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Mortality of *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) Adults Exposed to Different Concentrations of Filter Cake in Stored Wheat

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የሲልከን ውሁድ ያላቸው ግዑዝ ብናኞች የተከዘነ እህልን ከጎተራ ነፍሳት ከመከላከል አንጻር ከኬሚካሎች ይልቅ ለአካባቢ ተስማሚ ለጤናም ጉልህ ጉዳት የማያስከትሉ አማራጮች እየሆኑ መጥተዋል፡፡ ከዚህ ጋር ተያይዞ አንድ የሲሊከን ውሁድ ያለው የማጣሪያ ኬክ ብናኝ በጎተራ ነፍሳት ላይ የሚኖረውን የመቆጣጠር አቅም ለመፈተሽ በጎተራ ነቀዝ እና በትንሹ እህል ቦርቧሪ ጥንዚዛ ላይ ጥናት ተደርጓል፡፡ ጥናቱ የተከናወነው 1ኪ.ግ.፣ 0.75ኪ.ግ.፣ 0.5ኪ.ግ.፣ እና 0.25ኪ.ግ. የማጣሪያ ኬክ ብናኝ በአንድ ኩንታል ስንዴ ላይ በመጨመር ሲሆን የብናኙን ውጤታማነት ለመቆጣጠር ምንም ብናኝ ያልተደረገበት ስንዴ ከጎን ተጠንቷል፡፡ ጥናቱ ባጠቃላይ ለ14ቀናት የተከናወነ ሲሆን ብናኝ ባለው ስንዴ ውስጥ የገቡ ጉልምስ ነፍሳት የደረሰባቸው የሞት መጠን ተገምግሟል፡፡ በዚህም መሠረት በጎተራ ነቀዞች ላይ በሶስት ቀናት ብቻ ከ41.3% እስከ 70.0% የጉልምሶች ሞት የተከሰተ ሲሆን ይህ በትንሹ እህል ቦርቧሪ ጥንዚዛ ጉልምሶች ላይ ከ73.3% እስከ 93.3% ሞት አስከትሏል፡፡ ከ14 ቀናት በኋላ ሲታይ በጎተራ ነቀዝ ጉልምሶች ላይ እስከ 98.7% ሞት ያስከተለ ሲሆን በትንሹ እህል ቦርቧሪ ጥንዚዛ ላይ ግን እስከ 100% ሞትን አስከትሏል፡፡ ስለዚህ በዚህ ጥናት ላይ የተካተቱ የነፍሳት ዝሪያዎችን በማጣሪያ ኬክ ብናኝ ከምላጎደል መቆጣጠር እንደሚቻል መመልከት ይቻላል፡፡

Abstract

Silica based inert dusts are eco-friendly alternatives to control stored grain insect pests due to environmental and health concerns associated with use of synthetic insecticides. A study was conducted to determine the efficacy of filter cake (a silica-based inert dust) on the granary weevil, ***Sitophilus granarius (L.)*** *and lesser grain borer,* ***Rhyzopertha dominica (F.)*** *in stored wheat. Filter cake dust of ≤0.4 mm particle size was admixed with 500 g of wheat seed to provide nominal rates of 10000, 7500, 5000, and 2500 ppm (mg/kg), while the control treatment consisted of wheat seeds that were untreated. The bioassays were carried out using a liter-sized plastic jars in completely randomized design with three replications. Experiments were maintained at 23.1±1.7^{o}C and 61.0±4.3% relative humidity. Mortality data were collected at 3, 7, and 14 d after treatment. Results indicated that mean mortality rate at 3 d after treatment ranged from 41.3 to 70.0% in* ***S. granarius*** *and 73.3 to 93.3% in* ***R. dominica****. Mean mortality of* ***S. granarius*** *adults at 14 d in filter cake treatments was 84.0 to 98.7%, whereas that of* ***R. dominica*** *was 98.3 to 100%. The present results show that filter cake dusts can be used to control these two species in stored wheat. Filter cake has potential in protection of wheat from* ***S. granarius*** *and* ***R. dominica*** *infestations in storage.*

Introduction

Wheat is one of the major cereal crops grown in Ethiopia and it is well adapted to highlands which range between 1500 to 2800m above sea level (Gebre-Mariam *et al*., 1991), recently covering above 1.7 million hectares of land (CSA, 2017). Despite of the increased wheat yield due to use of improved production technologies and packages (Kotu and Admassie, 2016; Mann and Warner, 2015) the country remains a net importer of wheat.

