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Diatomaceous earth outperformed horsetail powder in laboratory assays on the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrychidae)

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Abstract

This study aimed at comparing the insecticidal and repellency activity of a commercial diatomaceous earth (DE) formulation (Silicosec®, Biofa AG) and a horsetail powder - *Equisetum telmateia* (Ehrh.) (Equisetales: Equisetaceae) against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). These substances were mixed with soft wheat grains at concentrations of 1000 and 2000 ppm and tested on unsexed adult beetles of mixed ages. Insecticidal assays were conducted at three exposure intervals (2, 7 and 14 days), after which the wheat samples were destructively sifted and mortality was recorded. The highest mortality was achieved with the DE, and the percentage of dead beetle significantly increased with exposure interval and concentrations. On the other hand, treatments with the horsetail powder did not significantly affect beetle mortality, regardless of the dose and exposure interval. Repellency tests, which were run according to Mohan and Fields's method, showed a higher activity of both doses of the DE in comparison to the horsetail powder. The difference between the two application doses of the DE was statistically supported only at 24 h of exposure, whereas no differences were detected between the two doses at 48 and 72 h. Based on our findings, the horsetail powder seems not to be a reliable alternative to DEs for the control of *R. dominica*. Considering both efficacy and cost-effectiveness, the 1000 ppm DE dose could represent the best compromise for the management of the lesser grain borer in commercial applications.

Key Words

Equisetum telmateia, integrated pest management, repellency, stored grain pest, wheat

Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), feeds on a wide variety of substrates including grains in the family of Poaceae and Fabaceae, packaging materials and stored pharmaceuticals (Potter 1935). It is a primary pest of grains since it breeds in corn, wheat, rice and food containing starch. Dry cereal grains are the most suitable substrates for *R. dominica*, with wheat often providing the best conditions for survival and reproduction (Edde 2012). Larvae and adults of the lesser grain borer are internal feeders that consume the kernel endosperm. Feeding and tunnelling activities result in the accumulation of frass composed of exuviae, faecal material, and other feeding by-products. Moreover, infestations may increase the temperature and relative humidity of the substrate promoting the growth of secondary pests.

Infestations by *R. dominica* in stored grains are commonly managed using chemical insecticides with residual activity and fumigants. However, strains of the lesser grain borer resistant to major chemicals for grain protection—including organophosphate insecticides and phosphine—have been reported (Boyer et al. 2012).

Moreover, the increasing request for food without chemical residues and the general awareness of environmental pollution have led pest managers to turn to natural insecticides such as inert dusts, i.e. diatomaceous earths (DEs), or alternative substances like plant powders (Rajapakse 2006; Korunić 2013; Korunić et al. 2016).

DEs are the fossilized shells of diatoms, unicellular aquatic algae (Korunić 1998). The main constituents of shells are amorphous hydrated silica and other mineral elements, like aluminium, iron oxides, magnesium, calcium hydroxide and sodium (Baldassari and Martini 2014). The insecticidal activity of DEs is based on their mechanical and physical features; in fact, DE particles adhere to the insect body and absorb lipids from the epicuticular waxy layer, so that insects lose water and dies because of dehydration (Baldassari et al. 2008). In addition, DE particles may abrade the digestive tract causing internal disorders and they can also plug the tracheal system causing insect suffocation (Korunić 2013; Korunić et al. 2016).

The use of DEs for protection of grains has many advantages since they are stable on grains, persistent depending on relative humidity and do not leave residues toxic to mammals (Nikpay 2006; Korunić 2013; Korunić et al. 2016). DEs also present some disadvantages such as the negative impact on physical and mechanical properties of bulk grain. Another limitation to DE use is the moisture content of the grains and the relative humidity, which can be exploited by insects to replace the lost water. For these reasons, DEs are mostly effective against insects when the commodity is dry (Nikpay 2006; Korunić 2013; Korunić 2016; Korunić et al. 2016).

The use of plant materials to protect stored food and commodities against insect pests has been practiced since ancient human civilizations (Isman 2006), and dry powders of many plant species have been tested in laboratory conditions to evaluate the efficacy in controlling infestations in stored food. Plant-based materials typically exhibit a combination of repellent and toxic activities against insects (Rajapakse 2006).

