Spanish Journal of Agricultural Research
15 (3), e1009, 8 pages (2017)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2017153-11172
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Comparison of insecticidal efficacy of four natural substances against granary weevil (*Sitophilus granarius* [L.]) adults: does the combined use of the substances improve their efficacy?

Tanja Bohinc and Stanislav Trdan

University of Ljubljana, Biotechnical Faculty, Dept. Agronomy, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Abstract

Laboratory tests were carried out to evaluate the insecticidal efficacy of different natural inert dusts (diatomaceous earth, wood ash, quartz sand) and the leaf powder of *Azadirachta indica* A. Juss. against granary weevil (*Sitophilus granarius* [L.]) adults. The efficacy of the substances was tested individually and in combination with each other. The substances were applied at different concentrations, and bioassays were carried out at four different temperatures (20, 25, 30 and 35°C) and two different relative humidity (RH) levels (55% and 75%). The adult mortality was recorded after the 7th, 14th and 21st days of exposure. The progeny production of individuals exposed to different combinations was also assessed. Wood ash proved to be the most efficient inert dust in our research. We detected 100% mortality in the treatment exposed to a higher concentration (5 w%) of wood ash at 35°C and 55% RH after 7 days of exposure. A lower RH level had also a negative impact on the progeny production. We can conclude that wood ash can be efficient in controlling granary weevil adults as a single substance or in combination with other substances. Further surveys should focus on the impact of the wood ash dose rates. Due to the high percentage of area covered with forest in some European countries, the main ingredient is present locally, but additional surveys are needed to help improve the practical use of wood ash.

Additional keywords: wood ash; inert dusts; botanical insecticide.

Authors' contributions: Designed the research and wrote the manuscript: TB and ST. Performed the counting of specimens, and statistically analysed the data: TB.

Citation: Bohinc, T.; Trdan, S. (2017). Comparison of insecticidal efficacy of four natural substances against granary weevil (*Sitophilus granarius* [L.]) adults: does the combined use of the substances improve their efficacy? Spanish Journal of Agricultural Research, Volume 15, Issue 3, e1009. https://doi.org/10.5424/sjar/2017153-11172.

Received: 07 Feb 2017. **Accepted:** 05 Jul 2017

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Tanja Bohinc: tanja.bohinc@bf.uni-lj.si

Introduction

The granary weevil (*Sitophilus granarius* [L.]) is an important primary stored product insect pest of cereals, and its economic impact has been controlled with various methods in different regions of the world. The application of synthetic insecticides has proven to cause negative impacts on the environment, non-target organisms and people's health, which has stimulated the search for alternative ways of controlling pests (Hamza *et al.*, 2016). For this reason, the control of stored product pests using inert dusts (diatomaceous earth, quartz sand, wood ash, zeolites) (Mohapatra *et al.*, 2015) and plant preparations, such as plant extracts, essential oils and powdered parts of plants (Bohinc *et al.*, 2013; Bouayad *et al.*, 2013; Mohapatra *et al.*, 2015) has been investigated. Diatomaceous earth stands out among the inert dusts by its extent of application (Korunic, 1998; Kavallieratos *et al.*, 2015), while research on the insecticidal properties of wood ash has been most frequently undertaken in African countries and India (Demissie *et al.*, 2008; Jean *et al.*, 2015). Research on insecticidal dusts made of powdered plant leaves is common both in Europe (Bohinc *et al.*, 2013) and elsewhere (Mkenda *et al.*, 2015).

The purpose of our research was to investigate the insecticidal effects of three inert dusts and one plant powder applied for the suppression of granary weevils independently and in combinations.

Material and methods

Natural substances, commodity and insects

Four different natural substances were used in the tests: diatomaceous earth, wood ash, quartz sand, and neem leaf powder. As the diatomaceous earth, we used the commercial product SilicoSec® (manufacturer: Biofa, Germany); supplier: Metrob d.o.o., Slovenia), and wood ash (from European beech [*Fagus sylvatica* L.]) was obtained from a local household (location: Logatec, Slovenia). We used local quartz sand (location: Moravče, Slovenia), and neem leaf powder (product Neem listni prah®; supplier Azimut-Vester, Ltd., Slovenia). The tested wheat grain was infestation- and pesticide-free.

