CONTACT EFFICACY OF SIX INERT DUSTS AGAINST ADULTS OF BEAN WEEVIL (*Acanthoscelides obtectus* [Say], Coleoptera, Chrysomelidae)

Monica NOVLJAN[1], Tanja BOHINC[2], Eva INDIHAR[3], Luka BATISTIČ[4], Stanislav TRDAN[5]

[1-5] University of Ljubljana, Biotechnical Faculty, Department of Agronomy

ABSTRACT

A set of laboratory experiments was conducted in 2023 in the Laboratory of Entomology of Biotechnical Faculty, University of Ljubljana, Slovenia, to study the insecticidal activities of six inert dusts against the adults of bean weevil (*Acanthoscelides obtectus* [Say]). The inert dusts used were wood ash of fir trees (*Abies alba* L.), wood ash of spruce trees (*Picea alba* L.), zeolite, quartz sand, Slovenian diatomaceous earth, and Silicosec®, a commercial brand of diatomaceous earth. Each of the inert dust was used in two concentrations, 10 and 20 g/m^2, and each combination was done in nine repetitions. Each dust was weighed directly into Petri dishes according to concentration, in which common beans (*Phaseolus vulgaris* L.) as the weevil's food source were then added and mixed with the dust. Fifteen adult weevils were introduced into each Petri dish to be in direct contact with the dust. An additional treatment of untreated beans was added as a negative control. Each experimental set was prepared four times, as they were stored in the dark condition in the four combinations of two storage temperatures of 20 and 25°C and two relative humidities (r.h.) of 55 and 75 % for two weeks. The mortality of the weevil then was counted on days 1, 2, 3, 4, and 7 to determine the mortality. On the eighth day of the experiment, the live weevils were transferred into new Petri dishes without dust treatment along with a new food source, and the mortality was counted again for days 8, 9, 10, 11, and 14 to determine the delayed mortality. The mortality and delayed mortality values were then adjusted with the mortality in the control with Abbott's formula (Abbott, 1925). This paper presents the detailed results of this experiment, for each treatment combination and each storage condition, and the potential use of inert dusts in controlling bean weevils in the future.

Keywords: alternative control, bean weevils, inert dust, plant dust, storage pest

[1] mag. ing. hort., Jamnikarjeva 101, SI-1000 Ljubljana, email: monica.novljan@bf.uni-lj.si
[2] znan. sod., dr., ibid.
[3] mag. ing. hort., ibid.
[4] mag. ing. agr., ibid.
[5] prof. dr., ibid.

IZVLEČEK

KONTAKTNO DELOVANJE ŠESTIH INTERTNIH PRAŠIV NA ODRASLE OSEBKE FIŽOLARJA (*Acanthoscelides obtectus* [Say], Coleoptera, Chrysomelidae)

V letu 2023 je bil izveden niz laboratorijskih poskusov v Laboratoriju za entomologijo Biotehniške fakultete, Univerze v Ljubljani, Slovenija, z namenom raziskati insekticidno delovanje šestih inertnih prahov na odrasle osebke fižolarja (*Acanthoscelides obtectus* [Say]). Uporabljeni inertni prahovi so bili lesni pepel jelke (*Abies alba* L.), lesni pepel smreke (*Picea alba* L.), zeolit, kremenčev pesek, slovenska diatomejska zemlja in Silicosec®, komercialna znamka diatomejske zemlje. Vsak od inertnih prahov je bil uporabljen v dveh koncentracijah, 10 in 20 g/m^2, vsaka kombinacija pa je bila ponovljena devetkrat. Prah je bil stehtan in dan v petrijevke glede na odmerjeno koncentracijo, v katere je bil dodan navadni fižol (*Phaseolus vulgaris* L.) kot vir hrane za fižolarja. V vsako petrijevko je bilo vnešenih 15 odraslih osebkov fižolarja, tako so bili neposredno v stiku s prahom. Dodatno obravnavanje netretiranega fižolovega zrnja je predstavljalo negativno kontrolo. Vsaka od štirih kombinacij je bila nato shranjena na temperaturni vrednosti 20 ali 25°C na dveh različnih vrednostih relativne vlage (55 in 75 %) dva tedna v temi. Smrtnost imagov se je ugotavljalo 1., 2., 3., 4., in 7. dan. Osmi dan eksperimenta so bili živi imagi prestavljeni v petrijevke brez prahu s svežim zrnjem fižola. Smrtnost se je nato ugotavljala 8., 9., 10., 11., in 14. dan. Vrednosti smrtnosti in zakasnjene smrtnosti smo kasneje korigirali z izračunom po Abbottovi formuli. V prispevku so predstavljeni podrobni rezultati eksperimenta za vsako obravnavanje in vsako kombinacijo ter potencialno uporabo inertnih prahov pri tretiranju fižolarja v prihodnosti.