Horsetails, *Equisetum* spp. (Equisetales: Equisetaceae), have been traditionally used as a natural insect deterrent, with dried plant material applied to repel or suppress insect pests due to high silica content. Horsetails accumulate large amounts of silica throughout their tissues, especially in the epidermis and cell walls, resulting in a continuous silica layer covering all aerial organs (Sapei et al. 2007; Currie and Perry 2009; Husby 2013; Guerriero et al. 2018). For example, the concentration of silica in *Equisetum hyemale* L. can reach up to 16% dry weight (Sapei et al. 2007); in *Equisetum telmateia* (Ehrh.) the absolute content of pure silica is about 23% (Neumann et al. 2010). Silica has several important functional and mechanical proprieties in horsetails providing resistance to fungal infections, herbivores and nutrient shortages (Guerriero et al. 2018). Moreover, silica could also be exploited by the plant as a reservoir of minerals (Sapei et al. 2007; Husby 2013).

Field horsetail (*Equisetum arvense* L.) powders have shown moderate insecticidal activity under laboratory conditions against stored-product insect pests such as the bean weevils *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) (Oliveira et al. 2007; Trdan and Bohinc 2011; Bohinc et al. 2013). In the present study, the insecticidal activity and contact repellency of *E. telmateia* powder were evaluated for the first time against *R. dominica* on soft wheat grains and compared with the commercially available DE SilicoSec®. Given that horsetails are widely distributed worldwide (Husby 2013), their powders represent promising and locally available options for grain protection, particularly in rural communities of low-income countries. It is therefore important to conduct a comprehensive evaluation of their efficacy against major global stored-grain pests, such as *R. dominica.*

Materials and methods

Insects rearing

Adults of *R. dominica* used in the experiments were obtained from a laboratory colony derived from crossbred strains originating from Department of Agricultural and Food Sciences (DISTAL) of the University of Bologna and the Università Cattolica del Sacro Cuore (Piacenza, Italy). The rearing had been maintained under laboratory conditions for more than 100 generations, without replenishment from wild individuals.

Insects were reared inside two-liter-Bormioli jars closed with a cap which had a 4.5 cm hole and a fine-mesh brass net to prevent escape and to allow air circulation. The jars were kept in a climatic chamber in the dark at 26 ± 1 °C. Insects were fed *ad libitum* with soft wheat.

Horsetail powder and diatomaceous earth

The horsetail powder was prepared from *E. telmateia* plants collected in uncultivated land in Bertinoro (Forlì-Cesena, Italy). Species identification was carried out on fertile stalks, whereas the sterile stems were used for the assays. Horsetail plants were gathered to form bundles and sun-dried for 15 days. At first, they were ground with a food processor of 700 W; after that, the material was milled into powder using the IKA M20 grinding machine. Horsetail powder was oven dried at 40 °C for 24 h and then sifted using two sieves of 40 and 140 mesh (ASTM, USA), in order to obtain particles less than 105 µm in size. The horsetail powder was immediately put into plastic containers.

The DE used in this investigation was the commercial product, SilicoSec® (Biofa AG, Münsingen, Germany), containing 84.47% of SiO_2 and with a declared median particle size between 8 and 12 µm.

The application rates tested for both horsetail powder and DE were 1000 and 2000 ppm (i.e. 1 and 2 g/kg soft wheat).

Experimental substrate

The soft wheat cv. Palesio, purchased from the Consorzio Agrario (Cesena, Forlì-Cesena, Italy), was used in both the insecticidal bioassays and the contact repellency tests. Before use, grains were kept at -18 °C for 48 h for disinfestation. Wheat was then mechanically screened in order to remove impurities like husk, broken seeds or seeds of other species. This procedure was carried out at the Seed Research and Testing Laboratories (DISTAL, Bologna, Italy) using the winnower (model LA-LS) and then the densimetric table (model LA-K Westrup ApS, Slagelse, Denmark).

Insecticidal assays

The experimental unit consisted of a 1-liter glass jar containing 500 g of soft wheat, to which each treatment was added and then carefully mixed by hand. Fifteen *R. dominica* unsexed adults of mixed age were introduced in each jar. The same procedure was followed for each treatment, dose rate and exposure interval. An additional jar containing untreated wheat was used as the control for each trial set.

All experiments were performed in a thermostatic chamber set at 26 ± 1 °C and 70 ± 5% RH. These parameters were kept constant by putting a tray with a saturated water solution of sodium chloride in the incubator. To record and check the environmental parameters within the chamber a thermohydrometer (RHT10, Extech Instruments, Nashua, NH, USA) was used. The assays were performed in the dark at three exposure intervals of 2, 7 and 14 days. For each trial (treatment, dose and exposure interval) 3 treated samples and 3 untreated controls were set up. At each exposure interval, the jars were destructively sifted, live and dead insects were collected, counted and then all discarded.