The granary weevils used in the tests were reared at the Laboratory of Entomology, Department of Agronomy, Biotechnical Faculty, University of Ljubljana. The age of the unsexed exposed granary weevils was unidentified.

Bioassay

Exposure studies were carried out in incubators set at 20°C, 25°C, 30°C and 35°C, 55% and 75% relative humidity (RH), and continuous darkness, and the mortality was assessed on the 7th, 14th and 21st days after exposure. We tested the insecticidal properties of the four substances mentioned above, applied individually or in combination with each other. For each application treatment, 500 g lots were prepared. All treatments are presented in Table 1. The lots of wheat grain were placed into a plastic container of 2 L, and a specific powder or powder combination was added to each plastic container (Trdan & Bohinc, 2013). The preparation of each individual treatment (in a 100-mL vial) was done according to Bohinc *et al.*, 2013). Untreated wheat served as control treatment. All bioassays were repeated three times, and after day 21, the progeny tests were performed. For each specific treatment exposed to specific parameters (temperature & RH), all 100 mL vials were stirred into 1000 mL vials. Then, after 56 days, the vials were opened, and then the numbers of progeny were counted. The number of progeny was expressed as an average number per treatment.

Chemical analysis of substances used in our research and their particle size

According to Rojht *et al.* (2010a), the diatomaceous earth that was used in our experiment, contained 87.47% SiO_2. The SilicoSec® particle size was between 8 and 12 µm (Vayias & Athanassiou, 2004). Quartz sand contained 99.24% SiO_2, and the other components present in quartz sand included 0.13% Al_2O_2, 0.06% Fe_2O_3, 0.02% CaO, and traces of MnO, P_2O_5, TiO_2, Na_2O and MgO that were less than 0.01% (Rojht *et al.*, 2010b). The particle size of all quartz sands used in this study was 12 µm. According to Kranjc *et al.* (2009), wood ash contained 25-26 w% (as weight percent) SiO_2. We sieved the wood ash and Neem listni prah® through a 1 mm mesh. The main active ingredient in neem is azadirachtin, which is a limonoid; it is available in up to 96% purity (Gahukar, 2014). Other limonoids in neem are melantriol, salannin, and nimbin (NRC, 1992).

Data analysis

The mortality counts were corrected according to Abbott's formula (Abbott, 1925). The data were analysed using repeated measure analysis (Statgraphics Centurion XVI, 2009) with the exposure interval set as the repeated factor, the insect mortality set as the response variable and the treatment, dose, temperature and RH set as the main effects. The associated interactions of the main effects were also included in the analysis. The means were separated using the

Table 1. Combinations of inert dusts tested in our research

Treatment	Substance(s)
T1	SilicoSec® 450 ppm
T2	SilicoSec® 900 ppm
T3	Wood ash 5 w%
T4	Wood ash 2.5 w%
T5	SilicoSec® (450 ppm) + Wood ash (2.5 w%)
T6	Neem listni prah® (1.25 w%)
T7	Neem listni prah® (2.5 w%)
T8	Neem listni prah® (2.5 w%) + Wood ash (2.5 w%)
T9	Quartz sand 450 ppm
T10	Quartz sand 900 ppm
T11	Quartz sand 450 ppm + Wood Ash (2.5 w%)
T12	SilicoSec® (225 ppm) + Wood ash (1.25 w%) + Neem listni prah® (0.0625%) + Quartz sand (225 ppm)
T13	Control

Tukey and Kramer HSD tests at $p<0.05$ (Statgraphics Centurion XVI, 2009).

Results

General mortality of *S. granarius*

All the main effects and associated interactions for the corrected mortality of *S. granarius* adults were significant at $p<0.005$ (Table 2).

The mortality of adults was significantly different within the exposure intervals, $(F_{2,2642}) = 88.34$, $p<0.0001$. All the main effects and associated interactions for the corrected mortality of weevil adults between exposure intervals (df = 880) are presented in Table 3.

Mortality of *S. granarius* after 7 days of exposure

After 7 days, more than 48% (48.52±1.49) of the weevil adults exposed to 2.5 w% wood ash at 20°C and 55% RH were dead (Table 4). The mortality rates of the granary weevils exposed to SilicoSec®, quartz sand and Neem listni prah® were significantly lower. The mortality of the individuals exposed to 75% and 20°C was significantly the highest in the treatments where 2.5 w% wood ash was used, and the mortality in vials exposed to 2.5 w% wood ash at 25°C and 55% RH reached > 90%. At 25°C and 55% RH, the mortality was significantly the highest for T11. At 25°C and 75% RH, the highest mortality was 24%, in T12.

