Material properties determining the insecticidal activity of highly divided porous materials on the pharaoh ant (*Monomorium pharaonis*)

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Abstract

BACKGROUND: Historically, inert insecticidal powders such as diatomaceous earth were researched for pest management applications, and it was revealed that these types of powders killed insects by desiccation. However, data on the critical material properties that affect their efficacy are sparse. The present study investigated the insecticidal effect of powdered materials on the pharaoh ant, a notorious domestic pest.

RESULTS: The insecticidal activity of 24 porous materials was tested. Eight of these materials performed better than the benchmark, diatomaceous earth. Zeolite Y and carbon black II performed best, inducing 50% mortality within 40 and 55 minutes, respectively. Statistical analysis of seven material properties revealed that macroporous surface area and Brunauer–Emmett–Teller (BET) specific surface area were most predictive of insecticidal activity. For zeolites and ordered mesoporous silica materials, the most important parameters were, respectively, BET and large mesopore surface area. Finally, gas chromatography–mass spectrometry (GC–MS) analysis confirmed the adsorption of epicuticular hydrocarbons onto the zeolite powders.

CONCLUSION: This study shows clear potential for the use of environmentally friendly, inert porous materials as insecticides against the pharaoh ant and identified the key material properties influencing insecticidal activity. The GC–MS data support the hypothesis that the mortality was caused by the removal of the protective epicuticular hydrocarbons.

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Supporting information may be found in the online version of this article.

Keywords: pest management; zeolites; activated carbon; desiccant powders; wax adsorbents; pharaoh ants

1 INTRODUCTION

Insect pests are a common problem, not only in agriculture, but also in households and the service industry. For household and agricultural applications, most insect pest control systems on the market today make use of hazardous pesticides.¹ Although the application of such pesticides is strictly regulated, there are still significant health and environmental concerns related to their use. In addition, many insects quickly build up genetic resistance to pesticides.² Together, these factors have led to a strong need for alternative pest control methods.³

Although biological pest control strategies based, for example, on the introduction of predators, parasitoids or pathogens of the target pest⁴ have been shown to have great potential for the control of many agricultural pests and have been very successfully deployed in the glasshouse industry, they can have undesirable side effects on local biodiversity^{5,6} when released in the environment.⁷ For specific pest species, the sterile insect technique, based on the release of sterile or genetically modified populations of the target pest, has also been shown to have great

promise.^{8,9} Neither of these approaches, however, is suitable to be deployed inside residential or service buildings.⁴ In households and service industries, a quick and cost-efficient solution is required to remove small pest populations without compromising the health of residents, clients or other animals. For such applications, the use of hazardous pesticides presents obvious health hazards. An alternative and increasingly popular approach is the use of inert, usually inorganic, powders that are lethal to insects but that are relatively harmless to humans and higher vertebrates and that have limited impact on the environment. These inert powders, such as diatomaceous earth^{10,11} and clays,^{12,13} are not toxic by

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ingestion and mostly classified as nuisance dusts, which are only considered harmful upon chronic exposure.¹⁴

Diatomaceous earth is one of the oldest inert powders known to kill insects. Based on the observation that birds take “dust baths” to free themselves of mites and other parasites, the Chinese had already discovered the merits of this material in pest control applications over 4000 years ago.¹⁵ Similarly, charcoal and ashes were used in Egypt in the Old and Middle Kingdoms (ca. 2575–1640 BC) to control fleas and pests of stored grain¹⁶ and the Aztecs of ancient Mexico (14th–16th Century) protected harvested maize using powdered lime (calcium hydroxide).¹⁷ In America, road dust was observed killing cotton worms as early as 1880.¹⁸ In the 1940s, the mechanism of action of such inert dusts was investigated, using various agricultural pests and cockroaches as target organisms.^{12,19–25} More recently, insecticidal powders have been researched for use in stored grain protection^{19,26,27} and domestic applications to target bed bugs.¹¹

While the exact mechanism of action of these materials was never formally proven, several modes of action such as blocking of the spiracles (suffocation)²⁸ and impairment of the digestive tract (starvation) were disproven.²⁷ Direct water uptake from the environment has also been shown not to be the main cause of death, as stated by Alexander in 1944, who found that, among the tested materials, hydrophobic powders were more effective than hydrophilic ones.²⁴ Instead, the most widely accepted theory now is that these powders adhere to the insect body and remove the insects’ protective epicuticular wax layer by adsorption or abrasion. To show this removal of the epicuticular molecules, B. Subramanyam published a transmission electron microscopy (TEM) image of a healthy rice weevil and one exposed to dusted concrete for 24 h, and showed that the latter displayed a greatly reduced epicuticular wax layer.²⁹ However, whether adsorption or abrasion is prominent depends on the properties of the powder used. Rough powders with sharp edges may mainly abrade the wax layer, while softer, porous powders probably have adsorption as the main mode of action.^{12,19–21,30} Most of the available research into the mechanism included solely inorganic powders (some natural and a few synthetic zeolites,^{31,32} clays^{13,23} and metal oxides^{22,33}). In addition, Majumder and Ebeling also included carbon samples in their studies.^{21,23} The physical properties of the powders and the mechanism of the observed insecticidal effect, however, have hardly been addressed.²¹ Ebeling and Wagner used supplier-provided particle size, pore diameter, specific surface area and oil adsorption capacities as well as in-house determined abrasive indices, beeswax adsorption and pH as possible determinants of the induced mortality. They found that a pore size of at least 2 nm was required to effectively adsorb wax, and suggested that microporous materials (pores <2 nm) were therefore not suited as insecticidal powders. They also noted that the adsorptive dusts included in their study (based on oil and wax uptake) were overall more effective than the abrasive, non-adsorptive dusts.^{12,21} David and Gardiner, in contrast, came to the conclusion that abrasive dusts were effective insecticides as well. They noted that particles should be small (< 10 µm) and preferably angular. Majumder *et al.* reported that activation treatment (which increases the specific surface area of the material) resulted in an improved insecticidal activity of charcoal and clay samples. They also stated that charcoal was less effective than the clay samples as a consequence of it being more hygroscopic.^{13,23} Korunic thoroughly researched several diatomaceous earth properties for stored grain protection across 32 samples. He measured adherence to wheat kernels, silica content, particle size, density and test weight reduction, and he

also took active surface and oil adsorption into account. He noted a significant correlation between induced mortality and density, silica content, test weight reduction and wheat kernel adherence of the diatomaceous earth.

