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Effects of different inert dusts on *Sitophilus oryzae* and *Plodia interpunctella* during contact exposure

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Abstract

The use of natural inert dusts against storage insect pests is increasing recently, as an alternative to conventional insecticides. Laboratory study was carried out to evaluate the contact effect of three inert dusts, diatomaceous earth (DE), kaoline (KA) and vermiculite (VE), at rates 5, 7.5, 10, 15 and 20 gm⁻², against adults of *Sitophilus oryzae* (L.) and larvae of *Plodia interpunctella* (Hubner). Insect mortality was evaluated 1, 2, 3 and 7 days after the exposure. Insect mortality varied depending on the species, concentrations and exposure periods. The DE and KA caused 86.7-98% mortality of *S. oryzae* after 2 days of exposure at the highest rates, while at 5 and 7.5 gm⁻², 100% mortality was achieved only after 7 days. The highest rates of inert dusts caused 42-50% (DE) and 60-75% (KA) mortality of *P. interpunctella* larvae only after 7 days. The mortality of moths increased gradually with the concentration and 100% was achieved 3 days after the contact with DE and KA (10, 15 and 20 g m⁻²). However, inert dusts induced faster pupation of *P. interpunctella*, while adult emergence was reduced and adults had smaller body-sizes, compared to control. The VE caused relatively low mortalities (7-11% of *S. oryzae* adults and 5-8% of *P. interpunctella* larvae) at all tested rates during the entire experiment. Our results have shown good insecticidal effect of DE and KA against *S. oryzae* and *P. interpunctella* at 10, 15 and 20 gm⁻². These products could therefore be used by small-scale farmers to protect stored grains against insect pest infestation.

Key words: Inert dusts, *Sitophilus oryzae*, *Plodia interpunctella*, contact exposure, diatomaceous earth

Introduction

In recent years, the use of contact insecticides and fumigants for controlling storage pests is under increasing restriction due to the presence of residues in food and development of insect resistance (Collins, 2000; Kljajić and Perić, 2005). These shortcomings have stimulated the need for testing and evaluation of non-toxic methods that can replace conventional insecticides in stored grains (Arthur, 1996). Recently, physical control methods, like the use of inert dusts, have become prominent (Field and Korunić, 2002). These materials are classified into different groups depending on their composition and particle size and include materials such as diatomaceous earth, silicophosphate, rock phosphate, sand, kaolinite, clay etc. (Golob, 1997). There is a growing interest especially in desiccant or absorptive dusts, among which, diatomaceous earth is the most widely used in practice worldwide (Golob, 1997; Korunić, 1998a; Subramanyam and Roesli, 2000) and in commercial storages in the developed world. On the other hand, non-silica dusts and those composed of coarse grain silicates, such as kaoline and sand, have been used traditionally as grain protectants by small-

scale farmers in the developing world (Golob, 1997). Inert dusts, regardless of the group, can control a variety of common storage insect pests. Korunić (2013) reported that there are three areas of DE use: a) admixture with grain, b) as structural treatment on walls and floors and c) addition of DE to the surface of grain bulks. However, inert dusts, primarily DE, was found to reduce the grain bulk density, affects the flow characteristics of bulk grain (flowability), and also leaves visible dust residues (Subramanyam et al., 1998; Golob, 1997; Korunić et al., 1998b) and health concerns (Korunić, 2016). Thus, DE is highly recommended for surface treatments and in general, it is thought that DE should be used primarily as a preventive measure for grain protection and not as a curative measure. In Australia, preparation based on DE (Dryacide) is frequently applied as a structural treatment in empty storages, and on grain bulk surfaces (Aleen, 2001). This work aimed to assess the contact efficacy of three different inert dusts (diatomaceous earth, kaolin and vermiculite) against *S. oryzae* adults and *Plodia interunctella* larvae, as additional preventive measure during storage.

Material and methods

Laboratory studies were conducted to evaluate the contact effect of three inert dusts: diatomaceous earth-DE (commercial preparation SilicoSec, produced by Biofa, uncalcinated diatomite), kaoline clay - KA and vermiculite dust - VE as contact insecticides against *Sitophilus oryzae* adults and 3rd instar larvae of *Plodia interunctella*. Inert dusts were applied on glass Petri dishes (surface area, 153.5 cm²) at rates 5, 7.5, 10, 15 and 20 gm⁻². The dusts were dispersed over the glass surface and 20 (2-4-weeks old) adult weevils or 3th instar larvae of *P. interunctella* were put into dishes. Mortality was evaluated after 24, 48, 72 h and 7 days of exposure. Clean Petri dishes served as the control. The LC₅₀ and LC₉₀ were calculated using Probit analysis in SPSS 21.