Data were analyzed using a generalized linear model (GLM) with binomial error distribution and probit link function. The dependent variable was the proportion of dead insects out of the total insects tested. Treatments (SilicoSec® at 1000 ppm, SilicoSec® at 2000 ppm, horsetail powder at 1000 ppm, horsetail powder at 2000 ppm and control) were used as a factor. Also, the exposure intervals (2, 7 and 14 days) were considered as a factor because of the destructive sampling. The interaction "treatment*exposure interval" was tested as well. If a significant factor effect was detected by the GLM, Bonferroni method ($p < 0.05$) was used for multiple comparisons among factor levels.

Contact repellency tests

Assays were conducted according to Mohan and Fields (2002) using cups made of plastic net: 2 × 2-mm mesh shaped into a cylinder of 8 cm diameter × 11 cm height and closed at the bottom. This cylinder was partially inserted into a clear plastic cup with an upper diameter of 9 cm and a height of 12.5 cm. For testing compounds, the experimental unit was placed in a polyethylene cylinder closed at both ends with a Petri dish (Fig. 1). In this way, the plastic cup collected the beetles escaped through the bottom of the perforated container and the Petri dish below the glass collected the insects escaped through the sides.

Assays were performed by carefully mixing soft wheat with each of the previously described experimental substances. The same doses of SilicoSec® and horsetail powder were used as well. From each treated or untreated grain lot, samples of 200 g each were obtained and poured into the mesh cylinder. Then 20 *R. dominica* unsexed adults of mixed age were introduced into each mesh cylinder. Controls without treatment were maintained to record natural movement. The assays were conducted at room conditions (24 ± 3 °C and 60 ± 10% RH). For each treatment and the control, 5 replicates were set up. The number of escaped adults was counted after 24, 48 and 72 h for each experimental unit without dismantling it.

Figure 1. Experimental arenas used to evaluate contact repellency.

Because of limitations on laboratory equipment, only three experimental arenas could be run simultaneously. Each set included one replicate for SilicoSec®, one replicate for horsetail powder either at 1000 or 2000 ppm and one replicate for untreated control. To compare SilicoSec® and horsetail powder, which were tested in different sets of experimental units, percentages of escaped insects were corrected with the Schneider-Orelli formula to account for escaping rates in the control groups. The corrected percentages, which met the assumptions for parametric models, were analyzed with mixed design ANOVA considering checking intervals (24, 48 and 72 h) as within-subject factor and treatments (SilicoSec® at 1000 ppm, SilicoSec® at 2000 ppm, horsetail powder at 1000 ppm and horsetail powder at 2000 ppm) as between-subject factor. Owing the significant effect of the interaction "checking intervals*treatments", percentages of escaped adults were analyzed separately in each checking interval by means of one-way ANOVA.

Multiple comparisons of treatments were carried out with the Ryan-Einot-Gabriel-Welsch-Q test ($p < 0.05$).

All statistical analyses were performed with the software package SPSS Statistics ver. 23 (IBM).

Results

Insecticidal assay

The mortality caused by different treatments showed the same pattern in each exposure period and this is reflected by the lack of significance of the interaction between the two factors (Table 1). For all exposure periods, the highest percentages of mortality were recorded in *R. dominica* individuals exposed to both concentrations of SilicoSec® (Fig. 2). A concentration-dependent effect was also observed as mortality in the jars treated with 2000 ppm SilicoSec® (85.93 ± 3.76%, mean among exposure intervals ± SE) was higher than in jars with 1000 ppm (69.63 ± 6.39%, mean among exposure intervals ± SE). On the other hand, no significant differences were detected between horsetail powder, at both high (5.19 ± 2.16%, mean among exposure intervals ± SE) and low concentrations (2.22 ± 1.11%, mean among exposure intervals ± SE), and the control (2.96 ± 1.61%, mean among exposure intervals ± SE).

Table 1. Results of the generalized linear model run on mortality data.

Factor	Wald χ^2	df	*p*
Treatment	386.67	4	< 0.001
Exposure period	141.69	2	< 0.001
Treatment* Exposure period	8.61	6	0.20