Since these early studies, porous materials science has greatly progressed and many safe synthetic porous materials are now available from other research fields, such as catalysts, pharmaceutical formulations and food additives. These developments triggered a renewed interest in the use of desiccant powders for insect pest control. Nanoparticles^{22,34} and zeolites³¹ are examples of material classes whose potential for agricultural pest management have recently been assessed. But established “natural insecticides” such as diatomaceous earths are also being revisited by researchers.¹⁰ The insecticidal effectiveness, however, of a vast array of synthetic environmentally friendly and non-hazardous porous materials remains unknown. Hence, the aim of this work was to investigate the insecticidal activity of a broad collection of currently available synthetic porous materials for deployment in insect pest control in domestic applications and the service industry, and determine which material properties are critical to achieve maximal activity.

The target pest for this study was the pharaoh ant (*Monomorium pharaonis*), a notorious, now almost cosmopolitan pest, which in temperate regions can be a nuisance in heated buildings,³⁵ can spread pathogens in hospitals³⁶ and can damage electrical equipment. As a result of their unicolonial lifestyle, colonies can be spread out over large areas and can contain hundreds of queens, which makes eradication of the pharaoh ant very hard, often requiring treatment to target the whole of the infested building.^{35,37–39} Conventional pharaoh ant control involves the use of attractant bait stations laced with slow-working insecticides, such as the metabolic inhibitors hydramethylnon (toxic, and toxic to the aquatic environment) and fipronil (toxic, hazardous for human health, and toxic to the aquatic environment)⁴⁰ or the insect growth regulator methoprene (harmful, and toxic to the aquatic environment),⁴¹ all of which are indicated as toxic or harmful in their respective material safety data sheets (MSDSs). As pharaoh ant colonies easily split when agitated, other methods such as spraying of foragers or the nest with toxins or repellents often result in scattering and relocation of the colony. This only increases the extent of the infestation.^{37,38} We propose that the use of insecticidal but environmentally non-toxic powders may offer an alternative and ecologically friendlier solution.

2 EXPERIMENTAL METHODS

2.1 Pharaoh ant colonies

One pharaoh ant colony was originally obtained from Purdue University in Indiana, and was then reared further in a climate room, where it was subdivided into different trays and kept at 27 °C and 70% relative humidity. Opaque plastic Petri dishes of which the bottom was covered with a layer of moisturized plaster was used to serve as nesting space. The ants were fed weekly with sugar water and protein-rich food such as mealworms, grasshoppers and fruit flies and were regularly provided with fresh water.

2.2 Test materials

The insecticidal properties of 24 different porous materials, selected based on commercial availability, price, and low environmental impact, including some with known effectiveness, were compared (Table 1). These materials included nine zeolites, six

Table 1. Porous material samples, their origin and composition, and the occupational exposure limit (OEL) as imposed by the National Institute for Occupational Safety and Health (NIOSH)

Material	Category	Sample name	Supplier	Idealized chemical formula	NIOSH OEL (mg/m ³)
H-ZSM22-57	Zeolite	NA	In-house	H ₂ (SiO ₂) ₅₇ (Al ₂ O ₃)	NA
Na-X-2.2	Zeolite	Na-X-2.2	Uetikon (Uetikon am See, Switzerland)	Na ₂₂ (SiO ₂) ₂₂ (Al ₂ O ₃) ₁₀	6
NH ₄ -ZSM5-30	Zeolite	CBV3024E	Zeolyst (Delfzijl, The Netherlands)	(NH ₄) ₂ (SiO ₂) ₃₀ (Al ₂ O ₃)	6
NH ₄ -MOR-38	Zeolite	CBV30A	Zeolyst	(NH ₄) ₂ (SiO ₂) ₃₈ (Al ₂ O ₃)	6
H-BEA-300	Zeolite	CP811C300	Zeolyst	H ₂ (SiO ₂) ₃₀₀ (Al ₂ O ₃)	6
NH ₄ -ZSM5-280	Zeolite	CBV28014	Zeolyst	(NH ₄) ₂ (SiO ₂) ₂₈₀ (Al ₂ O ₃)	6
H-BEA-30	Zeolite	H-BEA-30	Süd-Chemie (München, Germany)	H ₂ (SiO ₂) ₃₀ (Al ₂ O ₃)	6
H-Y-30	Zeolite	CBV720	Zeolyst	H ₂ (SiO ₂) ₃₀ (Al ₂ O ₃)	6
H-Y-80	Zeolite	CBV780	Zeolyst	H ₂ (SiO ₂) ₈₀ (Al ₂ O ₃)	6
COK-19-I	OMS	NA	In-house	SiO ₂ x H ₂ O	6
COK-12-I	OMS	NA	In-house	SiO ₂ x H ₂ O	6
COK-19-III	OMS	NA	In-house	SiO ₂ x H ₂ O	6
COK-19-II	OMS	NA	In-house	SiO ₂ x H ₂ O	6
COK-12-II	OMS	NA	In-house	SiO ₂ x H ₂ O	6
COK-19-IV	OMS	NA	In-house	SiO ₂ x H ₂ O	6
Calcium silicate I	Calcium silicate	Promaxon T	Promat (Tisselt, Belgium)	Ca ₆ Si ₆ O ₁₇ (OH) ₂	5
Calcium silicate II	Calcium silicate	Promaxon D	Promat	Ca ₆ Si ₆ O ₁₇ (OH) ₂	5
Diatomaceous earth (DE)	Natural	Food Grade DE	Lumino (Washougal, WA, USA)	SiO ₂ x H ₂ O	6
Milled quartz	Natural	White quartz	Sigma-Aldrich (Overijse, Belgium)	SiO ₂	0.05
Carbon black I	Carbon	Printex U	Degussa (Düsseldorf, Germany)	C	3.5
Activated carbon	Carbon	Anderson AX-31	Anderson (Adrian, MI, USA)	C	5
Carbon black II	Carbon	Vulcan XC72R	Cabot (Boston, MA, USA)	C	3.5
TiO ₂ -I	Metal oxide	Aeroxide P25	Evonik (Hanau-Wolfgang Germany)	TiO ₂	0.3
TiO ₂ -II	Metal oxide	TiO ₂	Umicore (Brussels, Belgium)	TiO ₂	0.3