Ključne besede: fižolar, inertni prahovi, nekemično zatiranje, rastlinski prahovi, skladiščni škodljivci

1 INTRODUCTION

The bean weevil (*Acanthoscelides obtectus* [Say]), Coleoptera: Chrysomelidae) is a species native to Central America and now is a cosmopolitan pest that feeds on vetches, beans, and other leguminous plants (Döring, 2022). Larvae and pupae of these weevils develop entirely within grain legumes. Adults emerge through a window cut in the testa, so heavy infestation can result in a large number of holed seeds, with adults moving across the stored commodity (CABI, 2021). In Slovenia, bean weevil is one of the harmful organisms with great economic significance (Trdan and Bohinc, 2019).
The methods to control bean weevils are done preventively by cultural control and sanitary methods and curatively by pesticides (CABI, 2021). In Slovenia, only one active ingredient is registered to control this species, which is based on aluminum phosphide and applied as a fumigant (Fito-info, 2024). The use of one active ingredient only can lead to pesticide resistance (Jasrotia, et al. 2022), and aluminum phosphide also have been reported as toxic to humans (Yadav et al., 2021, Mohammadinejad et al., 2022), therefore the use of non-chemical control of bean weevils should be encouraged. Some types of inert dust, such as kaolin and diatomaceous earth, have been reported to be effective in controlling stored product beetle pests (Stathers et al., 2004; Mikami et al., 2010; Viteri Jumbo et al., 2019; Wille et al.,2019), including *A. obtectus.*

Therefore, in this research, we studied the efficacy of six inert dusts against the adust of bean weevils. Most of the inert dusts we used were locally obtained, and can potentially be a sustainable control measure against bean weevil in Slovenia.

2 MATERIAL AND METHODS

2.1 Test insects

In the experiment, the adults of *A. obtecus* were obtained from the Laboratory of Entomology, Departement of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia. The insects were reared under dark conditions a room temperature (22 ± 2°C) and relative humidity (r.h.) of 55 ± 5%.

2.2 Experimental design

In this experiment, six types of inert dusts were used, which were wood ash of fir trees (*Abies alba* L.), wood ash of spruce trees (*Picea alba* L.), zeolite, quartz sand, Slovenian diatomaceous earth, and Silicosec®, a commercial brand of diatomaceous earth. Common beans (*Phaseolus vulgaris* L.) were used as a food source for the weevils. Each of the inert dust was used in two concentrations, 10 and 20 g/m^2, and each combination was done in nine repetitions.

Each of the inert dust according to the treatment and concentration was weighed directly into a Petri dish in which a sufficient amount of common beans were then added and mixed. Fifteen adult weevils then were introduced into the Petri dishes, and the dishes were sealed with sellotape. One treatment of untreated beans (without dust) was used as a control treatment. The whole set of 13 treatments then was stored under a certain storage temperature and relative humidity (r.h.) for two weeks. There were four different storage combinations used, which were the combinations of two temperatures (20 and 25 °C) and two r.h. (55 and 75%).

The numbers of weevils were counted every day on days 2, 3, 4, 5, and 8 to determine the percentage of direct mortality because of the dust. On day 8, the live weevils were transferred into a clean Petri dish with untreated beans and were stored again for a week. The numbers of weevils were counted again on days 9, 10, 11, and 12 to determine the percentage of delayed mortality. The direct and delayed mortality values were then adjusted with the mortality in the control with Abbott's formula (Abbott, 1925).

