 14, 16, 18, 20, 22, 24 h ranged from 20.96 ± 6.87 to 100 ± 0.00% and 24.40 ± 3.44 to 96.56 ± 3.44%, respectively.

Table 1. Probit estimates and concentrations required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 14 d after exposure to filter cake and Triplex for 12 h

Powder	**N**[a]	**Mean ± SE**		**LC (95%CL) (g/m^2)**		**x^2(df)**[b]
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	180	0.63 ± 0.03	0.56 ± 0.07	0.61 (0.37 – 0.82)	7.54 (4.65 – 18.60)	11.01 (16)
Triplex	270	0.13 ± 0.05	0.84 ± 0.07	2.63 (2.12 – 3.11)	23.46 (15.88 – 44.05)	18.88 (25)

[a] N = total number of adults used to generate the probit regression estimates
[b] x^2- values for goodness-of-fit of model to data were not significant ($P > 0.05$)

Table 2. Probit estimates and times required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 14 d after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex

Powder	**N**[a]	**Mean ± SE**		**LC (95%CL) (g/m^2)**		**x^2(df)**[b]
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	240	-2.12 ± 0.32	2.72 ± 0.35	4.42 (3.47 – 5.26)	21.92 (16.74 – 33.56)	23.49 (22)
Triplex	390	-1.61 ± 0.24	2.00 ± 0.22	4.29 (3.14 – 5.34)	39.62 (29.87 – 60.30)	23.28 (37)

[a] *N* = total number of adults used to generate the probit regression estimates
[b] x^2- values for goodness-of-fit of model to data were not significant ($P > 0.05$)

The x^2 values for goodness-of-fit of the model to corrected mortality data were not significant ($P > 0.05$) (Table 1 and 2) indicating good fit of model to data. The lethal concentrations to kill 50% of *S. zeamais* adults exposed to filter cake and Triplex were 0.61 g/m^2 and 2.63 g/m^2, respectively. Triplex had significantly higher LC_{50} value than filter cake (ratio [95% CI] = 4.33 (2.85 – 6.57). The lethal times to kill 50% of adults exposed to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 4.42 and 4.29 h respectively. There was no significant differences between the LT_{50} value of Filter cake and Triplex (ratio [95% CI] = 0.97 (0.70 – 1.35).

Mean number of adult progeny produced ± SE by *S. zeamais* adults 42 days after exposure to 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 g/m^2 concentrations of filter cake at 12 h ranged from 0.00 ± 0.00 to 135.00 ± 27.78. The corresponding mean number of adult progenies produced ± SE were ranged from 1.00 ± 0.58 to 123.67 ± 34.76 for Triplex at 1, 2, 3, 4, 5, 6, 7, 8 and 9 g/m^2 concentrations. Similarly, Mean number of adult progeny produced ± SE by *S. zeamais* adults after exposure to 3 g/m^2 of filter cake or 9 g/m^2 of Triplex for 1,2,4,6, 8, 10,12, 14, 16, 18, 20, 22, 24 h ranged from 0.00 ± 0.00 to 111.33 ± 7.62 and 24.40 ± 3.44 to 96.56 ± 3.44%, respectively. The percentage reduction of adult progeny produced relative to the control treatment was used to fit the model.

Table 3. Probit estimates and concentrations required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 7 d after exposure to filter cake and Triplex for 12 h

Powder	N^a	Mean ± SE		LC (95%CL) (g/m^2)		x^2(df)
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	180	1.41 ± 0.07	1.93 ± 0.28	0.18 (0.10 – 0.26)	3.00 (2.15 – 5.19)	6.31 (7)[b]
Triplex	270	-1.17 ± 0.28	2.76 ± 0.44	2.66 (1.98 – 3.30)	18.59 (11.91– 43.60)	470 (25)

[a] *N* = total number of adults used to generate the probit regression estimates
[b] x^2- values for goodness-of-fit of model to data were not significant ($P > 0.05$)

Table 4. Probit estimates and times required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 7 d after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex

Powder	N^a	Mean ± SE		LC (95%CL) (g/m^2)		x^2(df)[c]
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	210	-0.82 ± 0.19	1.89 ± 0.25	1.74 (1.10 – 2.32)	17.49 (12.50 – 30.42)	132.51 (19)
Triplex	390	-1.08 ± 0.16	1.93 ± 0.16	2.34 (1.75 – 2.91)	22.31 (18.23 – 29.12)	200.52 (37)

[a] *N* = total number of adults used to generate the probit regression estimates
[c] x^2- values for goodness-of-fit of model to data were significant ($P < 0.05$)