ordered mesoporous silica (OMS) materials, two calcium silicates, two naturally occurring materials (fresh water diatomaceous earth and crudely milled quartz) and three types of carbon materials. Two types of titanium dioxide nanopowders were added to increase the variety of the materials tested and their properties, even though these particular materials are known to be hazardous (see Section 2.3), and would be undesirable to be used in final applications. The OMS materials, COK-19 and COK-12, were synthesized in-house according to Kerkhofs *et al.*⁴² and Jammaer *et al.*⁴³ respectively. COK-12 synthesis performed at 0 °C (COK-12-I) instead of the original 20 °C (COK-12-II) generated an amorphous microporous material in which the pores were no longer ordered. COK-19 was synthesized at 20 °C (I), 50 °C (II), 70 °C (III) and 100 °C (IV) to obtain materials with different porous properties. The zeolite sample notation is structured A-B-X with A the cation exchanged in the zeolite, B the zeolite name and X the silica over alumina (SiO₂/Al₂O₃) ratio of the material.

2.3 Toxicity

The materials used in this study are mostly classified as nuisance dusts by the National Institute for Occupational Safety and Health (NIOSH) and Occupational Safety and Health Administration (OSHA) and in Table 1 we list the NIOSH occupational exposure limits (OELs) calculated for a 40-h work week with a maximum of 10 h exposure per day [Centers for Disease Control and Prevention (CDC); <https://www.cdc.gov/niosh/npg/>]. The NIOSH exposure limit was chosen over the OSHA limit, because it is often more restrictive and is also usually provided in the MSDS. The OMS materials as well as the non-fibrous zeolites and amorphous diatomaceous earth are all classified as amorphous and precipitated silica material. Quartz is classified separately as

pure crystalline silica material as it can cause silicosis, a severe lung problem, even at lower exposure rates, so exposure has to be monitored more closely.^{44,45} Titanium dioxide nanoparticles are also more harmful, as they can penetrate deep into the lung tissue because of their small size.⁴⁴ Thus, they are also subject to a low occupational exposure limit. Fibrous materials are most often linked to asbestos; however, not all fibrous particles are equally dangerous. Manmade vitreous fibers (MMVFs) such as the calcium silicate used in this study are often more soluble than asbestos and do not shed small fibrous particles. Their increased solubility reduces the residence time of the particles in the human body, making them significantly less hazardous.⁴⁴ For the fibrous calcium silicates, the fibrous mineral wool exposure limit was used, which is not much lower than the amorphous silica limit. Finally, for activated carbon, the generic NIOSH exposure limit for a nuisance dust applies, which is 15 mg m⁻³ total dust and 5 mg m⁻³ inhalable particles (CDC; <https://www.cdc.gov/niosh/npg/>).

To reduce the amount of inhalable particles and possible health effects, the powdered materials were pelletized to a size of 125–250 µm by compression and crushing, with the exception of the OMS materials (which were not available in sufficient amounts to be pelletized) and the carbon-based materials (which could not be pelletized without adhesive), which were used as a powder. Quartz was also milled to a particle size of 200 µm, which corresponds to the average pellet size of the other materials, thereby also avoiding any possible health hazards. Overall, for domestic applications, we would expect all materials tested in our study to be safe, with the exception of the two titanium dioxide nanoparticles, which were merely included for comparison.

However, there is an overall concern about the hazard of nanoparticles (< 100 nm), which are often found to be increasingly hazardous with decreasing particle size.^{44,46} Recently, L. Thomassen found that nano zeolites (Y and A) were not cytotoxic, as opposed to colloidal silica nanoparticles, indicating that toxicity of nanoparticles also depends on their composition.⁴⁷

2.4 Mortality tests

The powder samples were acclimatized in the ant climate room for a minimum of 48 h before the start of the experiment to avoid further water uptake during the experiment.

The experiments were conducted in cylindrical jars with a diameter of 3 cm and a height of 7 cm, coated with polytetrafluorethylene (Fluon, blades biological Ltd, Cowden, Edenbridge, UK) to prevent the ants from escaping. Fifty milligrams of the acclimatized powder was added to each jar and kept in the climate room for another 24 h. The use of an abundant amount of powder was recommended by Ebeling.²¹ Other researchers used samples with a fixed weight (g/kg food,²⁷ g/kg insects²² or g/m² surface²⁶). For these reasons, a relatively large fixed weight of powder and fixed number of 40 adult foraging ants were used in these experiments.

The experiment was conducted in the same climate room where the ants were bred and kept. The ants were obtained directly from the stock colonies. After transfer to the specimen jars for the experiment, the ants had no access to food or water. Each of the experiments was conducted in triplicate and a maximum of nine specimen jars were observed at any one time.

Deaths (immobile and unresponsive foragers) were recorded once every 30 minutes for 7 hours. For the more effective materials attaining 100 % mortality within 3.5 hours, the experiment was repeated with observations that were carried out every 5 to 10 minutes. Three blank measurements, with three containers without powder and 40 foragers each, were conducted during the course of the survival assays. No significant differences were observed among our different blank measurements (Cox proportional hazard model and Tukey post hoc tests, $P > 0.05$), indicating a good reproducibility of the results.

2.5 Material properties analysis

Each material was thoroughly characterized with the aim of linking the properties with the observed insecticidal activity. In particular, the following material properties were measured: Brunauer–Emmett–Teller (BET) specific surface area, pore diameter, surface area and volume (micropores, small mesopores, large mesopores and macropores), water adsorption capacity and particle size.