2.3 Statistical analysis

For data processing, we used the Statgraphics Centurion XVII program. General statistical analysis was performed using multifactor analysis of variance (MANOVA), in which the days, the type of dust, the dust concentrations, the storage temperatures, and the storage humidities acted as factors. The statistical differences within individual factors in the experiment were calculated using a one-way analysis of variance (ANOVA). The Duncan Multiple Range Test for multiple comparisons ($P \leq 0.05$) was used to statistically evaluate the differences in the average value for each parameter. The results of the experiment were graphically displayed using the Microsoft Excel program.

3 RESULTS AND DISCUSSION

3.1 The effect of the days, treatments, inert dust concentrations, storage temperatures, and storage humidities on the average of corrected mortality and delayed mortality of *A. obtectus*

The Manova results showed that the average corrected mortality (days 1-7) of *A. obtectus* is affected by days (*df*: 4, *F*-ratio: 925.18, *p*-value: 0.0000), concentrations (*df*: 1, *F*-ratio: 59.82, *p*-value: 0.0000), treatments (*df*: 5, *F*-ratio: 2622.57, *p*-value: 0.000), storage temperatures (*df*: 1, *F*-ratio: 219.40, *p*-value: 0.0000), and storage relative humidities (*df*: 1 , *F*-ratio: 4.83, *p*-value: 0.0280). Meanwhile, the average of corrected delayed mortality (days 8-14) is affected by days (*df*: 4, *F*-ratio: 8.40, *p*-value: 0.0000), concentrations (*df*: 1, *F*-ratio: 9.57, *p*-value: 0.0020), treatments (*df*: 5, *F*-ratio: 1131.84, *p*-value: 0.0000), storage temperatures (*df*: 1, *F*-ratio: 117.15, *p*-value: 0.0000), and storage relative humidities (*df*: 1, *F*-ratio: 117.15, *p*-value: 0.0000). Figures 1-6 showed the value of the mentioned in every factor studied.

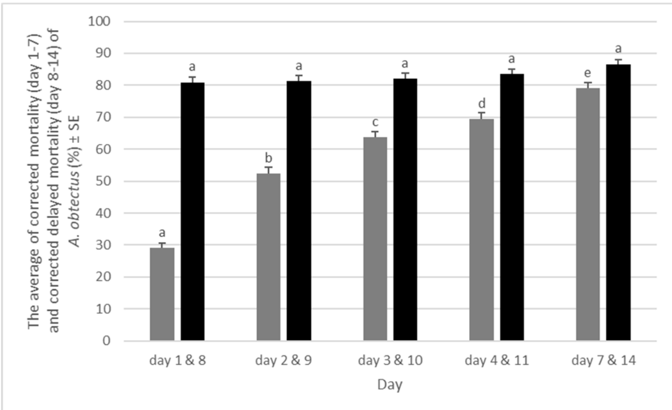

Figure 1: The average of corrected mortality (days 1-7) and the average of corrected delayed mortality (days 8-14) of *A. obtectus* between different days. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

In general, the results showed that both mortality (days 1-7) and delayed mortality (days 8-14) of *A. obtectus* are influenced by days/ length of exposure, treatments, concentration, and storage temperature, but are not influenced by storage r.h. (Figure 1-5). In general, in the first week, the weevil's mortality increased by the day and reached the maximum on the fifth day of exposure (Figure 1). In the second week, the delayed mortality still increased by day but not in a significant difference between the days. In the first week, the mortalities were the highest in the treatment with SilicoSec®, but after a week, the treatments with both wood ashes and Slovenian diatomaceous earth resulted in the same level of delayed mortalities compared to SilicoSec® (Figure

2). The efficacies of zeolite and quartz sand were both inferior compared to the efficacies of the mentioned four treatments.