The x^2 for goodness-of fit of the model to percentage adult progeny reduction data collected 42 d after exposure to filter cake and Triplex were not significant (P > 0.05) (Table 3). The effective concentration to decrease 50% of adult progeny production for *S. zeamais* 42 d after exposure to filter cake and Triplex were 0.18 and 2.66 g/m^2, respectively. Triplex had significantly higher EC_{50} value than filter cake (ratio [95% CI] = 14.41 (8.83 – 23.51). The x^2 for goodness-of fit of the model to adult progeny reduction data collected 42 d after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were significant ($P < 0.05$) (Table 4), indicating poor fit of model to data. The corresponding effective times to reduce 50% of adult progeny production of *S. zeamais* after 42 d were 1.74 and 2.34 h for filter cake and Triplex, respectively. Triplex had significantly higher ET_{50} value than filter cake (ratio [95% CI] = 1.81 (1.12 – 2.92).

4. Discussion

Contact insecticides have been used to protect stored commodities from pests for a long period of time (Ebling, 1971; Headlee, 1924). Such products have large proportion of silica which has high capacity to absorb epicuticular lipid from insects' body (Ebling, 1971; Malia et al., 2016; Subramanyam and Roseli, 2000). Silicon and oxygen are the major components of filter cake and Triplex (Tadesse and Subramanyam, 2018) which makes them similar to other inert dusts.

Our data demonstrated contact toxicity of filter cake and Triplex through concentration and exposure time responses of *S. zeamais* adults. The mortality of *S. zeamais* increased with increasing concentration of filter cake and Triplex. Accumulation of powder over insect's body is directly proportional to concentration of the powder (Le Patourel et al., 1989), and insect behavior such as mobility. These factors cause adverse effects to exposed insects (Malia et al., 2016). A positive correlation between oil absorption and silicon dioxide concentration was reported by Filipović et al. (2010) after testing oil absorption capacity of silica powders prepared from sodium silicate solution with different concentration of silicon dioxide. This is supported in our study where adult mortality tended to increase with increasing concentration of filter cake and Triplex. The same phenomenon was reported by Tesfaye and Subramanyam (2018) after unsifted filter cake and Triplex were tested at 2.5, 3.75, 5, 7, 7.5 and 10 g/m^2 concentrations of filter cake or Triplex against *S. zeamais*.

The x^2 values for goodness-of fit of the model to our data to determine LT_{50} and ET_{50} was significant. These heterogeneous responses could be related to sex as unsexed adults were used in the experiment. Heterogeneous responses of insects were reported by several researchers when exposed to temperature (Mahroof et al., 2003), Spinosad and Chlorpyrifos-Methyl Plus Deltamethrin (Seghal et al., 2013), spinosad (Subramanyam et al., 2014) and chlorine-dioxide (Xinyi et al., 2017). The LC_{50}, EC_{50} and ET_{50} values of filter cake were three time less than that of Triplex. This was confirmed by the ratio test which showed significantly higher toxicity of filter cake to *s. zeamais* than Triplex. A 7.54 g/m^2 concentration of filter cake was effective to kill 99% of s. zeamais adults within 12 hours and 21.92 hours were required to get the same mortality when 3 g/m^2 concentration of filter cake was used. A similar concentration (7.5 g/m^2) of filter cake was reported by Tadesse and Subramanyam (2018) to kill 100% of *s. zeamais* adults exposed for 24 h. However, more than three times the concentration of filter cake was required to kill *s. zeamais* adults after exposure to Triplex for 12 h. This could be related to the carbon content (atomic percent = 39.43 ± 12.63) of filter cake in the form of calcium carbonate (Tadesse and Subramanyam, 2018). Calcium carbonate at 1 and 2% (w/w) applied to maize caused 70.2 and 84.2% mortality of *s. zeamais* adults, respectively, 15 d after treatment (Silva et al., 2004). Filter cake was also effective in suppression of progeny production with more than 3 times less concentration than Triplex.

In conclusion, 7.54 g/m^2 of filter cake was effective in killing adults of *s. zeamais*, an economically important species. It also completely suppressed adult progeny production, indicating adults were killed before they lay eggs into the kernel. Three-fold higher concentration was required to get similar result on Triplex treatments. This indicated that filter cake was highly efficacious to *s. zeamais* than Triplex. This work was done under laboratory conditions. Therefore, it needs to be confirmed under practical field conditions such as traditional storage structures of smallholder farmers.

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Efficacy of 10 dusts on life cycle of *Tribolium castaneum*

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Silica dusts have a long history of use in agriculture for insect control. But the product has several problems that limit its overall use and effectiveness. The present study is investigating a form of silica that has not been used in agriculture to date. The study has been comparing both hydrophilic and hydrophobic forms of the silica against traditional silica products such as Dryacide. The study