At 30°C and 55% RH, the weevil mortality in T2 did not exceed 55.19% (50.19±5.00). The mortality was the highest in T4 (98.51±0.81%), T5 (99.25±0.49%), T11 (98.89±0.35%) and T12 (100.00±0.00%). At 30°C and 75% RH, the mortality of the weevil adults did not exceed 12%, and the highest mortality was established in T5. Finally, at 35°C, the mortality was lower in the treatments exposed to 75% RH in comparison with those exposed to 55% RH.

Mortality of *S. granarius* after 14 days of exposure

After 14 days, the mortality of the individuals after exposure to 20°C and 55% RH was significantly the highest in the T4 (93.33±2.72%) and T5 (93.70±2.32%) treatments. At 20°C and 75% RH, the mortality was significantly the highest in T11 (75.92±3.22%) and T12 (75.55±3.14%). At 25°C and 55% RH, the highest mortality occurred in T3, T4, T5, and T12, in which all weevils were dead. On the other hand, the mortality in the treatments exposed to 25°C and 75% RH was significantly the highest in T5 (58.15±2.99%). T5 (57.69±4.85%) also had the highest mortality at 30°C and 75% RH. In the case of 30°C and 55% RH, the mortality of the individuals was significantly the highest (100.00±0.00%) in T3, T4, T5, T8, T11 and T12. For 35°C, at 55% RH, mortality was significantly the lowest in T9 and T10 (both <90%) (Table 5). In all other treatments, the mortality reached 100%. At 35°C and 75% RH, the highest mortality (100.00±0.00%) was recorded in T1, T3, T4, T5, T6, T7, T8 and T9.

Mortality of *S. granarius* after 21 days of exposure

After 21 days, the mortality of the individuals exposed to 20°C and 55% RH was significantly the highest in the T4 (96.67±0.19%), T5 (96.30±0.25%) and T12 (98.51±0.23%) treatments (Table 6). At 20°C and 75% RH, the highest mortality was recorded in the vials exposed to T5 (98.51±1.13%) and T12 (93.66±1.97%). All individuals were dead in T1, T2, T3, T4, T5, T8, T11, and T12. At 30°C and 75% RH, the highest mortality was recorded in T5 (86.31±3.95%). At

Table 2. MANOVA parameters for main effects and associated interactions for mortality levels of *S. granarius* adults (df =2406).

Source	Df	F	*p*
Temperature	3	2277.63	<0.0001
Relative humidity	1	1602.22	<0.0001
Treatment	11	401.37	<0.0001
Exposure interval	2	677.81	<0.0001
Treatment × Temperature	33	47.48	<0.0001
Treatment × Relative humidity	11	25.56	<0.0001
Temperature × Relative humidity	3	386.82	<0.0001
Treatment × Exposure interval	22	6.55	<0.0001
Relative humidity × Exposure interval	2	6.67	0.0013
Temperature × Exposure interval	6	40.21	<0.0001

Table 3. MANOVA parameters for main effects and associated interactions for mortality levels of *S. granarius* adults between exposure intervals (for all intervals, error df =880).

Exp. interval	Source	Df	F	*p*
Day 7	Treatment	11	141.15	<0.0001
	Relative humidity	1	654.79	<0.0001
	Temperature	3	1604.15	<0.0001
	Treatment × Relative humidity	11	44.52	<0.0001
	Treatment × Temperature	33	17.42	<0.0001
	Temperature × Relative humidity	3	204.01	<0.0001
Day 14	Treatment	11	290.29	<0.0001
	Relative humidity	1	1321.93	<0.0001
	Temperature	3	1447.15	<0.0001
	Treatment × Relative humidity	11	25.17	<0.0001
	Treatment × Temperature	33	41.65	<0.0001
	Temperature × Relative humidity	3	309.34	<0.0001
Day 21	Treatment	11	202.58	<0.0001
	Relative humidity	1	581.08	<0.0001
	Temperature	3	607.82	<0.0001
	Treatment × Relative humidity	11	5.88	<0.0001
	Treatment × Temperature	33	39.36	<0.0001
	Temperature × Relative humidity	3	132.09	<0.0001

Table 4. Mean corrected mortality (%) of *S. granarius* adults exposed for 7 days to different natural substances (lowercase letters present differences between treatments when exposed to one combination of temperature and relative humidity level).