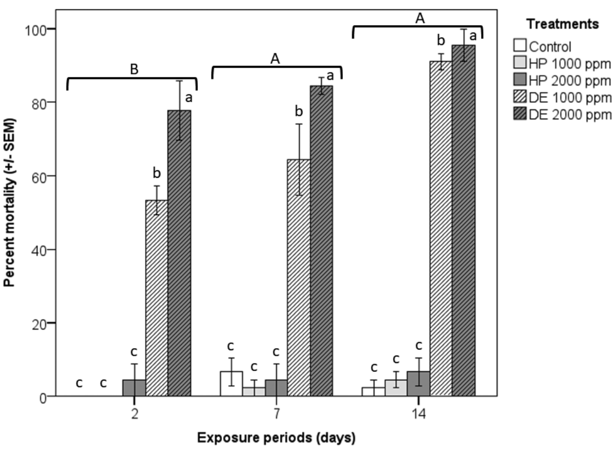

Figure 2. Mean (±SE) percent mortality of *Rhyzopertha dominica* adults in jars treated with diatomaceous earth (DE) and horsetail powder (HP) after 2, 7 and 14 days of exposure. Lower case letters indicate significant differences between treatments, whereas upper case letters above the brackets indicate differences between exposure periods (GLM followed by multiple comparison with Bonferroni methods, $p < 0.05$).

At increasing exposure periods, the mortality significantly increased as well. At 2-day exposure, the overall mortality was significantly lower than at the two longer exposure periods, but no significant differences could be detected between 7- and 14-day exposures.

Contact repellency tests

The results of this assay were overall consistent with the experiment testing the insecticidal activity. The corrected percentages of escaped insects, which were used as an index of contact repellency by the treatments, increased as a function of time (Table 2), with the exception of horsetail at 2000 ppm (Fig. 2). Most beetles left the jars treated with both concentrations of SilicoSec®, whereas the repellent activity of the horsetail powder was negligible, even after 72 h of exposure. Significant differences between SilicoSec® at 1000 and 2000 ppm were detected only at 24 h exposure when the highest concentrations forced more beetles to leave the grains. At 48 and 72 h exposures the percentage of escaped insects tended to level off and no significant differences between 1000 and 2000 ppm could be found (Fig. 3).

Table 2. Mixed design ANOVA results.

Factor	F	df	*p*
Within-Subject Effects			
Checking interval	15.80	1.30, 20.85[1]	< 0.001
Checking interval * Treatment	4.45	3.91, 20.85[1]	0.01
Between-Subject Effects			
Treatment	25.23	3, 16	< 0.001

[1]: degrees of freedom corrected with Greenhouse-Geisser method to accomplish for the lack of sphericity.

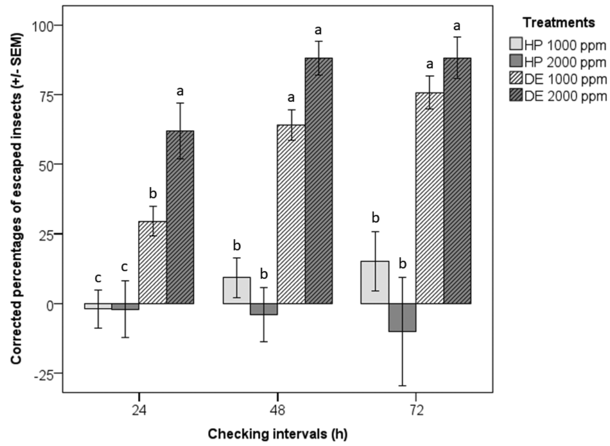

Figure 3. Mean (±SE) corrected percentages of *Rhyzopertha dominica* adults escaped from the experimental units treated with diatomaceous earth (DE) and horsetail powder (HP) after 24, 48 and 72 h. Letters indicate significant differences between treatments within each checking interval (one-way ANOVA within each checking interval followed by Ryan-Einot-Gabriel-Welsch-Q multiple comparison procedure, $p < 0.05$).

Discussion

This study confirmed the insecticidal activity of the commercial DE against the adults of *R. dominica* even after only a 2-day exposure, whereas at the experimental conditions tested the horsetail powder did not reduce beetle survival. To the best of our knowledge, no published data are available on the activity of horsetail powder on *R. dominica*. However, Bohinc et al. (2013) reported moderate insecticidal activity of the powder of *E. arvense* on adults of *A. obtectus* above 25 °C at 7-day exposures. In the same study, SilicoSec® DE was also evaluated and proved to be more effective than plant powders in controlling bean weevil populations below 30 °C. Although horsetail powder showed satisfactory activity at 30 and 35 °C, such temperature conditions are unlikely to occur under routine grain storage practices.