The surface area and porosity of the materials were derived from nitrogen adsorption isotherms at -196 °C determined using a Quantachrome Autosorb-1 instrument (Hartley Wintney, Hook, UK). The samples were degassed at 150 °C prior to the measurement. The BET surface area was estimated in the relative pressure P/P_0 range 0.01–0.3 according to the best linear fit. As a consequence of the limited quantitative relevance of the BET surface area for microporous materials,⁴⁸ supported by published discrepancies between Density Functional Theory (DFT) and BET surface area values for carbon materials⁴⁹ and zeolites,⁵⁰ the non-local Density Functional Theory (NLDFT) method was also applied to determine different pore surface areas. This approach was already suggested for carbon materials by Béguin and Frackowiak in 2010.⁵¹ To this end, pores were divided into three categories: micropores (< 2 nm), small mesopores (2–5 nm) and large mesopores (5–50 nm). The pore size distribution and pore volumes were

also obtained from NLDFT modeling. Barrett–Joyner–Halenda (BJH) analysis was executed with a Micromeritics (Norcross, GA, USA) Tristar instrument to determine the surface area of macropores with diameters from 50 to 240 nm. All of the abovementioned models are readily available in the software of the Micromeritics instruments.

The water adsorption capacity of the powders was used as an indication of hydrophobicity and was measured using a magnetic suspension balance (MSB, Robathern, Charveux, Belgium). The powders were degassed for 24 h at 150 °C and loaded in the MSB where they were exposed to a relative humidity of 73% at a temperature of 23 °C. The samples all reached water adsorption equilibrium within 48 h. To avoid strong correlation with the pore volume of the material, the ratio of the pore volume to the amount of water adsorbed was included as a parameter in the statistical model. The higher the value, the more hydrophobic the material is, provided that the pores of the material are accessible. In the past, other researchers have also used oil adsorption or water adsorption capacity for this purpose.^{10,12,13,21}

The average particle size of the materials was determined by scanning electron microscopy in a Nova NanoSEM450 (FEI, Eindhoven, The Netherlands). The samples were loaded on carbon tape and measured at low voltages (0.5–1 kV) (Fig. 1).

2.6 Statistical analysis

A nonparametric Cox proportional hazard model was used to compare ant survival across the experimental treatments. This was done using package survival and the function coxph in R version 3.2.3. In this model, replicates were taken into account using a cluster factor and material was included as a fixed factor. Differences in survival compared with the blank as well as with diatomaceous earth (which served as a benchmark) were obtained using Dunnett's post hoc tests carried out using the package lsmeans. From the Cox model, corresponding Kaplan–Meier survival curves were generated using the function survfit from the package survival. Median survival times [or median lethal times (LT50s), the time points at which half of the individuals had died] and hazard ratios in relation to the no-powder blank were also derived from this model. To determine which material properties were most predictive of insecticidal activity, an exhaustive model search was carried out using the R package bestglm, whereby the log₁₀-transformed hazard ratios were used as the dependent variable and seven measured material properties were included as independent variables, which could in principle be included as main effects in the final best model. Which properties were included in the model search was based on their variance inflation factors and relative significance of related parameters. The best model across all materials was chosen using leave-one-out cross-validation, whereas for the analysis of the subsets of zeolite and ordered mesoporous materials, the Bayesian information criterion (BIC) was used. The overall predictive value of the obtained models was measured on the basis of the adjusted R^2 and η^2 was used as a measure of the variance explained by each individual variable in the models.

2.7 Epicuticular wax adsorption

To analyze the epicuticular wax uptake of the materials, the subclass of zeolites was selected, as this was the largest group of similar materials that were tested. For this analysis, 15 cylindrical jars, identical to the ones in Section 2.4, were used and were filled with 50 mg of material and 120 foragers (or no foragers for the blanks). After 1 h of exposure, the ants were removed from the jars and

Figure 1. SEM image of diatomaceous earth (a), zeolite H-Y-30 (b), activated carbon (c), calcium silicate I (d), TiO₂-II (e) and COK-19-IV (f).

500 mg of the total amount of powders was extracted using 3 ml of *n*-hexane [high-performance liquid chromatography (HPLC) grade, 95%; Acros Organics, Geel, Belgium]. The mixture was sonicated for 30 minutes, then centrifuged at 1843 *g* for 15 minutes. A volume of 1.5 ml of the supernatant *n*-hexane solution was pipetted into a gas chromatography (GC) vial and both the powder and the GC vial were left open to evaporate the *n*-hexane (the wax molecules from the epicuticle are long alkanes, which are not volatile and therefore left as a residue in the container). A second identical extraction was performed on the powder to extract any remaining components from the 500 mg of powder. The 1.5 ml of *n*-hexane supernatant solution was added to the same GC vial and evaporated. For the reference sample, 1200 ants were put in 1.5 ml of *n*-hexane and vortexed for 2 minutes to extract the epicuticular compounds. The solution was then left to evaporate. Finally, 500 µl of fresh *n*-hexane was added to the GC vials, after

which they were vortexed for 1 minute and analyzed with gas chromatography–mass spectrometry (GC–MS).

GC–MS analysis was performed on a Thermo Trace 1300 gas chromatograph (Thermo Fisher Scientific, Asse, Belgium), linked to a Thermo Scientific ISQ mass spectrometer (Thermo Fisher Scientific) and an electron ionization source (70 eV). The component separation occurred in a 30-m-long MXT-5 column (Restek, Middelburg, The Netherlands) with a diameter of 0.25 mm coated with a 0.25-µm phase, consisting of 5% diphenyl and 95% dimethyl polysiloxane. A split injection was used with a 1/3 split ratio, an injection volume of 1 µl, an inlet temperature of 320 °C and a helium gas flow of 0.9 ml/min. The column temperature was programmed as follows: a 2-min hold at 40 °C, increase to 120 °C at 20 °C/min, increase to 200 °C at 10 °C/min, increase to 250 °C at 7 °C/min, increase to 350 °C at 5 °C/min, and a 4-min hold at 350 °C. The transfer line and the ion source were kept at 300 °C, the solvent

Figure 2. Pharaoh ant survival rate measurements modeled with Kaplan–Meier upon exposure to different materials. The key is arranged according to the type of powder used and, per powder type, from least to most effective.