Treat.	RH 55%				RH 75%			
	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
T1	2.59±0.50b	1.79±0.97b	11.53±4.10bc	95.11±0.31d	5.18±1.94b	2.22±0.08c	1.03±0.12d	91.47±2.56c
T2	4.81±0.81c	0.33±0.21a	50.19±5.00d	100.00±0.00f	7.41±1.21c	5.55±0.38d	5.66±0.27f	98.29±0.85d
T3	7.78±1.58d	87.54±4.34e	87.36±3.77e	100.00±0.00f	22.22±2.48ef	13.33±0.32f	2.02±0.11e	99.43±0.39de
T4	48.52±1.59g	90.94±2.77f	98.51±0.81f	100.00±0.00f	24.81±2.78f	23.70±0.52h	9.34±0.41h	98.86±0.75d
T5	25.18±2.84e	83.01±1.88e	99.25±0.49f	100.00±0.00f	58.15±3.00g	31.48±0.51i	11.15±0.22i	100.00±0.00e
T6	0.00±0.00a	0.49±0.25a	11.90±1.36c	97.78±0.21e	0.00±0.00a	0.00±0.00a	0.00±0.00a	97.16±1.23d
T7	0.00±0.00a	1.96±0.94b	7.43±1.36b	92.44±0.27c	0.00±0.00a	0.37±0.37a	0.00±0.00a	97.16±0.89d
T8	0.00±0.00a	69.80±3.12d	93.31±7.29ef	100.00±0.00f	0.00±0.00a	8.14±0.38e	0.68±0.07c	99.43±0.56de
T9	0.00±0.00a	1.04±0.54ab	1.73±2.77a	58.22±0.87a	0.00±0.00a	0.00±0.00a	0.00±0.00a	78.41±5.02b
T10	0.00±0.00a	4.39±1.21c	7.14±0.34b	71.56±0.83b	0.00±0.00a	0.37±0.06b	0.34±0.05b	28.92±6.86a
T11	30.37±4.69f	98.11±0.99f	98.89±0.35f	100.00±0.00f	13.33±2.78d	15.18±0.43g	8.12±0.30g	76.14±2.82b
T12	22.96±4.29e	88.30±2.40ef	100.00±0.00g	100.00±0.00f	19.26±2.34e	24.07±0.46h	8.12±0.30g	89.21±2.47c

35°C, we recorded 100% mortality in nearly all treatments, exposed to both RH values, except in T12 (93.54±2.82%), which was exposed to 35°C and 75% RH.

Progeny production of *S. granarius*

We recorded no progeny production on wheat exposed to 35°C, and there was no impact of the RH value. Regarding temperatures 20°C, 25°C and 30°C number of individuals was the highest in control treatments (non-treated grain). If we compare number of weevils born in vials treated with SilicoSec® to number of vials treated with wood ash (applied alone), we can confirm that progeny production was influenced by wood ash (applied alone) All values are presented in Table 7.

Table 5. Mean corrected mortality (%) of *S. granarius* adults exposed for 14 days to different natural substances (lowercase letters present differences between treatments when exposed to one combination of temperature and relative humidity level).