Poor post-harvest handling of wheat grains that results in quality and weight loss due to insect and mold damages (Boxall, 1998) is important among other factors that contributed to shortage of wheat supply. Stored product insects are important factors that cause quality and weight loss of wheat and other cereals under smallholder conditions in the country. Major stored product insect species on wheat grain include *Sitophilus granarius, S. oryzae* and *S. zeamais* whereas *Tribolium castaneum*, *T. confusum* and *Ephestia cautella* on wheat flour (Gebre-Mariam *et al*., 1991). Generally, *S. granarius* is a major pest in the higher altitudes while heavy damages by *R. dominica*, which is a primary pest in many regions of the world, were also reported (refer to review by Tadesse *et al*., 2008).

Silica based inert dusts are becoming eco-friendly alternatives to control stored grain insect pests due to environmental and health concerns associated with chemical use. Previous studies highlighted susceptibility of insects to silica based inert dusts as affected by insect species, grain moisture, and storage temperature (Gana *et al*., 2016; Fields and Korunic, 2013; Prasantha *et al*., 2015; Vayias and Stephou, 2009; Stathers *et al*., 2004). Cook and Armitage (2000) reported that granary weevil (*S. granarius*) population was totally suppressed within 12 weeks after surface application of 3-5g/kg Dryacide on wheat grain of 16% moisture content at 75% RH and 15°C. However, it took about 22 weeks to achieve a 100% suppression of *Oryzaephilus surinamensis* population under similar conditions. Sousa *et al*. (2013) also concluded that lower doses are more effective at high temperatures and longer exposure times for the *S. zeamais* control. Kavallieratos *et al*. (2015) underscored that *T. confusum* was the least susceptible species to three diatomaceous earth formulations compared to *R. dominica* and *S. oryzae.*

Demissie *et al.* (2008) evaluated a material namely filter cake and found that it is effective against the maize weevil at rate of 1% or higher. A recent study showed that the composition (w/w) in filter cake dust has considerable amount of silicon dioxide (Tadesse and Subramanyam, 2018). However, reports on efficacy of filter cake against storage insect pests are limited in the literature. Efficacy of the inert dust at lower doses also needs investigations since applying at rate of >1% (10000ppm) may be too high since commercial formulations of the diatomaceous earth are normally used at rates as low as 3500ppm (0.35%) (Shah and Khan, 2014). This study, therefore, was initiated to investigate the efficacy of filter cake dust against adult populations of *S. granarius* and *R. dominica* in stored wheat.

Materials and Methods

Test insects

Test insects of *S. granarius* were collected from farmers' stores in Ofla district, Tigray, Ethiopia, at altitude of about 2509m above sea level and geographic position of N12°30.939' E039°30877' in early June, 2016. Approximately 200 live adult insects of different age and sex were recovered from wheat grain samples and placed in 1liter plastic jar filled with around200g of disinfested wheat grain. The jar was then covered with cotton fabric for raring under room condition at College of Agriculture and Environment Sciences, Bahir Dar University, Ethiopia. Original insects were then removed after 15 days (assuming that adequate number of eggs could have been laid during the due time). Additional disinfested grain was added to plastic jars to avoid food limitation to the growing insects. Progenies with age of about three to six weeks were collected and used for the bioassay during end of September, 2016.

Adult insects of *R. dominica* were recovered during end of August 2016 from certified seed samples purchased from the Ethiopian Seed Enterprise, Bahir Dar Center. About 150 to 200 adults were reared in plastic jars filled with 200g of disinfested wheat grain under similar conditions with that of granary weevil. Additional disinfested grain was added to plastic jars to avoid food limitation to the insects. Original adults were not removed in the case of lesser grain borer, but sifted at the end of incubation period (42 days). Then, newly emerged progenies were collected for about two weeks (Kavallieratos *et al*., 2015) until sufficient number was obtained for the bioassay.

Temperature and relative humidity of the room during the period between July and end of September was recorded in hourly interval using Hobo® temp/RH data logger, Onset® Computer Corporation, USA, and the average room temperature was about 23.1±1.7°C and 61.0±4.3% relative humidity.

