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Relative Susceptibility of Four Coleopteran Stored-product Insects to Diatomaceous Earth SilicoSec®

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Abstract: Laboratory bioassays were conducted to evaluate the insecticidal effect of diatomaceous earth (DE), SilicoSec against *Callosobruchus maculatus* (F.), *Rhyzopertha dominica* (F.), *Sitophilus zeamais* (Motschulsky), and *Tribolium castaneum* (Herbst) in cowpea, maize and wheat, at 25-32 °C and 54-68% relative humidity (r.h.). SilicoSec was applied at rates: 0 (untreated control) 250, 500, 750 and 1,000 mg/kg of commodity. Adult mortality was measured after 3 to14 days of exposure. Progeny production was assessed after 40 or 56 days. The tested species varied in sensitivity to SilicoSec, with *S. zeamais* being most susceptible, but no significant differences in mortality levels observed between *R. dominica, C. maculatus* or *T. castaneum* after three days of exposure. After 5 days of exposure, all *C. maculates* adults died on cowpea treated at 1,000 mg/kg. Similarly, after 14 days of exposure all adults of the other species died on grains treated at 1,000 mg/kg, and were ranked in decreasing order of susceptibility *S. zeamais* > *T. castaneum* > *R. dominica.* SilicoSec treatments suppressed progeny production (30 to 100%) in all the tested species. The implications of these findings to DE-based control strategy are discussed.

Key words: Diatomaceous earth, insect species, susceptibility, control strategy.

1. Introduction

The use of fumigants and conventional neurotoxic insecticides as grain protectants are unsuccessful in controlling store product pests because of environmental problems such as pollution and mammalian toxicity [1]. Resistance of pests to residual insecticides and the demand for residue-free food have led researchers to evaluation of new-reduced risk insecticides to control stored product pest [2, 3].

One of the most well-studied and most promising alternatives to traditional neurotoxic grain protectants is the use of DE [4-8]. Diatomaceous earths are naturally occurring siliceous sedimentary mineral compound formed from the fossils of tiny phytoplanktons (diatoms) which absorb the epicuticular lipids of the insect cuticle, causing death through desiccation [9]. They are known to protect commodities against stored-product pests in two ways. First, DE particles are picked up by the insect as they walk through the commodity. The body of adult stored-product insects is made up of an exoskeleton covered with waterproofing waxes and lipids. The wax layer on the insect's epicuticle is damaged and insects lose water through the cuticle [10, 11]. In addition, another mode of action of DE is its ability to repel insects [12].

While several DE formulations have been evaluated and shown to be effective, chiefly as grain protectants, and many of them are now commercially available in many parts of the world [13], many researchers underline the fact that DE efficacy can be affected by several biotic or abiotic factors e.g. temperature [2, 14-16], moisture content or relative humidity [15, 17-19], and type of grain commodity [4, 20, 21]. Laboratory experiments have also revealed significant effect of insect species [15], insect strain [22, 23],

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stage [19, 24]. Apart from the importance of the strain used, other authors underlined the importance of the age of the test insect [25].

Previous studies document that *R. dominica* and *T. castaneum* were among the most tolerant species to DE. *Sitophilus* spp. were reported to be moderately tolerant to DE. However, there was few published information regarding the tolerance of *C. maculatus* in relation to other well studied species. This study was therefore undertaken to evaluate the relative response of four coleopteran stored product species to SilicoSec and determine the relative susceptibility of *C. maculatus*.

2. Materials and Methods

2.1 Insects

The insects tested were *C. maculatus, R. dominica, S. zeamais* and *T. castaneum.* These were collected from gain stores in Maiduguri and subsequently cultured in the laboratory on cowpea, wheat, maize and wheat flour, respectively, for several generations in the laboratory.

2.2 Grains

Three grain types were used in the experiments: cowpea, maize and wheat. The grains were cleaned and disinfested in an electric oven set at 55 °C for three days, then allowed to equilibrate under ambient conditions for 10 days prior to the experiments.