Treat	RH 55%				RH 75%			
	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
T1	8.52±1.58c	11.85 ±0.29d	79.91±3.78c	100.00±0.00b	8.89±2.42d	4.81±1.26c	8.63±3.41c	100.00±0.00c
T2	24.07±3.64d	37.20±0.35f	99.21±0.52d	100.00±0.00b	22.59±3.59e	13.70±1.79d	21.56±4.76d	91.63±3.19b
T3	59.63±6.10e	100.00±0.00i	100.00±0.00e	100.00±0.00b	42.22±4.55f	52.60±3.23f	35.69±5.99e	100.00±0.00c
T4	93.33±2.72h	100.00±0.00i	100.00±0.00e	100.00±0.00b	42.22±5.06f	52.22±2.89f	41.64±4.06d	100.00±0.00c
T5	93.70±2.32h	100.00±0.00i	100.00±0.00e	100.00±0.00b	64.55±3.01g	58.15±2.99g	57.69±4.85f	100.00±0.00c
T6	3.71±2.69b	3.62±0.32b	77.16±2.22c	100.00±0.00b	0.00±0.00a	0.00±0.00a	0.00±0.00a	100.00±0.00c
T7	0.00±0.00a	4.66±0.15c	65.74±4.79b	100.00±0.00b	0.37±0.37a	0.37±0.37a	0.00±0.00a	100.00±0.00c
T8	77.04±5.54f	70.50±0.45g	100.00±0.00e	100.00±0.00b	3.33±0.96c	16.67±1.67e	9.16±1.57c	100.00±0.00c
T9	0.74±0.49b	1.45±0.13a	30.31±5.15a	88.89±10.01a	1.48±0.81b	0.00±0.00a	0.00±0.00a	100.00±0.00c
T10	1.48±0.58b	14.36±0.37e	31.10±3.69a	88.89±10.01a	0.37±0.37a	1.11±0.55b	0.68±0.42b	78.71±7.45a
T11	82.96±5.10g	99.62±0.06h	100.00±0.00e	100.00±0.00b	75.92±3.22h	48.51±3.04f	25.39±1.30d	97.87±2.13bc
T12	89.63±3.16g	100.00±0.00i	100.00±0.00e	100.00±0.00b	75.55±3.14h	48.15±2.83f	25.39±1.30d	91.49±4.64b

Table 6. Mean corrected mortality (%) of *S. granarius* adults exposed for 21 days to different natural substances (lowercase letters present differences between treatments when exposed to one combination of temperature and relative humidity level).

Treat.	RH 55%				RH 75%			
	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
T1	21.85±0.29e	72.74±3.40d	100.00±0.00e	100.00±0.00a	13.43±2.36d	17.17±4.44d	2.43±3.70ab	100.00±0.00b
T2	50.00±1.36f	76.32±2.88d	100.00±0.00e	100.00±0.00a	34.71±1.49e	39.93±2.77f	65.01±1.87e	100.00±0.00b
T3	74.81±1.16g	100.00±0.00e	100.00±0.00e	100.00±0.00a	46.28±5.74f	89.53±2.83h	76.04±3.18f	100.00±0.00b
T4	96.67±0.19i	100.00±0.00e	100.00±0.00e	100.00±0.00a	55.60±4.97g	97.01±1.53i	74.14±3.70f	100.00±0.00b
T5	96.30±0.25i	100.00±0.00e	100.00±0.00e	100.00±0.00a	98.51±1.13i	31.48±3.20e	86.31±3.95g	100.00±0.00b
T6	8.89±0.53d	8.21±0.87a	99.33±0.11d	100.00±0.00a	0.00±0.00a	2.28±0.96b	0.16±0.11a	100.00±0.00b
T7	4.07±0.21b	8.40±2.03a	78.67±0.84c	100.00±0.00a	0.29±0.29a	1.54±0.60a	0.63±0.46a	100.00±0.00b
T8	95.92±0.45h	67.82±2.99c	100.00±0.00e	100.00±0.00a	2.86±0.86bc	80.22±3.03g	28.51±3.15c	100.00±0.00b
T9	1.11±0.08a	15.07±10.32b	53.34±1.19a	100.00±0.00a	1.83±0.68b	1.91±0.85a	1.94±0.91ab	100.00±0.00b
T10	6.29±0.27c	74.07±5.02d	56.67±1.00b	100.00±0.00a	2.61±0.00c	4.35±1.50c	3.25±1.01b	100.00±0.00b
T11	95.55±0.25h	100.00±0.00e	100.00±0.00e	100.00±0.00a	93.28±1.76h	96.27±1.03i	46.39±4.11d	100.00±0.00b
T12	98.51±0.23j	100.00±0.00e	100.00±0.00e	100.00±0.00a	97.76±1.58i	93.66±1.97h	46.39±4.11d	93.54±2.82a

Dicussion

The insecticidal effects of different inert dusts have been for the last decades a subject of numerous studies (Fields *et al.*, 2001). These studies have focused primarily on the research of diatomaceous earth (Korunic, 1998; Vayias *et al.*, 2009; Kavallieratos *et al.*, 2015). A growing body of research now focuses on the study of the combined effects of inert dusts (diatomaceous earth, kaolin, etc.) and essential oils (Campolo *et al.*, 2014), as well as the effects of environmentally more acceptable insecticides in combination with diatomaceous earth and entomopathogenic fungi (Athanassiou *et al.*, 2016) on the mortality rates of stored product insect pests. In view of the presented information, our research tested the combined use of one or more inert dusts, since we have not come across any studies applying this approach.