Treatment setup

Wheat seed samples were sifted and disinfested through refrigeration at 5°C for two weeks. Grain samples were acclimatized for 48 hours at room conditions of 23.1±1.7°C temperature and 61.0±4.3% relative humidity. Damaged grains were removed and only intact grains were used for the experiment.

Filter cake dust (which passed through 0.4mm meshes) and grains (12.5% moisture content) were homogeneously mixed with spoon at rates of 10000ppm, 7500ppm, 5000ppm, 2500ppm, and 0ppm/control/. A 500g of treated wheat was then placed in 1000ml plastic jar covered with woven cotton fabric. Adult *S. granarius* and *R. dominica* of different age and sex were used for this experiment. From individual species, 50 and 20 insects, respectively, were placed in each of the jars. The jars were slightly moved forward and backward for 5 seconds to facilitate entrance of insects into the grain mass. The jars were then placed in the laboratory under ambient conditions, with 23.1±1.7°C temperature and 61.0±4.3% relative humidity, in completely randomized design with three replications.

Data collection

Insect mortality was examined at 3, 7, and 14 days after treatment. Dead insects were removed at every time of counting. All live insects were returned to the jars until the end of data collection i.e. 14 days. Daily temperature and relative humidity were recorded every hour. Mortality data were corrected based on the following equation provided by Rosenheim and Hoy (1989)

$$Pcorr = 1\left(\frac{(1-\mathrm{T})(1-K)}{1-\mathrm{C}}\right)x\ 100 \text{ and } \mathrm{K} = \left(\frac{Var(\mathrm{C})\mathrm{t}^2}{(1-\mathrm{C})^{2\mathrm{nC}}}\right)$$

Where, *Pcorr*=corrected proportion of dead adults, *T*=mortality in treated grain and *C*=mortality in untreated grain, n_c=number of replications used for estimating *C*, and *t*=value of *t* distribution *nc*-1 degrees of freedom at α=0.05. Percentage data were arcsine transformed using the equation: $\text{asin}\ (\sqrt{r})$ where *r* is the mortality rate.

Data analysis

One way analysis of variance and mean separation were carried out using the GLM Procedure of the SAS System (Version 9.0). Mean separation employed the Ryan-Elinot-Gabriel-Welsch Multiple Range Test (REGWQ) using the transformed data and means were reversed to percentage data using the equation: $100 * \sin{(t)}^2$ where *t* is the transformed mean. Linear regression analysis and contour plotting were carried out using the SigmaPlot Version 12.5 (Systat Software, San Jose, CA). Binomial log-logistic regression analysis was carried out using the “drc” package (Ritz *et al*., 2015) for R Version 3.4.1 (R Core Team 2017). The binomial log-logistic analysis was carried out using all data frames for the treatments and controls without any transformation.

Results and Discussion

Insect mortality at different filter cake concentrations

The mortality of *S. granarius* and *R. dominica* adults in the control treatment ranged from 0.7 – 13.3 %. Analysis of variance showed that there was statistically significant difference (*P<0.05*) among treatments on mortality of *S. granarius* at 3 and 14 days after filter cake application (Table 1). Differences in mortality of *S. granarius* were statistically not significant among various rates of filter cake after 7 days. However, the coefficient of variation was very high for mortality measured 7 days after treatment.

For *R. dominica*, average adult mortality rates for all concentrations of filter cake were statistically similar (p>0.05) at 3, 7, and 14 days after treatment. The coefficient of variation at 3 days after treatment was high showing that the observations could be less reliable.

Table 1. Mean±SE mortality (%) of *Rhyzopertha dominica* and *Sitophilus granarius* adults exposed to different concentrations of filter cake at three exposure times (each mean is based on n=3; df = 3, 8 for all; at each exposure time, means followed by the same letter are not significant (P>0.05, REGWQ test)

Filter cake concentration (ppm)	*Sitophilus granarius*			*Rhyzopertha dominica*		
	Days after filter cake treatment					
	3	7	14	3	7	14
10000	70. 0±4.0a	95.3±2.9	98.7±1.3a	93.3±6.7	98.3±1.7	100±0.0
7500	62.7±1.8ab	91.3±5.2	98.7±0.7ab	80.0±7.6	95.0±2.9	100±0.0
5000	52.7±1.8b	85.3±2.4	90.7±2.8bc	78.3±4.4	93.3±3.3	100±0.0
2500	41.3±2.4c	74.0±4.0	84.0±3.1c	73.3±7.2	93.3±1.7	98.3±1.7
Mean	56.7	86.5	93.0	81.3	95.0	99.6
F-value	21.05	3.59	9.40	2.53	0.89	1.00
CV%	5.64	11.84	6.87	15.86	10.3	4.19
P-Value	0.00	0.07	0.01	0.13	0.49	0.44