2.3 DE Formulation

The DE formulation used was SilicoSec (Biofa GmbH, Munsingen, Germany). SilicoSec is a relatively new DE formulation of freshwater origin containing 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3, and 1% Na_2O with average particle size between 8 and 12 µM [23]. The SilicoSec sample was obtained from Diatom Research and Consulting Inc., Canada. The DE was stored in the laboratory at ambient conditions in airtight polyethylene sachet until commencement of experiments.

2.4 Experimental Procedure

Four concentrations of DE were used (250, 500, 750 and 1,000 mg/kg). Glass bottles (250 mL capacity) were filled with 50 g of cowpea, wheat or maize. The appropriate amount was weight and placed into each bobble. Untreated grains served as control. Bottles were sealed and shaken manually for 3 min to distribute DE in whole grain mass. Twenty 1-48 h old adults *C. maculates* were placed in each bottle containing cowpea, *S. zeamais*—on maize, while *R. dominica* and *T. castaneum*—on wheat. These three species were aged 7-21 days. Each species was treated separately and each treatment was repeated three times. The bottles were kept under ambient laboratory conditions. Total numbers of living and dead adults were recorded after 3 and 5 days for *C. maculatus*, and after 3, 7 and 14 days in the cases of *R. dominica, S. zeamais* and *T. castaneum*. This was done very carefully to avoid loss of eggs and larvae that are external to the grain kernels. The temperature and relative humidity were measured by using Omson's hygrometer.

After the 5 and 14 d mortality count all adults (dead and alive) were removed from the bottles, and the bottles were left at the same conditions for an additional period of 35 days in the case of *C. maculatus* and 42 days in the cases of the three other species. The number of emerged adults of each species was then counted.

2.5 Data Analysis

Where the mortality exceeded 5%, counts were corrected using Abbott's [26] formula. The data on mortality and progeny were arcsine and square root transformed, respectively prior to analysis. All data were analyzed using the GLM Procedure of Statistix 8.0, with insect mortality and number of progeny as the response variables and DE dose rate and exposure interval as the main effects. In all cases, means were separated by using the Tukey-Kramer (HSD) test, at $P \leq 0.05$.

3. Results

The mean adult mortalities of *C. maculatus, S. zeamais, R. dominica* and *T. castaneum* after three days of exposure (Fig. 1) illustrates their relative susceptibility to SilicoSec. Within this exposure period > 50% of all exposed adults died. *S. zeamais* appeared to be the most susceptible species, while differences in mortality levels among the three other species were not significant.

Adult mortality among in the beetle species *S. zeamais, R. dominica* and *T. castaneum* was significantly affected by both DE and exposure period (Fig. 2). In all cases mortality was significantly lower in the untreated control than in treated grains in which case it increased with increase in dose rate and exposure period. After the three days exposure mortality level of *S. zeamais* was higher than of the other species, exceeding 95% on maize treated at 1,000 mg/kg of SilicoSec. With *R. dominica* and *T. castaneum* exposed to the same DE dose rate on wheat, mortality levels did not exceed 75% (Fig. 2a). After seven days of exposure to DE treated commodities mortality in all the three species increased exceeded 50% when SilicoSec was used at 250 mg/kg. Within this exposure period wide differences in mortality levels were noted between species, especially when the DE was applied at 500 and 750 mg/kg. However, at 1,000 mg/kg such differences were not noted between *R. dominica* and *T. castaneum* (Fig. 2b).

Increase in exposure period further increased mortality levels. Thus 14 days of exposure to grains treated with the lowest dose rate (250 mg/kg) of SilicoSec resulted in death of > 80% of exposed adults of the three insect species. In the case of *S. zeamais* all adults died on maize treated at ≥ 500 mg/kg of SilicoSec. In a similar manner, complete mortality of *T. castaneum* and *R. dominica* adults was recorded at ≥ 750 mg/kg and 1,000 mg/kg, respectively (Fig. 2c). The same trend as observed for the preceding species was noted for *C. maculatus*. That more beetle died with increase in DE dose rate and exposure period. After 3 days of exposure adult mortality increased from 29.9 ± 3.35 at 250 mg/kg to 70.8% at 1,000 mg/kg, while after five days of exposure, the corresponding values were 63.2% and 100%, respectively (Fig. 3).