delay time was set at 3 minutes and 40 seconds, the examined mass range of the scans was 33–720 amu and the scan time was 0.304 seconds. A linear C7 to C40 linear alkane ladder standard (49452-U; Supelco, Bellefonte, PA, USA) at three different concentrations (0.001, 0.01 and 0.1 µg/mL) was also run, which was used to quantify all hydrocarbons as well as to calculate their retention index. Peak areas in the total ion chromatogram were integrated using an in-house developed R script (see supplementary information in the paper by Parmentier *et al.*⁵²) and retention indices were calculated using cubic-spline interpolation based on the elution times of the external alkane ladders.⁵³ As we previously verified that the relationship between peak area and concentration was linear on a log–log scale,⁵² quantification of all hydrocarbon compounds (total amount adsorbed or total amount extracted from 1200 ants) was performed using interpolation on a log–log scale, using the peak areas of the closest eluting *n*-alkane of our external alkane ladders for each compound. In all cases, compounds recovered from the blank were subtracted from our measured sample amounts, and any peaks that were not present in our reference ant extract were omitted from further analysis. Cuticular compounds were identified on the basis of their expected mass spectra and retention indices,⁵² and via comparison with other studies, standards and published spectra.^{54–56}

3 RESULTS

3.1 Mortality and material properties

The survival rates of the pharaoh ants as a function of time exposed to the different desiccant powders that were tested are presented in Fig. 2. The median survival times (Table 2) ranged from 40 minutes to >7 hours, with the benchmark, diatomaceous earth, being ranked in ninth place with a median survival time of 95 minutes. The eight materials that performed better than the diatomaceous earth benchmark, ranked from most to least insecticidal activity, were: zeolites H-Y-30 and H-Y-80, carbon black II, OMS material COK-19-IV, activated carbon and zeolites H-BEA-30, NH₄-ZSM5-280 and H-BEA-300. The microporous

ordered mesoporous materials COK-12-I and COK-19-I as well as milled quartz did not show any significant insecticidal effect when compared to the blank.

An overview of the tested materials and their properties is presented in Table 2. The discrepancies between the BET and DFT surface areas can be attributed to the limited quantitative accuracy of the BET surface area calculation for microporous and carbon materials.⁴⁸ The sample collection contains materials with particle sizes ranging from 21 nm to 200 µm, BET specific surface areas in the range of <1 m²/g to 2242 m²/g and a wide variety of pore surfaces, spread in different proportions over micropores, small and large mesopores and macropores. The hydrophobicity, estimated as the ratio of pore volume (ml) to measured water adsorption capacity (g), ranged from 0.59 ml/g for diatomaceous earth to 5.94 ml/g for NH₄-ZSM5-280.

The naturally occurring materials consisted of dense, coarsely milled quartz (particle size 200 µm) and the benchmark, diatomaceous earth, a little porous, hydrophilic material, made up of large particles/diatoms (10 µm). While the use of milled quartz resulted in survival rates that were not significantly different from the survival rates in the blanks, diatomaceous earth proved to be quite effective, placing the benchmark in ninth place.

Zeolites are microporous (pore diameter < 2 nm), crystalline aluminosilicates per definition, but otherwise they have a wide range of textural properties (BET, pore surface area, particle size and hydrophobicity). The hydrophobicity increases with increasing silica to alumina ratio of the zeolite materials, because the amount of acidic sites in the zeolites then decreases. These materials also showed a wide range of effectiveness, with ultra-stable Y (USY; H-Y-30 and H-Y-80) being the most insecticidal material and H-ZSM22-57 being the fifth least insecticidal material. Two kinds of ordered mesoporous (2–50 nm pore diameter) silica materials were used (COK-12 and COK-19), synthesized under different conditions. This generated a collection of samples with a limited difference in particle size and morphology but a wide range of pore surface areas (649–1951 m²/g) and micropore/mesopore surface area proportions (0.23–6.1). The survival rates observed

Table 2. Overview of tested samples and their properties, BET surface area, DFT micropore surface area (Sp_{micro}), DFT mesopore surface area (Sp_{meso}), for small and large mesopores, according to their diameter (D_p), BJH macropore surface area (Sp_{macro}), pore volume (Vp_{tot}) over water adsorption (water ads), particle size, total pore volume (Vp_{tot}) and the median survival time (t_{surv}), as well as the hazard ratio compared with the blank with 95% confidence interval (CI)