Treatment of wheat grain with filter cake resulted in average mortality of 56.7%, 86.5%, and 93.0% at 3, 7, and 14 days after treatment, respectively on granary weevil. Mean mortality of *S. granarius* at 3 days after filter cake treatment ranged from 41.3% to 70.0%. Lowest mortality rate was recorded at 2500 ppm filter cake treatment.

At 7 days after treatment, almost 95.3% of *S. granarius* adults were dead at filter cake rate of 10000ppm while the mean mortality rates at all levels of filter cake treatment were statistically similar. Decreasing filter cake concentration from 10000ppm to 2500ppm reduced mean mortality of *S. granarius* by 21.3%. However, mean mortality of 74.0% at 2500ppm of filter cake concentration can be of a considerable effect.

Mean mortality of *S. granarius* adults was as high as 98.7% at 14 days after treatment with both 7500ppm and 10000 ppm filter cake dust (Table 1). Earlier report by Demissie *et al.* (2008) indicated that filter cake dusts at rate of 1% could achieve 100% mortality of *S. zeamais* at 15days after treatment. Difference in mean mortality between highest and lowest concentrations of filter cake treatment was around 14.7%. Halving filter cake concentration (5000ppm) resulted in 90.7% mortality at this time. The lowest filter cake concentration (2500ppm) resulted in 84.0% mean mortality of the pest. There is no previous report on efficacy of filter cake dust against the granary weevil, but studies on other silica based dust variants indicated that longer exposure period is required to achieve complete mortality *S. granarius* adults (Cooks and Armitage, 2000). In the present study, maximum mortality achieved at rate of 10000ppm filter cake dust was 98.7% after two weeks. This could be attributed to higher tolerance of *S. granarius* adults to desiccant dusts (Desmarchelier and Dines, 1987).

Average mortality rates of 81.3%, 95.0%, and 99.6% were recorded at 3, 7, and 14 days after treatment. Increasing filter cake concentration usually increased mean mortality rate. At 3 days after treatment, mean mortality rate ranged from 73.3% to 93.3% for the lowest and highest rates. This range was narrowed to 5.0% and 1.7% at 7 and 14 days after treatment, respectively. At 7 days after filter cake treatment, the mean mortality of *R. dominica* adults was around 95.0%, which was higher than that of *S. granarius* by 8.5% for the same time. At 14 days after treatment, filter cake concentrations of 5000ppm and 10000ppm resulted in 100% mortality in *R. dominica* adults. It was observed that *R. dominica* can be controlled at lower rates of filter cake, which is in agreement with previous findings on diatomaceous earth (Kavallieratos *et al.*, 2015).

R. dominica exhibited more average mortality rate than *S. granarius*. This is in line with a previous finding by Desmarchelier and Dines (1987) who concluded that *R. dominica* adults were more sensitive to silica based dust. Silica based dust increases rates of body water loss through the cuticle impairing the individual's water balance leading to faster mortality than in the untreated weevil (Malia *et al.*, 2016). Lower sensitivity of *S. granarius* to filter cake dust could be due to less damaging effect to the integument and in enhancing water loss from the body of individual weevils compromising their water content and leading to quick death by desiccation (Malia *et al.*, 2016).