Results

The effect of DE, KA and VE applied at 5, 7.5, 10, 15 and 20 gm⁻² on *S. oryzae* adults are presented on Figs. 1-3. In all the inert dust treated jars, insect mortality increased with the increase in concentration and exposure period. After 24 h, DE caused significant mortality at rates 10 (68%), 15 (73.5%) and 20 gm⁻² (98%), respectively. The mortality increased after 48h of exposure and ranged from 45% at the lowest rate (5 gm⁻²), 67% at 7.5 gm⁻² to 100% at the highest rates. After 72 h, 100% mortality was achieved in treatments with 10, 15 and 20 gm⁻² of DE, while satisfactory mortality was also obtained in treatments with 5 and 7.5 gm⁻² DE (68 and 93%, respectively). After 7 days of exposure mortality was 88-100%. The difference between mortalities depending on the type of inert dust and rates, within the same exposure period (24, 48, 72 h and 7 days) was statistically highly significant (F=57.66**, 102.12**, 93.02** and 145.29**, respectively, p<0.01). earth.

The KA caused significant mortality after 24h only at rates 10, 15 and 20 gm⁻² (75, 98 and 100%, respectively). However, after 48 h of exposure, the increase in mortality was recorded at all rates, namely 53, 65, 89, 97 and 100%, respectively, while after 72 h it ranged from 97.8-100%. The total mortality (100%) was caused by KA after 7 days for all the rates applied. Like in the case of DE, the difference between mortalities caused by different inert dusts and rates, was statistically highly significant within the same exposure period i.e. after 24, 48, 72 h and 7 days (F=103.09**, 123.54**, 107.23** and 225.62**, respectively, p<0.01).

VE caused low mortality of *S. oryzae* adults irrespective of the exposure periods. After 7 days, mortality ranged from 10-17.5% depending on the rate of VE applied. The difference between mortalities was not significant at all exposure periods (F=7.11NS; 0.96NS, 0.74NS and 14.01NS, respectively, p>0.05).

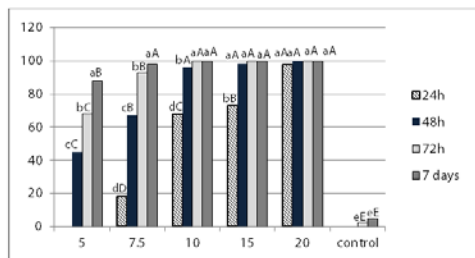


Fig. 1 The effect of DE on *S. oryzae* adults depending on the concentration and exposure period (small-case letters present differences within treatment between different days after exposure, upper-case letters present differences within day of exposure between different concentrations).

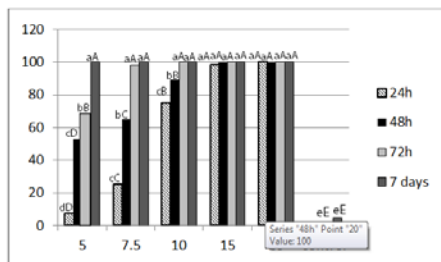


Fig. 2 The effect of KA on *S. oryzae* adults depending on the concentration and exposure period (small-case letters present differences within treatment between different days after exposure, upper-case letters present differences within day of exposure between different concentrations)

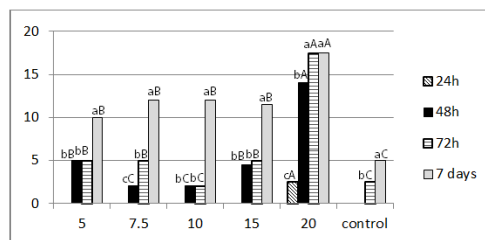
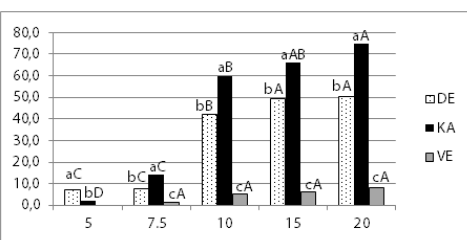


Fig. 3 The effect of VE on *S. oryzae* adults depending on the concentration and exposure period (small-case letters present differences within treatment between different days after exposure, upper-case letters present differences within day of exposure different concentrations)



Graph. 3. The effect of DE, KA and VE on *P. interpunctella* larvae depending on the concentration after 7 days (small-case letters present differences within treatment between different inert dusts, upper-case letters present differences within day of exposure between different concentrations).