However, when the susceptibility of *S. zeamais, R. dominica* and *T. castaneum* were compared after 14 days of exposure, a different outcome was obtained. In this case, *R. dominica* was the most tolerant, followed by *T. castaneum* and *S. oryzae* remained the most susceptible (Fig. 4).

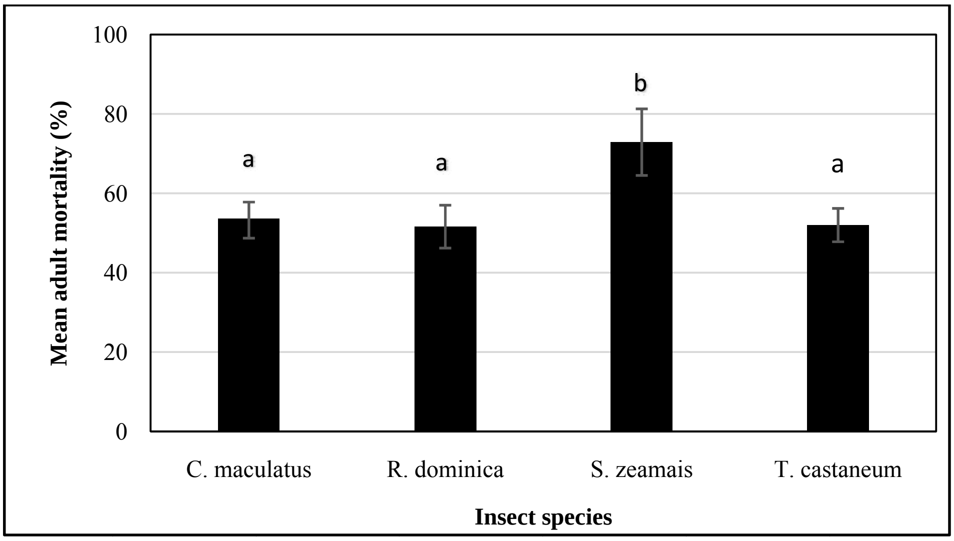
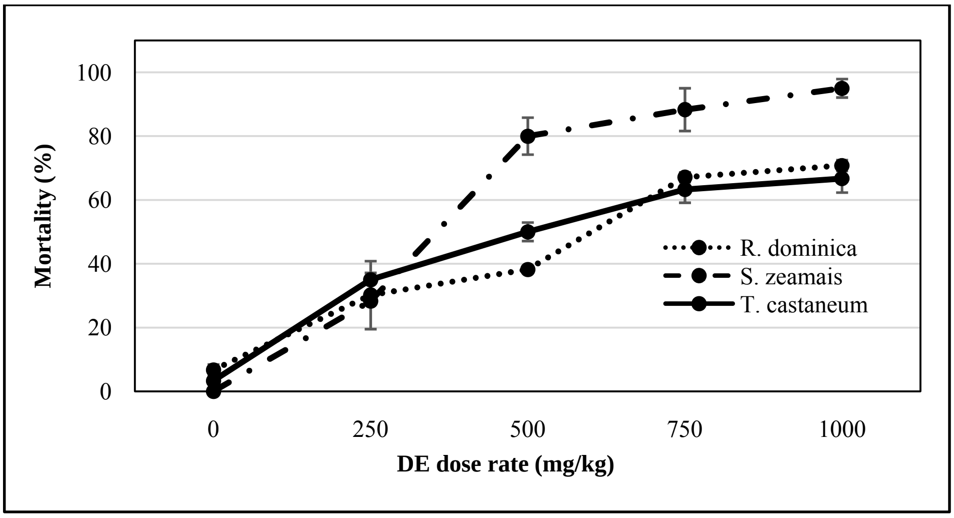
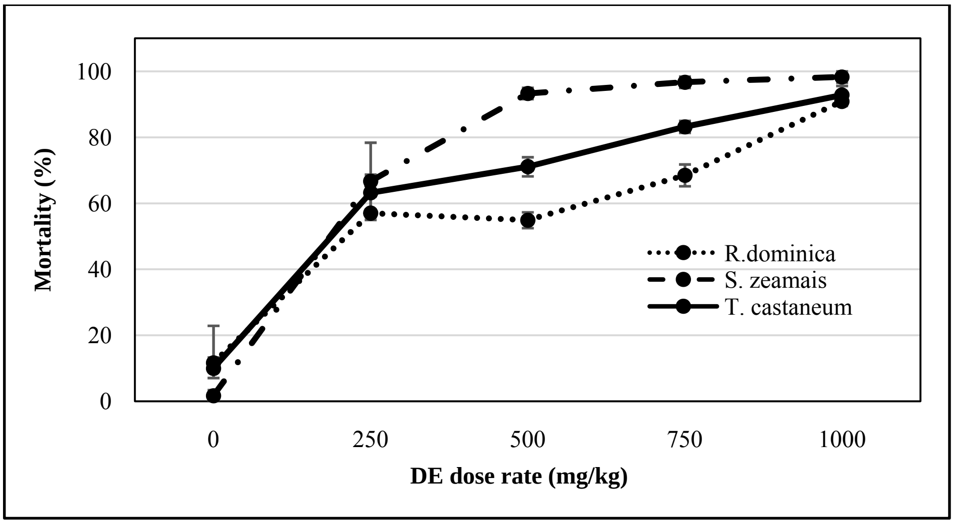