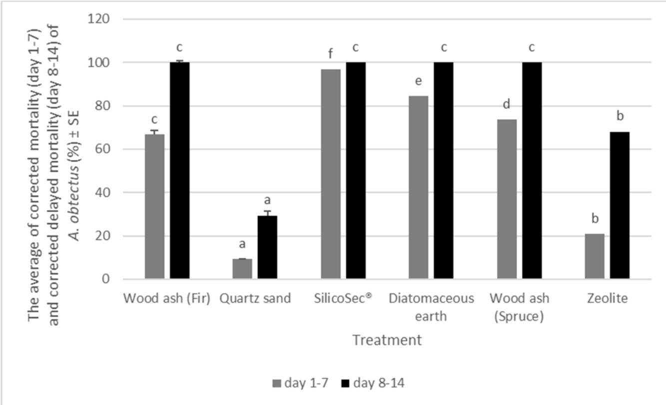

Figure 2: The average of corrected mortality (days 1-7) and the average of corrected delayed mortality (days 8-14) of *A. obtectus* between different treatments. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

The concentrations of dust influenced the mortality but not the delayed mortality (Figure 3). The concentration of 20 g/m^2 resulted in higher mortality in the first week compared to the concentration of 10 g/m^2, but the final delayed mortality results after two weeks were the same between both concentrations. Meanwhile, the temperature of 25 °C resulted in higher mortality and delayed mortality of the weevils as compared to 20 °C (Figure 5).

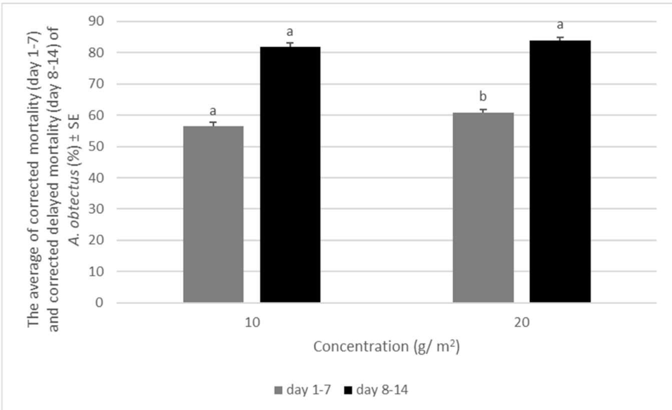

Figure 3: The average of corrected mortality (days 1-7) and the average of corrected delayed mortality (days 8-14) of *A. obtectus* between different inert dust concentrations. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

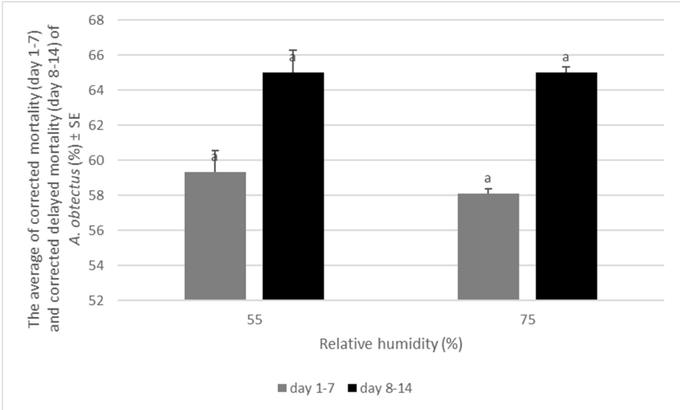

Figure 4: The average of corrected mortality (days 1-7) and the average of corrected delayed mortality (days 8-14) of *A. obtectus* between storage temperatures. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