Material	Median t_{surv} [95% CI] (min)	Hazard ratio compared with blank [95% CI]	BET (m^2/g)	Sp_{micro} (m^2/g)	$Sp_{\text{small meso}}$ ($D_p \leq 5 \text{ nm}$) (m^2/g)	$Sp_{\text{large meso}}$ ($D_p > 5 \text{ nm}$) (m^2/g)	Sp_{macro} (m^2/g)	Vp_{tot} water ads (ml/g)	Particle size (μm)	Vp_{tot} (ml/g)
H-Y-80	40 [40, 40]	1.8E + 06 [5.5E + 05, 5.7E + 06]	874	793	84	57	17	1.61	1	0.421
H-Y-30	40 [40, 40]	1.4E + 06 [4.5E + 05, 4.2E + 06]	830	861	85	59	23	1.55	1	0.449
Carbon black II	55 [55, 55]	1.9E + 05 [6.5E + 04, 5.8E + 05]	214	150	21	25	22	4.06	0.05	0.249
Activated carbon 60	60 [60, 60]	8.6E + 04 [2.9E + 04, 2.5E + 05]	2242	1846	64.4	4.3	9.13	1.16	20	0.996
COK-19-IV	60 [60, 60]	8.6E + 04 [2.8E + 04, 2.6E + 05]	561	106	70	389	13	1.17	1	0.872
H-BEA-30	80 [75, 80]	2.0E + 04 [7.0E + 03, 5.6E + 04]	624	1028	56	139	28	1.74	0.2	0.455
H-BEA-300	85 [80, 90]	1.3E + 04 [4.3E + 03, 3.8E + 04]	781	973	81	83	16	2.47	0.3	0.475
NH4-ZSM5-280	85 [80, 90]	1.3E + 04 [4.2E + 03, 4.1E + 04]	440	936	123	9.8	16	5.94	2	0.268
DE	95 [90, 100]	7.0E + 03 [2.7E + 03, 1.9E + 04]	34	0	17	9	13	0.59	10	0.057
Carbon black I	120 [120, 125]	2.6E + 03 [1.0E + 03, 6.8E + 03]	99	33	16	21	21	3.25	0.025	0.173
COK-12-II	120 [115, 150]	2.3E + 03 [6.5E + 02, 8.5E + 03]	418	654	234	169	12	1.05	0.5	0.654
TiO2 II	140 [135, 150]	1.2E + 03 [5.1E + 02, 3.0E + 03]	32	7	12	9	16	2.08	0.021	0.052
COK-19-II	150 [150, 160]	6.7E + 02 [2.8E + 02, 1.6E + 03]	1737	1206	451	258	11	3.92	1	1.062
COK-19-III	180 [180, 210]	3.1E + 02 [1.1E + 02, 8.5E + 02]	917	255	324	319	14	1.47	1	0.818
NH4-MOR-38	180 [180, 210]	2.6E + 02 [1.1E + 02, 6.0E + 02]	613	1249	89	68	5	2.41	1.5	0.402
TiO2 I	195 [180, 210]	2.3E + 02 [8.3E + 01, 6.4E + 02]	111	0	44	27	16	4.87	0.021	0.181
NH4-ZSM5-30	240 [210, 240]	1.0E + 02 [4.4E + 01, 2.3E + 02]	550	1431	55	34.8	10	2.87	2	0.312
Na-X-2.2	330 [330, 360]	2.0E + 01 [9.6E + 00, 4.3E + 01]	735	2202	43	16	17	2.06	5	0.364
Calcium silicate II	360 [360, 390]	1.3E + 01 [5.7E + 00, 2.8E + 01]	53	27	17	15	13	1.71	4	0.086
H-ZSM22-57	390 [360, 420]	1.2E + 01 [5.2 + 00, 2.8E + 01]	52	43.1	15	14.6	19	1.03	2	0.055
Calcium silicate I	390 [360, 420]	1.1E + 01 [4.6E + 00, 2.5E + 01]	36	11	13	11	15	1.38	4	0.060
COK-12-I	NA	3.0E + 00 [9.9E-01, 9.4E + 00]	497	635	86	18	10	1.41	2.5	0.244
COK-19-I	NA	2.0E + 00 [8.5E-01, 4.8E + 00]	594	584	118	54	11	1.49	1	0.296
Milled quartz	NA	8.7E-01 [2.5E-01, 3.0E + 00]	0	0	0	0	4	0	200	0

DE, diatomaceous earth. NA, not applicable; the median is > 7 h (420 min) and thus exceeds the duration of the experiment.

for the amorphous microporous materials COK-19-I and COK-12-I were not significantly different from those of the blank measurements, whereas COK-19-VI, which contained a large amount of large mesopores, performed better than the benchmark diatomaceous earth. The calcium silicate samples contain particles with an average length of 4 μm , forming larger aggregates (Fig. 1d). They are overall little porous and show little insecticidal effectivity (fourth and sixth least insecticidal materials). Finally, the carbon black samples consist of little porous (< 200 m^2/g combined micro- and mesopore surface area), small hydrophobic particles ($\leq 50 \text{ nm}$) and activated carbon is characterized by highly porous (> 1750 m^2/g pore surface area), large particles (20 μm) and more hydrophilic behavior. Activated carbon, with a very high BET specific surface area of 2242 m^2/g , and carbon black II each performed better than the benchmark. The final group of materials comprised titanium (metal) dioxide nanoparticles of 21 nm. The measured pore volume and surface areas were low and neither of the samples showed more insecticidal effectivity than the benchmark, diatomaceous earth.

Overall, the most insecticidal materials were both samples of the ultra-stable zeolite Y (H-Y-30 and H-Y-80) and carbon black II.

3.2 Statistical analysis

To link the seven measured material properties (micropore, small mesopore, large mesopore and macropore surface areas, BET surface area, particle size and the ratio of pore volume to the

amount of water adsorbed at 73% relative humidity) with the induced mortality, we used an exhaustive model search. The best fit, obtained using leave-one-out cross-validation, consists of several covariates, their coefficients, standard errors and P -values. The power of 10 of the coefficient describes by how much the hazard value would increase or decrease if the covariate increases by 1 unit when compared with the reference value. For example, in Table 3, when the BET specific surface area of the material increases by 1 when compared with the average, the hazard of pharaoh ants dying increases by 0.9% when compared with the average, thereby indicating that a material with a larger BET specific surface area would show more insecticidal activity. The standard errors associated with these coefficients are a measure of the accuracy of the estimate and the P -value indicates the significance of the covariate in the model. Finally, the η^2 is a measure of the variance explained by the covariate.

By applying this analysis to all data, an optimal model was obtained with an overall adjusted R^2 of 0.35 (Table 3). This model thus explains over one-third of the variation in the hazard ratio. As shown in Table 3, the hydrophilicity, calculated as pore volume to water adsorption capacity, and particle size did not show significant predictive value and were thus not included in the optimal model. The macroporous surface area and the BET specific surface area (strongly correlated to the total pore volume) were the only properties included in the model, generating an η^2 of 0.28 and 0.22, respectively. In addition, partial effect plots were

Table 3. List of properties of the optimal model including all materials: covariate coefficients, their power of 10, standard errors (SEs), *P*-values (significant <0.05), the *t*-values from which the *P*-values were calculated and η^2 , a measure of the explained variance for the covariates

Term	Coefficient	10 ^{coefficient}	SE	<i>t</i>	<i>P</i>	η^2
(Intercept)	−0.45	0.355	0.98359	−0.458719	0.651148	NA
BET	0.003866	1.009	0.00058	2.806111	0.010584	0.223804
Sp _{macro}	0.15966	1.444	0.05585	3.132243	0.005034	0.278849

Sp_{macro}, macropore surface area; NA, not applicable. **Figure 3.** Partial effects of the covariates for the optimal model including all materials (points, partial residuals; shaded areas, 95% confidence intervals).

generated, which show the partial influences of the covariates on the hazard ratios, keeping all other covariates constant (Fig. 3). From these plots, it is clear that the insecticidal effectivity increased with increasing macroporous and BET specific surface areas. *A priori* this was expected, as a material with a high specific surface area allows for more epicuticular compounds to be adsorbed.