Fig. 1 Mean adult mortality of four insect species after three days of exposure to SilicoSec treated grains; means accompanied by different letters are significantly different (Tukey-Kramer HSD test, *P* > 0.05).



(a)



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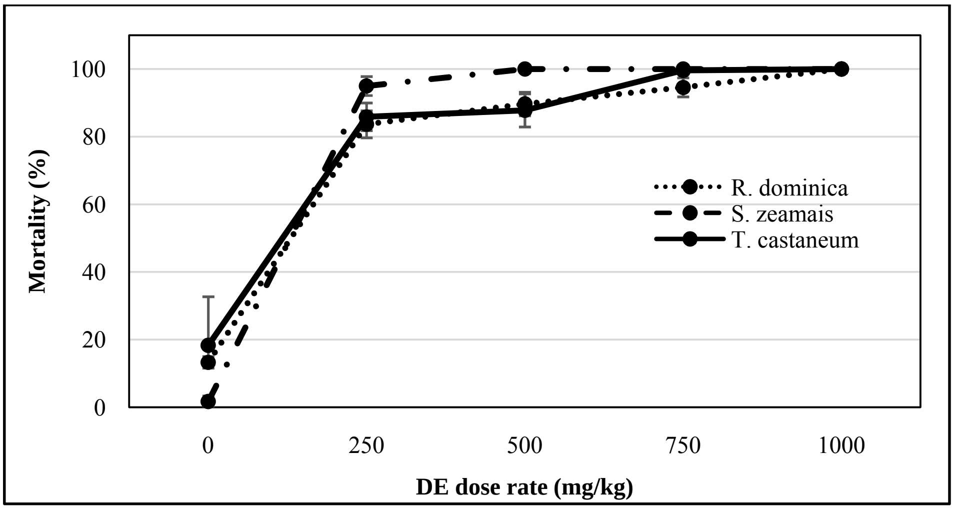

(c)

Fig. 2 Percentage mortality of *R. dominica, S. zeamais* and *T. castaneum* adults after 3 (A), 7 (B) and 14 (C) days of exposure to different dose rates of SilicoSec.