After 7 days of a combined application of diatomaceous earth and wood ash at 30°C and 55% RH, we achieved 99.99% mortality of the granary weevil adults. The insecticidal effects of wood ash used independently were very pronounced. Our findings are congruent with those reported by Tadesse & Basedow (2005), *i.e.*, wood ash

Table 7. Total number of progeny generation of *S. granarius* adults after 56 days.

Treat.	RH 55%				RH 75%			
	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
T1	177	442	256	0	200	870	980	0
T2	38	366	23	0	180	235	110	0
T3	10	0	0	0	70	240	70	0
T4	20	15	0	0	30	205	60	0
T5	12	12	0	0	20	112	60	0
T6	412	588	113	0	502	1221	517	0
T7	350	455	283	0	370	1343	355	0
T8	8	311	0	0	10	524	98	0
T9	321	252	812	0	302	456	1006	0
T10	55	313	700	0	277	476	908	0
T11	86	25	0	0	99	961	12	0
T12	5	1	0	0	22	113	0	0
Control	494	629	913	0	581	1509	1704	0

was in vitro very effective in suppressing stored product pests. These authors proved the satisfactory insecticidal effects of wood ash on the species *Sitophilus zeamais* Motsch.

After 14 days at 55% RH at temperatures 25-35°C, we achieved 100% mortality of individuals in all treatments with wood ash. This proves that the temperature and relative air humidity are important factors in the beetle mortality rates, which has also been established by previous studies (Athanassiou *et al.*, 2006; Bohinc *et al.*, 2013; Throne & Waver, 2013). Like the above authors, we too proved that a combination of higher temperature and lower relative air humidity contributes to the higher mortality of beetles. The diatomaceous earth that we used in our research is one of the commercially available types of this product, which produces satisfactory insecticidal effects at concentrations below 1000 ppm (Kavallieratos *et al.*, 2015), which was also the case in our research.

Using appropriate natural substances (dusts), we achieved 100% mortality after 21 days at lower relative air humidity (Athanassiou *et al.*, 2006). In our research this holds true for wood ash and diatomaceous earth or preparations related to these substances.

Tadesse & Basedow (2005) reported a very high mortality of *S. zeamais* beetles that were exposed to leaf powder of the plant *Azadirachta indica*. Our research did not confirm the insecticidal effects of the leaf powder made of *A. indica* on the beetle species *S. granarius.* Several factors can influence the effectiveness of this leaf powder, among them the geographic origin of the tree and the tree part from which the preparation is made (Gahukar, 2014). The concentration of azadirachtin is the highest in the seeds of the trees (Gahukar, 2014), and the concentration of azadirachtin is also higher in trees growing in alkaline soil.

The mortality of the beetles was also, in our research, conditioned by the length of exposure to inert dusts, which was reported in different studies (Athanassiou *et al.*, 2006 for other species from the genus *Sitophilus, e.g.*, for the species *S. oryzae*. The great importance of the length of time that harmful pests are exposed to wood ash was also recognised in the study by Demissie *et al.* (2008), in which a longer exposure significantly increased the mortality of beetles of the species *S. zeamais*. We detected no influence of the amount of ash on the beetle mortality in relation to the length of exposure. The higher mortality of the beetles after treatments with wood ash was, in our research, influenced primarily by the higher temperature and longer exposure.

Quartz sand did not provide satisfactory insecticidal effects for the suppression of some stored product pests, as was already proven by Rojht *et al.* (2010b), who investigated on rice weevils. On the basis of the results of our study, we can confirm their findings.