In two additional analyses, we also carried out model selection on just the zeolite and OMS materials. In this way, we could limit our analysis to materials with inherent similarities, thereby avoiding any other unmeasured confounding variables, linked for example to differences in composition, material structure or crystallinity, affecting our results, eventually leading to more accurate models with a higher adjusted R^2 and a higher predictive value. For the zeolite materials, material Na-X-2.2 was excluded, as the silica to alumina ratio was significantly lower and polarity higher for this material than for all the other zeolites used. In addition, because of the decrease in sample size from 24 to eight or six samples (for the zeolites and OMS materials, respectively), leave-one-out cross-validation was no longer possible and was replaced by model selection based on the BIC.

The optimal zeolite model had an adjusted R^2 of 0.93, indicating that this model explained most of the variance in the hazard ratio and provides an accurate prediction of the hazard based on the included material properties (Table 4). This optimal model contained the same covariates as the optimal model, but with the difference that all of the covariates not appear significant as indicated by their *P*-values. The BET specific surface area explained the highest proportion of the variance ($\eta^2 = 0.25$) (Table 4). A note has to be made that, for the zeolite materials, the total pore volume (the sum of the micropore and mesopore volumes) was strongly correlated with the BET specific surface area (Pearson correlation = 0.93; $P = 0.00034$). In contrast to the model for all materials, the variance explained by the micropore surface area increased at the expense of the variance explained by the macropore surface area when compared with the model of all materials. Effect plots were

generated and similar trends were observed when compared to the model containing all materials (Fig. 4). A high BET specific surface area and macropore surface area also caused a high insecticidal effectivity in the zeolite materials. The influence of the micropore surface area on the hazard ratio was negative in this model, while the small mesopore surface area was positive, indicating that a zeolite with larger (small) mesopore and macropore surface areas was linked to a higher hazard ratio. A sidenote can also be made here that the influence of the micropore surface area can indicate that zeolites containing larger micropores are more effective in adsorbing the epicuticular compounds. Larger micropores decrease the micropore surface area while keeping the total pore volume constant (which is strongly correlated to the BET specific surface area).

Analogously, the optimal model for the six OMS materials had an even higher explained variance (adjusted $R^2 = 0.97$) and contained only three covariates, all of which had a highly significant influence on the induced mortality, namely the BET specific surface area, the micropore surface area and finally, most importantly, the large mesopore surface area, which had an η^2 of 0.75 (Table 5). The microporous materials COK-12-I and COK-19-I had a negligible large mesopore surface area and a very low hazard value. The partial effect plots showed an increased effect with decreasing BET specific surface area, increasing micropore surface area and increasing large mesopore surface area (Fig. 5). As a consequence of the presence of mostly microporous materials as well as mostly mesoporous materials, no statement can be made about the influence of the small mesopore surface area.

3.3 Epicuticular wax adsorption

The zeolites, of which ultra-stable H-Y-30 and H-Y80 were the most effective insecticidal materials, were also selected to investigate adsorption of epicuticular compounds. For this purpose, 1800 ants were exposed to 750 mg of zeolite per material for 1 h, after which 500 mg of recovered material was extracted in hexane and analyzed via GC–MS, which therefore corresponded to the

Table 4. List of properties of the optimal model including only zeolites: covariate coefficients, their power of 10, standard errors (SEs), *P*-values (significant <0.05), the *t*-values from which the *P*-values were calculated and η^2 , a measure of the explained variance for the covariates

Term	Coefficient	10 ^{coefficient}	SE	<i>t</i>	<i>P</i>	η^2
(Intercept)	−1.14431	0.072	0.854745	−1.33877	0.251668	NA
BET	0.005482	1.013	0.00102	5.372653	0.005797	0.245662
<i>Sp</i> _{micro}	−0.00185	0.996	0.000409	−4.52686	0.010603	0.174404
<i>Sp</i> _{macro}	0.090209	1.231	0.0313	2.882082	0.044919	0.070693
<i>Sp</i> _{smallmeso}	0.02524	1.060	0.007055	3.577704	0.023215	0.108936

*Sp*_{smallmeso}, small mesopore surface area (*D*_p > 5 nm).

Figure 4. Partial effects of the covariates for the optimal model for the zeolite materials (points, partial residuals; shaded areas, 95% confidence intervals).

amount absorbed by ca. 1200 ants. In total, 37 compounds were identified, of which five were alkaloids (all monomorphines, of which some are known to act as one of the trail pheromones of this species⁵⁵), ten were linear alkanes (with chain lengths from *C*₂₃ to *C*₃₂), four were linear alkenes (with chain lengths from *C*₂₇ to *C*₃₃), two were linear alkadienes (with chain lengths of *C*₂₉ and *C*₃₃), nine were monomethylalkanes (with chain lengths from *C*₂₅ to *C*₃₃), five were dimethylalkanes (with chain lengths from *C*₂₇ to *C*₃₃) and two were trimethylalkanes (with chain lengths from *C*₂₈ to *C*₃₂) (Fig. 6; Supporting Information Table S1). The types of compounds that were adsorbed showed clear specificities across the different zeolites (Fig. 6). For example, the zeolite Na-X-2.2, which was more polar as a result of its high silica to alumina ratio, was the only zeolite that adsorbed some of the monomorphine alkaloids. Excluding this zeolite with quite different material properties, it was also the case that the total amount of cuticular hydrocarbons (CHCs) that was absorbed tended to correlate with the induced mortality (Spearman rank *R* = 0.36; *n* = 7), although with the number of materials investigated not significantly so (*P* = 0.43). It is likely, though, that the absence of a significant correlation is mainly a consequence of a lack of power, as a Spearman rank

correlation of 0.36, which according to Cohen (1992) qualifies as a “medium-sized effect”, would require a sample size of 58 materials to be investigated to achieve a statistical power of 80% at the α = 0.05 level. At present, achieving such a sample size in terms of number of zeolites that would have to be investigated is out of reach.