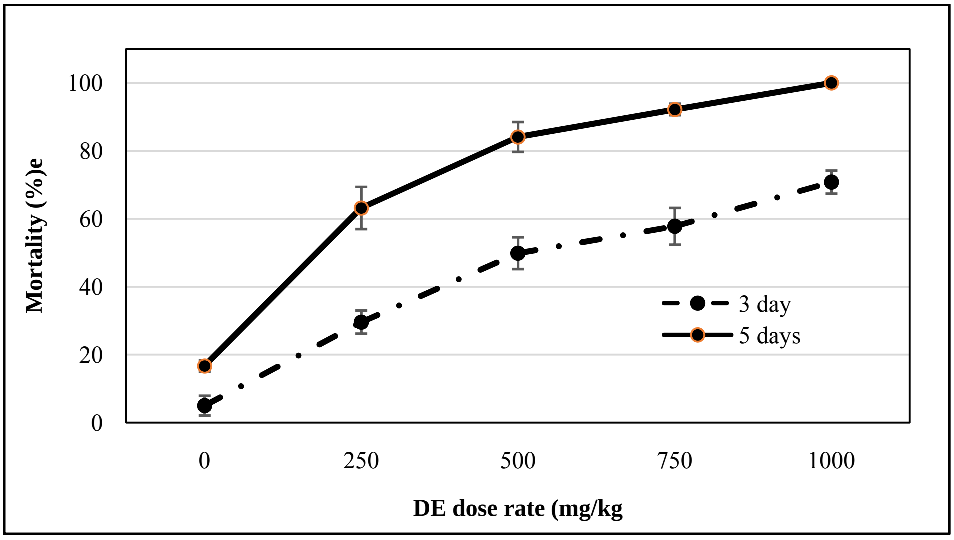

Fig. 3 Mortality of *C. maculatus* adults after three and five days of exposure to different dose rates of SilicoSec.

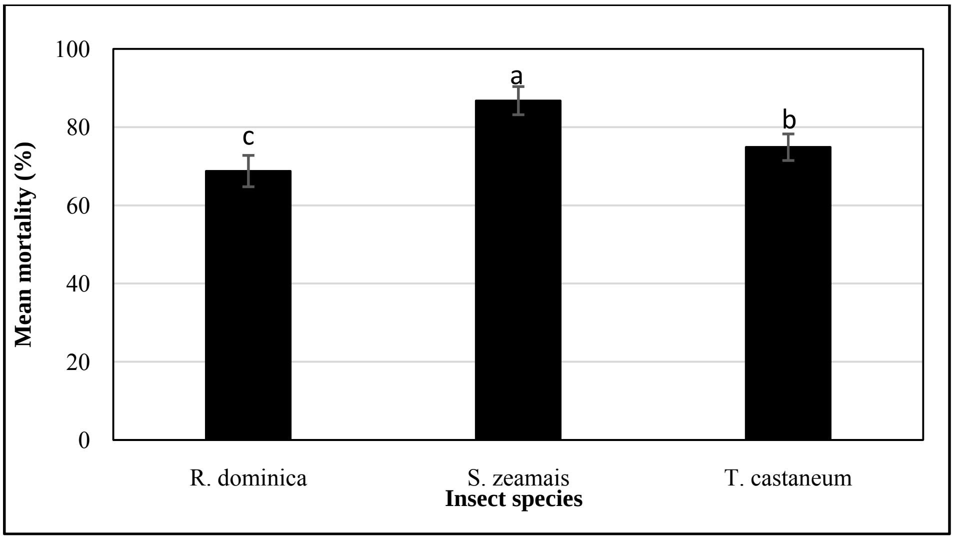

Fig. 4 Mean cumulative mortality of three insect species after 14 days of exposure to DE SilicoSec; means accompanied by different letters are significantly different (Tukey-Kramer HSD test, *P* > 0.05).

Progeny production in all the four species tested was significantly affected by DE treatment. Very high number of *C. maculatus* adults emerged in the untreated cowpea than in treated ones. This was followed by *S. zeamais* and *R. dominica* and the least by *T. castaneum* (Table 1 and 2). With all the tested species, however, the number of progeny in the untreated control was significantly higher than in the treated grains. Generally, progeny production in the treated grains was very low and did not exceed 8 individuals in grains treated at 250 g/kg of SilicoSec. With *T. castaneum* no adult progeny emerged in wheat treated at 750 mg/kg or more. Moreover, substantial proportions of emerged adults of *S. zeamais*, *R. dominica* and *T. castaneum* were dead at the time of progeny counts. Progeny suppression relative to the untreated increased with increase in DE dose rate. This was particularly very high on grains treated with 750 mg/kg of SilicoSec or more. With the exception of *S. zeamais* on maize treated at 250 mg/kg more than 50% of potential F1 progeny were suppressed (Tables 1 and 2).