Previous studies have shown that certain spice powders exhibit toxicity against beetles infesting stored grains (Devi and Devi 2013; Swami et al. 2013). These studies indicate that insecticidal activity varies with plant species, doses, and the duration of insect exposure to treatments. However, the effects of spice powders on stored-grain pests are largely attributed to essential oils and other bioactive secondary metabolites (Nikolaou et al. 2021; Li et al. 2025), which are present only in very minor amounts in horsetail powders (Radulović et al. 2006; Marin et al. 2019). In contrast, the insecticidal activity of horsetail and other inert powders appears to be strongly influenced by their silica content and particle size. Amorphous SiO_2 is directly associated with a desiccant mode of action, as it disrupts the lipid layer of the insect cuticle, leading to increased water loss and eventual mortality (Vayias and Athanassiou 2004). Particle size also plays a critical role by determining the extent of contact between insects and treated surfaces (Vayias et al. 2009); smaller particles ($\approx$ 10 µm) provide a high surface-to-volume ratio, improve dust coverage on grain kernels, and increase adherence to the insect body, thereby enhancing cuticular abrasion and lipid removal (Korunić 1998). Therefore, beyond differences in substrates, target insects, and experimental procedures, the discrepancies between our findings and those of previous studies on horsetail powders may be related to differences in silica content and particle-size distribution. These factors warrant further investigation.

According to our findings, the DE dose rate is crucial for insecticidal activity, in fact, significantly higher beetle mortality was found at 2000 ppm than at 1000 ppm. This is in line with Vayias and Athanassiou (2004) and Mortazavi et al. (2020). Similar results were also reported for the commercial DE Insecto® (Natural Insecto Products Inc., Costa Mesa, CA, USA) against *R. dominica* adults (Beriş et al. 2011).

A longer exposure period of the lesser grain borer to DEs resulted in overall increased mortality for both the dose rates tested. However, the mortality improved negligibly between 7 and 14 days. Similarly, Beriş et al. (2011) detected comparable mortalities of lesser grain borer adults treated with Insecto® DE at 7- and 14-day exposures. These results were also in line with a previous study testing the efficacy of SilicoSec® and Protector® (Biogard CBC, Grassobbio, Bergamo, Italy) at 500 and 1000 ppm against *R. dominica* adults (Baldassari and Martini 2014), in which the mortality for both DEs tested at 7- and 14-day was almost the same for each dose. However, 100% mortality of beetles was not achieved even at 2000 ppm and at the longest exposure interval. This was likely due to the mode of action of DEs, which slowly kill insects by dehydration after attachment to the body (Wakil et al. 2013). Beetle movements enhance the activity of DEs by favoring contact with the insects (Vayias and Athanassiou 2004).

Both dose rates of DE also exerted contact repellency on adults of *R. dominica* at all exposure periods. At 24 h exposure, the number of escaped adults was higher for 2000 ppm than for 1000 ppm, whereas no differences were detected between the two doses at 48 and 72 h exposures. Similar outcomes were reported for the commercial product Insecto® and other DEs tested at 1000 ppm that repelled up to 86% of treated individuals of *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) (Okonkwo et al. 2018). Rather than using DEs for disinfestation, the tendency of beetles to avoid treated grains may be exploited to prevent infestations by the lesser grain borer. This could be realized by layer application of DEs at the dose of 1000 ppm, which should simultaneously avoid excessive reduction in the grade value of the grain (Korunić and Mackay 2000) and achieve a satisfactory control of *R. dominica* infestations.

On the contrary, horsetail powder did not drive away the beetles from the treated wheat with both doses and even at the longest exposures. This is in line with the findings by Oliveira et al. (2007) who reported no repellent effect on adults of *Zabrotes subfasciatus* (Say) (Coleoptera: Bruchidae) when the powder of *E. arvense* was mixed at 3% w/w with dried beans. As for insecticidal activity, differences in contact-mediated repellency between DEs and silica-accumulating botanical powders such as *Equisetum* are likely driven by variation in silica content and particle size.

The results of this study clearly demonstrate that the commercial DE formulation is highly effective against *R. dominica* adults, exhibiting both insecticidal activity and contact-mediated repellency. In contrast, powder of *E. telmateia*, when tested at the same dose rates as the DE, showed negligible insecticidal or repellent effects against the lesser grain borer. These findings indicate that horsetail powder in this form is not a viable alternative to DEs for the control of *R. dominica* in stored wheat. However, to better assess the possible contribution of plant-derived silica for the management of stored-food pests, future research should examine insecticidal efficacy under conditions where silica concentrations are standardized between horsetail powders and commercial diatomaceous earth formulations, given that DEs typically contain approximately fourfold higher SiO_2 levels. For practical management of the lesser grain borer, the 1000 ppm dose of DE commercial formulation may represent a promising compromise, offering a strong repellent effect and substantial insecticidal activity while minimizing the negative impact on the grain's bulk properties and keeping treatment costs lower.

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