4 DISCUSSION AND CONCLUSIONS

In this study, 24 different materials were tested for their insecticidal effectivity against the pharaoh ant. Of these materials, eight performed better than the benchmark, diatomaceous earth. Statistical model selection revealed that, overall, the macroporous and BET surface areas were the most important parameters, explaining 35% of the variance in insecticidal effectivity, while for the zeolite and the ordered mesoporous materials these were, respectively, the BET surface and the large mesopore surface area. In agreement with these results, Ebeling previously also noted a positive correlation between the specific surface area, the resulting wax adsorption and the target insect mortality of his tested materials.²¹ Significantly better predictions of insecticidal activity were obtained

Table 5. List of properties of the optimal model including only ordered mesoporous silica materials: covariate coefficients, their power of 10, standard errors (SEs), *P*-values (significant <0.05), the *t*-values from which the *P*-values were calculated and η^2 , a measure of the explained variance for the covariates

Term	Coefficient	10 ^{coefficient}	SE	<i>t</i>	<i>P</i>	η^2
(Intercept)	−1.0541	0.088	0.422633	−2.49412	0.130109	NA
BET	−0.00378	0.991	0.000618	−6.10576	0.025791	0.222904
<i>S</i> _{p_{micro}}	0.00453	1.010	0.000815	5.559148	0.030868	0.18478
<i>S</i> _{p_{largemeso}}	0.019028	1.045	0.001701	11.18488	0.007899	0.747999

*S*_{p_{largemeso}}, large mesopore surface area (*D*_p > 5 nm).

Figure 5. Partial effects of the covariates for the optimal model for the ordered mesoporous silica materials (points, partial residuals; shaded areas, 95% confidence intervals).

when our models were limited to one material class, such as zeolites or ordered mesoporous materials, with explained variances >90%, as these materials have inherent similarities, thereby limiting the possibility for unmeasured confounding variables to influence their efficacy. The obtained correlation of the specific surface area of the materials with the observed pharaoh ant mortality was also clearly indicative of adsorption as a mode of action of these powders. This assumption was also backed by a GC–MS analysis performed on the zeolite materials, revealing adsorption of the compounds from the pharaoh ants' epicuticular wax layer, which would then be expected to lead to rapid dessication.^{12,19–21,30}

In this study, many different types of microporous materials were used. Previously, it has been suggested that micropores with pore diameters <2 nm would be expected to be too narrow to accept epicuticular wax compounds,^{20,21} because, at room temperature, penetration of these molecules into micropores would be expected to be unfavorable for thermodynamic and kinetic reasons. For the COK-12-I and COK-19-I materials this theory would appear to hold, as they were the second and third least effective materials. By contrast, in their ordered mesoporous counterparts (COK-12-II and COK-19-II, -III and -IV), the pores are

ordered and wider (classified as mesopores).⁴² These mesopores, therefore, would be expected to enhance the adsorption of the epicuticular wax molecules. In line with this theory, the most mesoporous material, COK-19-IV, was in joint fourth place when considering insecticidal effectivity. However, for the carbon materials with slit-shaped pores and the highly ordered zeolite materials, containing acidic sites, this appears not to be the case. In all likelihood, the presence of slit-shaped instead of cylindrical micropores and the presence of acidic adsorption sites⁵⁷ alters the thermodynamics of hydrocarbon adsorption in these materials, causing them to readily adsorb epicuticular compounds, and causing them to display high insecticidal activity. Zeolites H-Y-30 and H-Y-80 were the top performing materials and carbon black II as well as activated carbon performed well, being ranked third and fourth in terms of insecticidal activity. In previous research, Majumder claimed that activated carbon quickly lost its effectiveness because of its hygroscopic properties.^{13,23} Nevertheless, in this work the carbon samples that were used were equilibrated at 70% relative humidity before being tested, and still showed a very high insecticidal activity. The observed ineffectivity of milled quartz can be explained by its dense nature and large

Figure 6. Heatmap showing the cuticular profile composition of *M. pharaonis* workers (top) and the adsorption of the different epicuticular compounds by eight zeolite materials as determined by GC–MS analysis. Materials are sorted by induced mortality, from high at the top to low at the bottom.

particle size (200 µm). As David and Gardiner already stated, for an inert powder to abrade the epicuticular wax layer of insects, the particle size should be <10 µm.³⁰

The results of this study indicate a high potential of adsorbent powders for pharaoh ant abatement. However, as the epicuticular wax composition is similar for many types of insects, including harmless ones, application of adsorbent powder should be restricted to confined spaces as would be the case for the management of stored product pests and pest management in residential and service buildings.⁵⁸ For open air agricultural applications, where large areas would have to be treated, unintended consequences for other non-target insect species, including beneficial ones, would be likely, and for such applications more targeted approaches would be advised. Also, when applying finely divided powder in buildings, exposure of people to inhalable particles should be controlled. While most of the inert insecticidal dusts are only classified as nuisance dusts and do not pose an extra risk arising from their composition, some specific types of dusts require special attention (CDC; <https://www.cdc.gov/niosh/npg/>). In our study, the risk of inhalation was taken into account and the maximum amount of inhalable particles was reduced by pelletizing the materials to a grain size of 125 to 250 µm.

The artificial conditions of the performed experiments resembled as much as possible conditions in real-life applications. The relative humidity of 70% and temperature of 27 °C used in the survival assay led to a conservative estimate of pharaoh ant mortality, as 70% relative humidity is quite high when compared with the value in occupied buildings. Some conditions in our

experiment, however, did deviate from expected conditions during real-life application. For example, pharaoh ants would typically have access to food and water and would be able to move away from the powder. Further optimization of effective deployment under real-life conditions would therefore be required if the aim was to obtain similar time-to-death figures as observed in our experiments. Further work in this direction is in progress.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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