4. Discussion

This study demonstrated that the DE formulation, SilicoSec was effective against *C. maculatus,* *S.zeamais*, *R. dominica* and *T. castaneum* on stored cowpea, maize and wheat. The initial 3 days' adult mortality showed that *S. zeamais* was the most susceptible species, while the responses of the three other insect species to SilicoSec were similar. However, after 14 days of exposure, *R. dominica* was the most tolerant, followed by *T. castaneum* and *S. oryzae* remained the most susceptible. It is a known fact that stored product beetles vary widely in their susceptibility to DE [27]. *Sitophilus* spp. are usually ranked midway in susceptibility between the small mobile insects such as *Cryptolestes* spp. or *Oryzaephilus* spp. and the lesser grain borer *R. dominica* and *Tribolium* spp. which are more tolerant to insect dusts [11, 27, 28]. Our results with regards to *S. zeamais* are well in agreement these reports. Moreover, Korunic and Fields [29] found that *S. zeamais* was the most susceptible to DE among the *Sitophilus* spp.

The most interesting finding in this study is the relative susceptibility of *C. maculatus,* which was not widely reported in previous literatures. Based on the 3

Table 1 Effect of different doses of SilicoSec on progeny production of three stored product insect species.

Insect species	DE dose rate (mg/kg)	No. of progeny (mean ± SE)	Percentage of dead progeny	Progeny suppression (%)
R. dominica	0	43.7 ± 4.7a	8.6 ± 1.2b	-
	250	21.3 ± 10.4ab	64.5 ± 12.3ab	51.3
	500	3.0 ± 1.7b	55.6 ± 19.4ab	93.2
	750	3.3 ± 1.9b	95.2 ± 4.8a	92.5
	1,000	4.0 ± 1.5b	100 ± 0.0a	90.8
	F	11.6	6.5	
	P	0.0009	0.0076	
S. zeamais	0	43.3 ± 4.8a	10.7 ± 2.2c	-
	250	30.0 ± 10.0ab	43.1 ± 5.3b	30.7
	500	7.7 ± 2.4bc	83.5 ± 8.4a	82.2
	750	4.0 ± 2.0c	95.8 ± 4.2a	90.8
	1,000	5.7 ± 2.6c	100 ± 0.0a	86.8
	F	12.3	61.3	
	P	0.0007	< 0.0001	
T. castaneum	0	24.0 ± 7.0a	10.7 ± 1.7b	-
	250	8.7 ± 2.2a	95.2 ± 4.8a	63.8
	500	1.3 ± 0.7b	83.3 ± 16.7a	95.8
	750	0.0 ± 0.0b	100 ± 0.0a	100
	1,000	0.0 ± 0.0b	100 ± 0.0a	100
	F	25.5	24.0	
	P	< 0.0001	< 0.0001	

Means within a species and a column followed by the same letter are not significantly different (Tukey-Kramer HSD, $P > 0.05$).

Table 2 Effect of different doses of SilicoSec on progeny production of *C. maculates*.

DE dose rate (mg/kg)	No. of progeny (mean ± SE)	Progeny suppression (%)
0	152.0 ± 12.5a	-
250	13.30.9b	91.3
500	6.0 ± 2.1bc	96.1
750	2.7 + 0.9cd	98.2
1,000	0.7 ± 0.7d	99.5

Means within a column followed by the same letter are not significantly different (Tukey-Kramer HSD test, $P > 0.05$).

day mortality *C. maculatus,* appeared to share the same level of tolerance to DE, in particular to SilicoSec with *R. dominica* and *T. castaneum.* Tolerance of *C. maculatus*to DE may be attributed to the presence of hairs on the cuticle. As hairy insects tend to be less susceptible than insects with fewer hairs because hairs prevent dust particles from coming in contact with the cuticle [11]. In addition other factors such as size, rate of feeding (which does not apply to *C. maculatus,* as the adults do not feed) cuticular waxes, adhesion of DE to cuticle, absorbance of water from the hind gut or tolerance to low internal water are involved in tolerance to DE. However the exact mechanism of tolerance in *C. maculatus* is not yet established.