According to Probit analysis (Tab. 1), the lowest LD₅₀ and LD₉₀ values were achieved by KA (6.63, 9.66, respectively) indicating at higher toxicity of kaoline to *O. oryzae* adults compared to DE (10.15, 16.52, respectively).

Table 1 Toxicity of tested inert dusts

Inert dust	LC ₅₀	LC ₉₀	Fiducial limits	Slope	Relative potency
DE	10.15	16.52	9.98-14.13	-9.83	65.32
KA	6.63	9,66	4.95-10.71	- 6.43	100.0
VE*	/	/	/	/	/

*Probynt analysis was not performed for VE due to the lack of relevant data;

Relative potency = (LC50 of the most toxic inert dust / LC50 of candidate inert dust) × 100

The effects of DE, KA and VE applied at 5, 7.5, 10, 15 and 20 gm⁻² on *P. interpunctella* larvae are presented in Fig. 4. The highest rates of DE (10, 15 and 20 gm⁻²) caused significant mortality of *P. interpunctella* larvae only after 7days, 42-50%, and the differences between the number of dead larvae was highly significant only after the last exposure period (F=9.12NS; 4.11NS; 0.79NS and 307.54**, respectively, p>0.05/p<0.01).

Discussion

According to El-Sayed, lower concentrations (0.1 and 0.2% w/w) of DE caused low mortality (16.7, 32.0%) of *S. oryzae* adults after 24 h of exposure while after 48 h the mortality increased (86.7 to 100%), regardless on the concentration. These results are in accordance with the results of this work. The high efficacy of DE (SilicoSec used in this work), was also proved by Korunić et al. (2011) reporting that this preparation was the most effective against *S. oryzae* of all other tested types of diatomaceous earth.

The results of this work are partially in agreement with those of Permul and Patourel (1990) who found that *S. oryzae* was relatively tolerant to activated kaolin (8% w/w) when exposed for 72 h on treated paddy. After 96 h mortality was 90%, and after 7 days the complete mortality was reported by mentioned authors, which was also proven in our work. Similar results were presented by Swamiappan et al. (1976) who report that kaoline clay activated by acid and heat treatments caused 100% mortality of several storage pests among which *S. oryzae* L. within 24 h, even at the minimal dose of 10 mg per Petri dish. Present findings are in agreement with observations of Verma et al. (1976). According to Jadhav (2006) kaoline was more effective than other tested materials (sand, sawdust, ash, Neem seed dust etc.), which was also proven in this work. The opposite results than those obtained in this work, were presented by El-Sayed et al. (2010) stating that, in general, DE was more effective than the kaolin against *S. oryzae*.

Results of Vukajlović et al. (2018) indicate that DE, originating from Serbia, exhibited low larvicidal efficacy against *P. interpunctella* 5th larval instar, (23.5-34.5%), while high insecticidal potential was expressed on 3rd and 4th instars larvae. Subramanyam et al. (1998) tested the efficacy of DE product Insecto against *P. interpunctella* 5th larval instar. In the same application rates as in our study authors reported that efficacy was significantly lower (10-70%) compared to the efficacy on 1st larval instar (99.5-100.0%), indicating at higher susceptibility of younger larvae to insecticidal effects of DE products than mature larvae. Susceptibility of insects to DEs can be attributed to their anatomy and physiology. Smaller insects are more susceptible because their surface area in relation to their body volume is larger than in bigger insects and therefore they lose great amounts of water from their body (Korunić, 1997). Results of many studies confirm that efficacy of inert dusts increases with the duration of exposure (Athanasios et al., 2008; 2014; 2016; Kljajić et al., 2011; Andrić et al., 2012), which was also proven in this work.

The highest rates of KA (10, 15 and 20 gm⁻²) caused significant mortality (60-75%) of *P. interpunctella* larvae only after 7 days of exposure (F=115.07**, p<0.01). However, VE caused relatively low mortalities (5 to 8%) to *P. interpunctella* larvae at all tested rates during the entire experiment. The difference between mortalities in different exposure periods (24, 48, 72 h and 7 days) was not significant (F=11.04NS; 4.50NS, 0.79NS and 7.11NS, respectively, p>0.05).