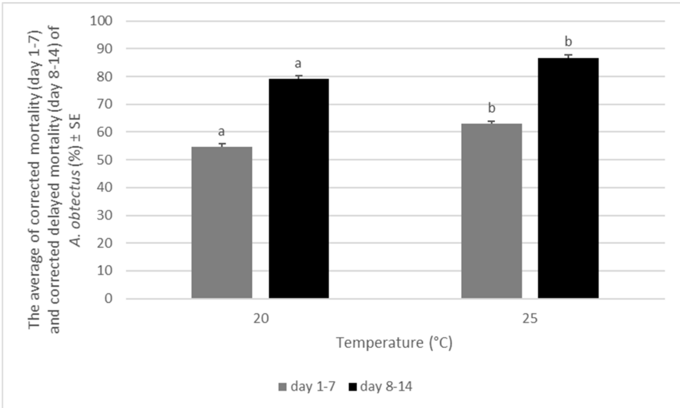

Figure 5: The average of corrected mortality (days 1-7) and the average of corrected delayed mortality (days 8-14) of *A. obtectus* between storage temperatures. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

Previously, a similar study by Bohinc et al. (2013) assessed the efficacy of diatomaceous earth against *A. obtectus*, specifically in five different temperatures between 15 and 35 °C, two relative humidities of 55 and 75%, and four different concentrations between 100 and 900 ppm. They reported that the increase in temperature increased the efficacy of diatomaceous earth, while the relative humidity did not have a significant effect on the weevil's morality. The study recommended the use of diatomaceous earth in controlling *A. obtectus*, as this inert dust showed high efficacy in lower concentrations. These mentioned results in general were very similar to our findings, in particular as shown in Figure 3-5.

Wille et al. (2019) studied two types of diatomaceous earth from the brewing industry, which were called conventional and residue diatomaceous earth, in controlling *A. obtectus*. A conventional diatomaceous earth means that it hasn't been used as a filter for the beer clarification process, whereas a residue explains the diatomaceous earth that was previously used in the filtering process. The study showed that higher concentrations of inert dust and a longer exposure time increased the mortality of *A. obtectus.* The study stated that the smallest dust concentrations of 0.5 g/ kg of beans of conventional and residue diatomaceous earth, respectively, resulted in 96 and 59% mortalities of *A. obtectus.* Additionally, after 7 days of exposure, both types of diatomaceous earth resulted in 100 and 90% *A. obtectus* mortalities, respectively.
Another study was also reported by Viteri Jumbo et al. (2019), stating that the dose of diatomaceous earth, temperature, and period of exposure positively affected the mortality of *A. obtectus.* It was found that between the range of temperature tested (25-35 °C), the temperature above 30 °C caused a mortality of at least 90%. Additionally, regardless of the dose and temperature used, all treatments with diatomaceous earth reduce the offspring production of *A. obtectus* to over 95%.

3.2 The corrected mortality of *A. obtectus* in each storage combination

Based on all the results mentioned in the previous chapter, diatomaceous earth can be recommended as an alternative control tool for *A. obtectus.* However, in our research, we discovered that wood ash of fir and spruce trees was also as effective as Slovenian diatomaceous earth and SilicoSec®. The performance of every single inert dust we tested in causing mortality of *A. obtectus,* particularly in every single storage condition, is shown in Figure 6-9.

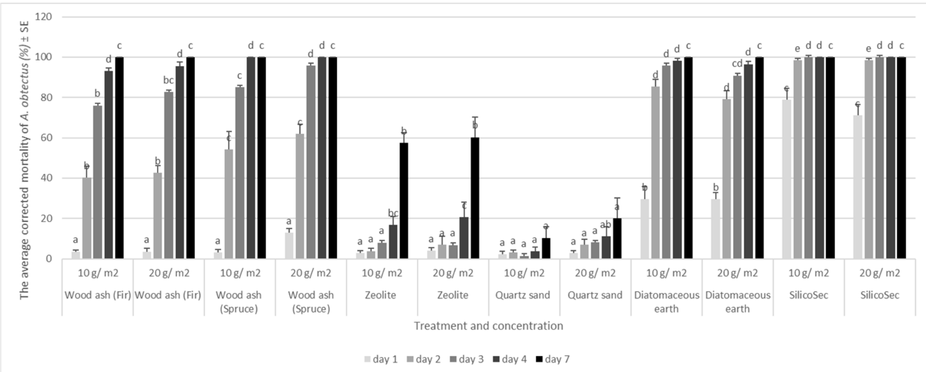

Figure 6: The average of corrected mortality (days 1-7) of adult bean weevil A. obtectus in the storage combinations of 20 °C 55% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test (p ≤ 0.05).