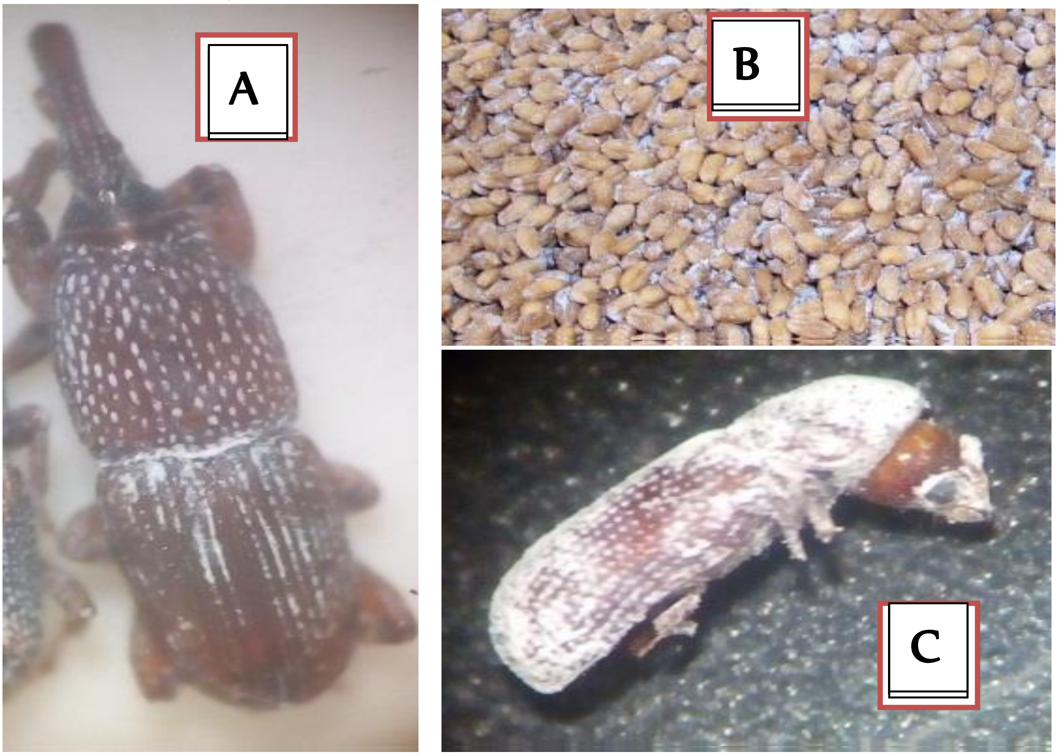

Figure 1. Dead adults of *Sitophilus granarius* (A) and *Rhyzopertha dominica* (C) recovered from wheat grain treated with 10000ppm filter cake (B) at 3 days after treatment. Dust particles adhered to insect bodies with higher density on *R. dominica*

Time-dose-response relationships

A regression analysis for the relationship between adult insect mortality (%) and filter cake application rate demonstrated a strong linear association. For every increase in parts per million of filter cake application rate, there was an increase in mortality rate of *S. granarius* adults by 0.00384% (R^2=0.873, F-value=76.7), 0.0028% (R^2=0.621, F-value=19.0), and 0.00208% (R^2=0.68, F-value=24.4) at 3, 7 and 14 days after application, respectively (Figure 2A). However, to achieve 100% mortality within the range of one to two weeks, one has to apply 7500 to 10000ppm of filter cake dust, i.e. 0.75 to 1.00g of filter cake dust for every kilogram of wheat grain (Figure 2B). Doses that result in 100% mortality within shorter period are important to continual protection of grains. Slow effects may allow adequate time for oviposition resulting in certain level of damages from progenies. In fact, higher doses such as 10000ppm may result in total mortality within eight days but this may not be attractive to flour plants. In the present case, use of 7500ppm may be adequate to cause 100% mortality within less than ten days (Figure 2B).

As exposure time increased from 3 to 14 days, constants of the regression equation increased from 32.7% to 80.0%. This could be attributed to the fact that only mortality (but not a new emergence of progenies) is considered as population parameter (in this study); and hence once exposed, *S. granarius* adults are subject to death in a given course of time regardless of the filter cake concentration.