The mortality of moths increased gradually with the increase of concentration and 100% was achieved 72 h after the contact with DE and KA at 10, 15 and 20 gm⁻². Also, a faster pupation was registered for larvae exposed to inert dusts, while adult emergence was reduced and adults had smaller body-sizes, compared to control. Shah and Khan (2014) also report that almost all tested larvae made the silken-web cocoon around their body and fastly entered the diapause or changed into the pupa stage after only two days after the exposure to maize kernels treated with 1.5 gkg⁻¹ of DE. The physical properties of DE induced faster than usual pupation or diapause. The authors explain that in this way, larvae protect themselves from different negative effects of DE, such as abrasion of the cuticle, absorption of cuticular waxes from the epicuticle surface, damage to the digestive tract, blockage of the spiracles and tracheae, surface enlargement combined with dehydration and repellence caused by the physical presence of the dust.

As proven in this work, storage insects express different susceptibility to inert dusts due to morphological, physiological and ecological characteristics of each species. All insect characteristics which affect the efficacy of dusts are related to a mode by which insects sustain optimal content of water in the organism, because it has been confirmed that insects with thinner and gentler wax

layer are more susceptible to inert dusts, as insects whose body surface is smaller. Also, it was found that insects from different parts of the world show different susceptibility to dusts (Golob, 1997; Korunić, 1998a; Subramanyam and Roesli, 2000; Vayias *et al.*, 2009). Among the representatives of Coleoptera order the most susceptible to diatomaceous earth are species from the genus *Cryptolestes*, somewhat less from *Sitophilus* and *Oryzaephilus* genus, while *R. dominica* and species from the genus *Tribolium* are the least susceptible (Korunić, 1997; Arthur, 2002; Athanassiou *et al.*, 2007).

The results of this work indicate a good potential of DE and KA (10, 15 and 20 gm⁻²) to be used as a surface treatment in grain stores for prevention of infestation by *S. oryzae* adults. However, since the contact effect on *P. interpunctella* larvae was not satisfactory within the first days of exposure, the prevention from this pest should not be relied only on the application of inert dusts as surface treatments, but other measures should be involved as well.

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Biopesticidal potential of green chemicals against *Callosobruchus analis* (f.) (Coleoptera: Bruchidae)

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Abstract

Pulses have 20-27% proteins which is 2- 3 times higher than traditional cereals. These constitute the main source of proteins for developing countries, like India where per capita consumption of the animal protein is low, thus they are rightly considered the **poor man's meat**. India is largest pulse consumer, importer and producer country of the world occupying an area of 228.47 lakh hectares with the production of 17380 million tones every year. With the United Nations declaration of 2016 as International Year of Pulses to replace the social evil of malnutrition by legume, the research pertaining to the biology and bio intensive management of bruchids pests has become increasingly important. Therefore, laboratory bioassay of essential oils which are regarded as "Green Chemicals" extracted from *Zanthoxylum armatum* DC., *Rabdosia rugosa* Wall. ex Benth, *Artemisia maritima* Linn. and *Colebrookea oppositifolia* Sm. by hydro distillation was carried out against *Callosobruchus analis* (F.) to evaluate biopesticidal potential in terms of oviposition and progeny deterrence and ovicidal activities. There was a significant difference in the number of eggs laid on treated and control sets and among the different treatments of essential oils. *Z. armatum* at 100 µl/ml allowed the bruchid to lay only 19.15±3.6 eggs as compared to 82.35±4.5 in control and proved to be most effective treatment with 76.74% oviposition deterrence. *R. rugosa* and *A. maritima* oil were found most effective in reducing the egg hatchability to 48.00±3.2 and 49.52±2.2% respectively at a lowest dose of 10 µl/ml. Egg hatching inhibition percentage increased with an increase in concentration of all the treatments. *R. rugosa* oil at 100 µl/ml proved to be most effective in reducing the adult emergence with 85.48% progeny deterrence followed by *A. maritima* showing 81.67% deterrence. All the tested essential oils revealed a wide range of bioactivities against the bruchid pest.

Keywords: Oviposition deterrence, essential oils, bruchid pest, progeny emergence, ovicidal activity.