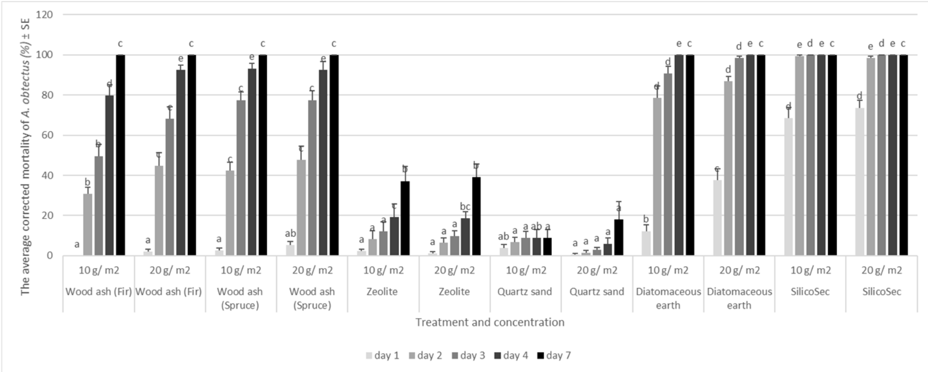

Figure 7: The average of corrected mortality (days 1-7) of adult bean weevil A. obtectus in the storage combinations of 20 °C, 75% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

A closer look at every storage combination (20°C-55%, 20°C-75%, 25°C-55%, and 25°C-75%) showed a consistent trend in weevil mortality and delayed mortality. SilicoSec® treatment was effective right from the first day, and the mortality in general had reached 100% by the second day. Meanwhile, the mortalities by wood ashes and diatomaceous earth increased every day and reached 100% on the fourth or fifth day. The mortalities by the zeolite and quartz sand treatments were also increasing by day, but overall the mortalities were lower compared to other treatments. Previously, the longer period of exposure had been known to increase *A. obtectus* mortality (Chapter 3.1).

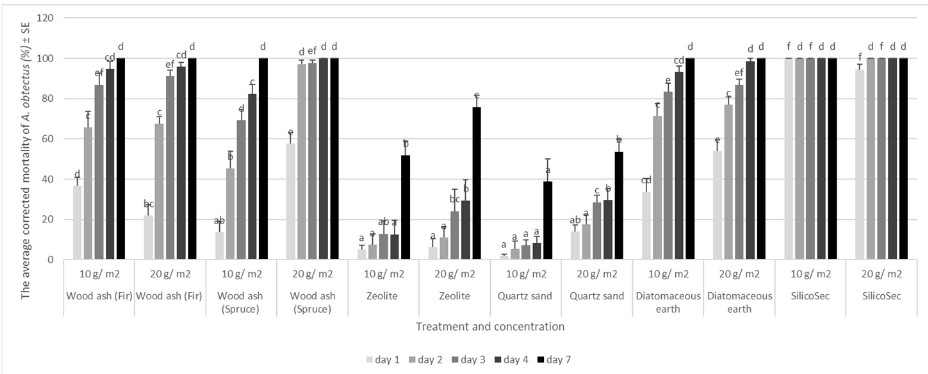

Figure 8: The average of corrected mortality (days 1-7) of adult bean weevil A. obtectus in the storage combinations of 25 °C, 55% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