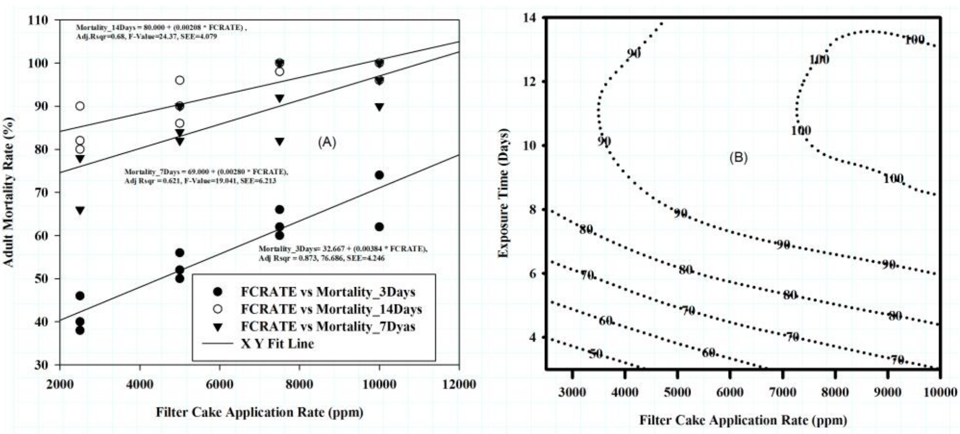

Fig. 2: Linear regression (A) and contour plot (B) of adult mortality rate of *Sitophilus granarius* at different rates of filter cake application and exposure time in stored wheat. FCRATE (Filter Cake application rate in parts per million), SEE (Standard Error of Estimates).

Log-logistic relationship

Effective doses for median mortality (ED_{50}) and 95% mortality (ED_{95}) were estimated using the log-logistic two-parameter model as suggested by Ritz *et al*. (2015) for dose response binomial data. Estimated effective dose for ED_{50} of *S. granarius* after 3 days of exposure was 3917.8±407.7ppm (~3.9g of filter cake dust per kilogram of wheat grain) (Figure 3). Both parameters (ED_{50} and the slope) were highly significant ($p<0.001$) with t-values of 9.562 and 5.5691 that *S. granarius* mortality data at 3 days exposure time has fit to the two parameter log-logistic model. The estimate for ED95 was 57473.5±25364.0ppm with 95% CL of 2677.8ppm to 112269.3ppm.

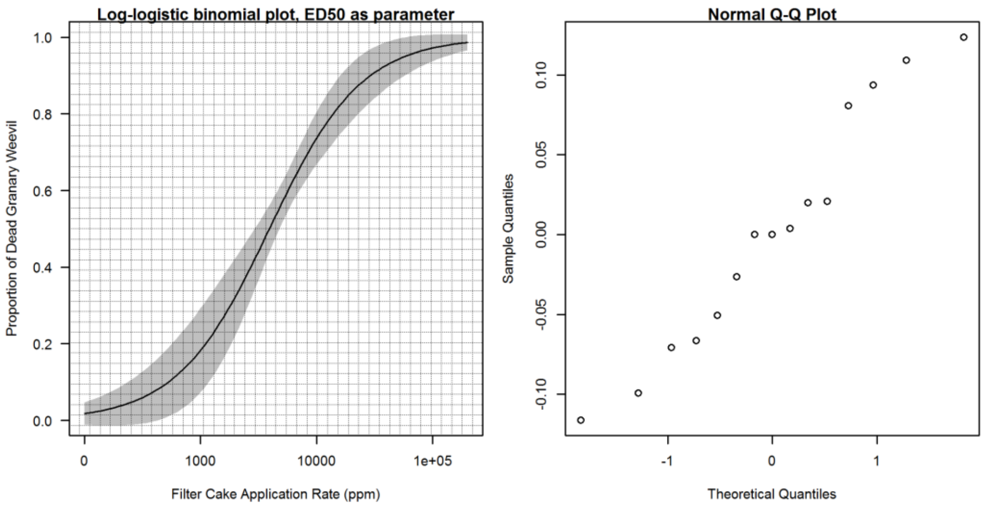

Figure 3. Log-logistic binomial regression of mortality rate of adult insects of granary weevil (*Sitophilus granarius*) exposed to different application rates of filter cake for three days. Slope (b) =-1.096±0.197 (t-value =-5.5691, p<0.001); Residual Standard Error =3.91; ED50 (e) =3917.8±409.7(t-value=9.562, p<0.001, 95%CL=3032.7-4803.0); ED95 =57473.5±25364.0 (95%CL = 2677.8-112269.3)

Conclusion and Recommendations

The present study revealed that filter cake is effective against *S. granarius* and *R. dominica* adults. Filter cake adheres more to the cuticle of *R. dominica* body than *S. granarius* and might cause impairment of water balance in a similar manner that was observed with other silica based products *(*Malia *et al.*, 2016). Reducing filter cake concentration up to 5000 ppm (0.5%) did not show any significant reduction in effectiveness after 14 d in both species. However, further studies should be undertaken to understand effects of grain moisture on efficacy of filter cake. Filter cake has the potential for control of *S. granarius* and *R. dominica* in stored wheat.

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