Our results showed that *R. dominica* was more tolerant than *T. castaneum* after 14 days of exposure. Results from previous studies comparing these species were somewhat inconsistent. For example, Kostyukovsky *et al.* [12] showed that *T. castaneum* was more tolerant than *R. dominica* to Detia Degesch Diatomaceous Earth (DDDE). Similarly, Baldassari *et al.* [30] reported higher mortality of *R. dominica* compared to *T. castaneum* on wheat treated with Protector®. However, Fields and Korunic [15] showed reported same level of tolerance to DEs by these two species. Yet, Kabir *et al.* [31] worked with same strains of insects used in the present study and found that more adults of *T. castaneum* than that of *R. dominica* died following exposure to wheat and maize grains treated with raw DE.

It is not known whether the results obtained in this study would hold true under different conditions. Perhaps different results may be obtained if the DE were to be tested on grains other than wheat or maize because DE efficacy is known to vary among grain types [4, 5, 16, 32]. The susceptibility of a given species to DE might be influenced by the insect strain [33]. For instance, Rigaux *et al.* [22] and Vayias *et al.* [23] found considerable variations in susceptibility levels *T. castaneum* and *T. confusum* populations respectively, obtained from different locations. This may explain the lower tolerance of *T. castaneum* compared to *R. dominica* to SilicoSec noted in the present study. Kavallieratos *et al.* [33] noted that knowledge of the exact species to be controlled may help the correct design of a DE-based control strategy. Our results suggest that in addition to species, information on the susceptibility of the strain concerned may help improve a given strategy.

SilicoSec treatment reduced progeny production in all the four species tested. With the exception of *T. castaneum* complete progeny suppression was not achieved. Progeny suppression was attributed to the fact that DE limits insects mating activity by physically hindering movement [34]. Few adult progeny emerged because the females might have laid eggs before being killed by the DE. Mewis and Reichmuth [35] working with *S. granarius* exposed to DE treated wheat similar observations. They reported that the adults died within a few days, which was time enough to produce progeny. In this regard the speed of parental mortality is important, so that insect die fast with very few eggs laid intreated commodity. Complete suppression of *T. castaneum* progeny in wheat treated SilicoSec at 750 or 1,000 mg/kg may be explained by the fact this species is an external feeder, with all life stages found external to the grain kernels. Thus, the larvae were exposed to DE particles. Kostyukovsky *et al.* [12] reported that larvae of *T. castaneum* are very susceptible to DE. *Tribolium castaneum* is a secondary pest which thrives well on processed cereals or damaged grains. Also the larvae of *T. castaneum* need grain dust and damaged kernels to feed on, but these were substantially removed in the process of clearing. This partly explains the lower number of *T. castaneum* adults in the untreated control relative to other species tested. It is also shown that adult stage of insects are more tolerant to DE than larval stages [24, 36]. Although the immature stages of *C. maculatus*, *S. zeamais*and to some extent of *R. dominica* were not directly affected by DE particles, high mortality were recorded as they emerged as adults as evidenced by the increasing percentages of dead progeny in treated grains. This also supports early reports that presence of DE in commodity gradually eliminates population of insects [28, 33].

In conclusion, the present study demonstrated that SilicoSec applied at 1,000 mg/kg could control *C. maculatus, S. zeamais, R. dominica* and *T. castaneum* in stored cowpea, maize and wheat. Initial mortality showed that *C. maculatus* adults exhibited same level of tolerance to SilicoSec as *R. dominica* and *T. castaneum,* whereas *S. zeamais* was the most susceptible species. Based on the results of the present study and available literature, knowledge of species composition and strain sensitivity to DE are important in devising a DE-based integrated management of stored product insects.

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