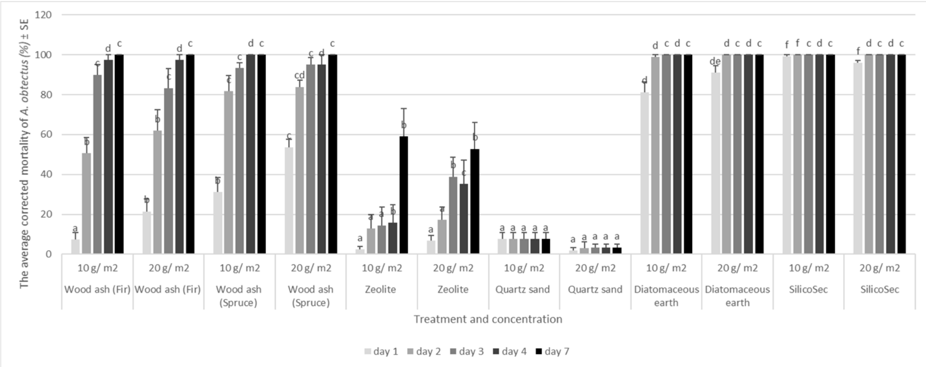

Figure 9: The average of corrected mortality (days 1-7) of adult bean weevil A. obtectus in the storage combinations of 25 °C, 75% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

3.3 The corrected delayed mortality of *A. obtectus* in each storage combination

Continuously, after assessing the effect of inert dust in causing mortality of *A. obtectus,* we studied the effect of those dusts on *A. obtectus* delayed mortality. The performance of every single inert dust we tested in causing delayed mortality of *A. obtectus,* particularly in every single storage condition, is shown in Figure 10-13.
In the second week and after the transfer of treated weevils to the untreated food source, it was observed that the delayed mortalities in wood ashes, diatomaceous earth, and SilicoSec® treatments had already reached 100%. The treatment with zeolite caused 100% delayed mortalities only at 25°C and less mortality at 20 °C, while the treatment with quartz sand never caused 100% delayed mortality. Previously, the higher storage temperature had been known to increase *A. obtectus* mortality (Chapter 3.1).

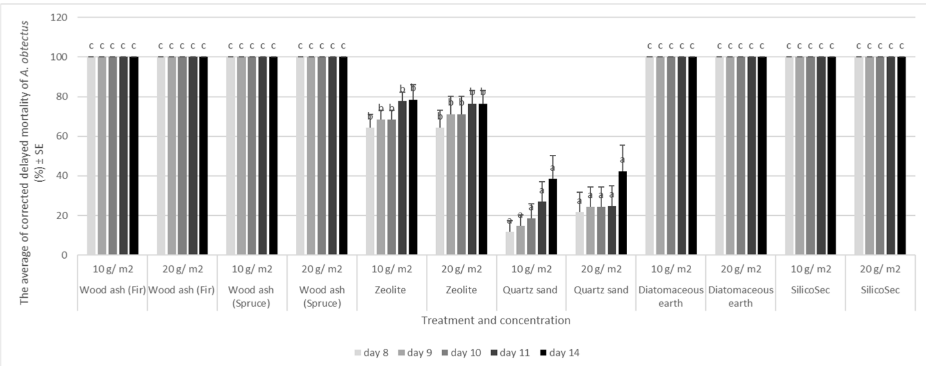

Figure 10: The average of corrected mortality (day 1-7) of adult bean weevil *A. obtectus* in the storage combinations of 20 °C, 55% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

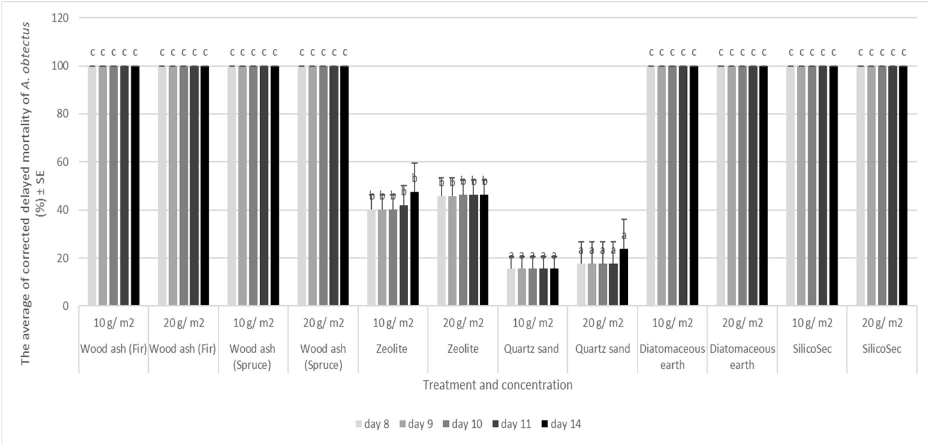

Figure 11: The average of corrected mortality (day 1-7) of adult bean weevil *A. obtectus* in the storage combinations of 20 °C, 75% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

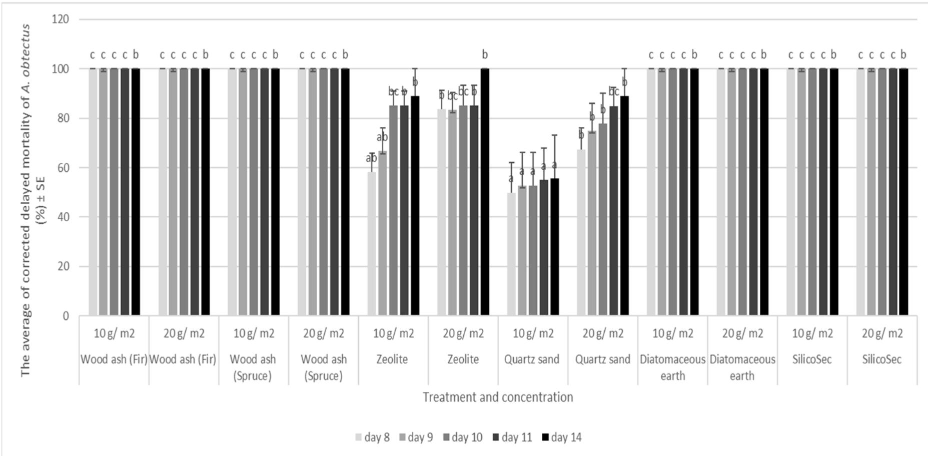

Figure 12: The average of corrected mortality (day 1-7) of adult bean weevil *A. obtectus* in the storage combinations of 25 °C, 55% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).
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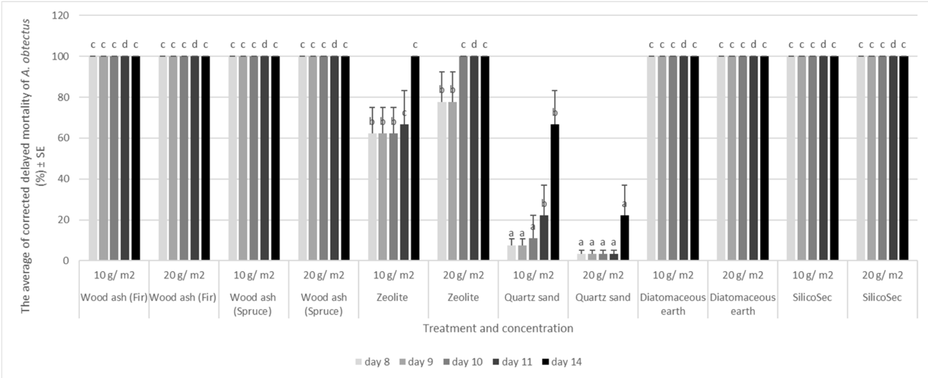

Figure 13: The average of corrected mortality (day 1-7) of adult bean weevil *A. obtectus* in the storage combinations of 25 °C, 75% r.h. Above the bars with the same color, the same letter shows no differences by Duncan's multiple-range test ($p \leq 0.05$).

4 CONCLUSIONS

The efficacy of wood ashes of fir and spruce trees and Slovenian diatomaceous earth in controlling bean weevils with contact method is as high as that of a commercial diatomaceous earth SilicoSec®. All these treatments are recommended as a non-chemical method to control bean weevils *A. obtecus.* A lower concentration of 10 g/m^2 of wood ashes, diatomaceous earth, and SilicoSec® is sufficient enough to control *A. obtectus* effectively. Additionally, the higher temperature of 25°C caused higher mortality and delayed mortality of *A. obtectus* compared to 20°C.

5 ACKNOWLEDGEMENT

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