The sizes of the individual particles in the dusts used in our research differ considerably. The concentration of SiO_2 was significantly the highest in quartz sand (Rojht *et al.*, 2010a,b), yet its insecticidal effects also depend on its shape and size (Vayias *et al.*, 2009). Diatomaceous earths contain SiO_2 in amorphous form, while the SiO_2 in quartz sand is also available in crystal form (Subramanyam & Roesli, 2000). The inert dusts used in our research all work in the same way. It is known that diatomaceous earth (Korunić, 1998; Rojht *et al.*, 2012) and quartz sand (Rojht *et al.*, 2010b) can affect harmful pests on the basis of their abrasiveness, dehydration or absorption of waterproof epicuticular waxes. The absorption of waterproof epicuticular waxes is also attributed to wood ash (Hakbijl, 2002), while dusts made of powdered parts of plants are primarily associated with repellent effects (Bohinc *et al.*, 2013). Though Golob (1997) reports that the amount of wood ash applied is important in suppressing stored product pests, our research determined that satisfactory results can also be achieved by applying a lower amount of ash.

In our research, different substances influenced the progeny production differently, which was already confirmed by Kavallieratos *et al.* (2015). Wood ash has all the characteristics of an appropriate natural insecticide because it also reduced the progeny of granary weevils. Diatomaceous earth also diminishes the progeny production of stored product pests (Kavallieratos *et al.*, 2015). Wood ash has been most frequently used for the suppression of stored product pests in Africa and Indonesia (Hakbijl, 2002; Tadesse & Basedow, 2005; Demissie *et al.*, 2008), yet its significance in the protection of plants against harmful pests is today relatively small, despite the fact that it is one of the oldest known insecticides (Hakbijl, 2002).

Ash, a traditional insecticide, caused higher beetle mortality in all treatments in our research, which is encouraging for further research in which we would like to stimulate its use in the protection of stored cereals against harmful insects. By adding wood ash to quartz sand and the leaf powder of the plant *A. indica*, we only improved the effects of the latter. The usefulness of wood ash as fertiliser has been studied much more extensively (Brais *et al.*, 2015) than its insecticidal effects. The results of some studies (Boiteau *et al.*, 2012; Maltas & Sinaj, 2014) indicate that wood ash can also be used to adequately suppress the Colorado potato beetle.

Our research thus puts wood ash side by side with diatomaceous earths, which is today a standard alternative way of suppressing stored product pests. Its insecticidal properties are also satisfactory in combination with other dusts. Some recent studies have reported the negative effects of diatomaceous earth application on stored cereals (Korunić, 2016), which puts the independent use of wood ash into a new perspective. Due to the lack of research on the methods of application (concentration of dust, etc.) and the importance of the ash's chemical composition, researchers in this field will still have to do a lot of work. Wood ash, which is in Europe due to the large forest areas in some countries (Scandinavia, Slovenia etc.) a readily accessible raw material, thus undoubtedly represents a substance that will have to be researched more thoroughly in relation to plant protection, as the results of our and numerous other studies have shown that its significance in the suppression of stored product pests has been underrated.

Acknowledgements

Jaka Rupnik is acknowledged for technical assistance.

References

Abbott WS, 1925. A method of computing the effectiveness of an insecticide. J Econ Entomol 18: 265-267. https://doi.org/10.1093/jee/18.2.265a

Athanassiou CG, Kavallieratos NG, Dimizas CB, Vayias BJ, Tomanovic Z, 2006. Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec® against adults of the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculinonidae). Appl Entomol Zoo 41: 201-207. https://doi.org/10.1303/aez.2006.201

Athanassiou CG, Rumbos CI, Sakka MK, Vayias BJ, Stephou, VK, Nakas CT, 2016. Insecticidal effect of the combined application of spinosad, *Beauveria bassiana* and diatomaceous earth for the control of *Tribolium confusum*. Biocontrol Sci Techn 26: 809-819. https://doi.org/10.1080/09583157.2016.1159657

Bohinc T, Vayias B, Bartol T, Trdan S, 2013. Assessment of insecticidal efficacy of diatomaceous earth and dusts of common lavender and field horsetail against bean weevil adults. Neotrop Entomol 42: 642-648. https://doi.org/10.1007/s13744-013-0168-7

Boiteau G, Singh RP, McCarthy PC, MacKinley PD, 2012. Wood ash potential for colorado beetle control. Am J Potato Res 89: 129-135. https://doi.org/10.1007/s12230-012-9234-7

Bouayad N, Rharrabe K, Ghailani NN, Jbilou R, Castañera P, Ortego F, 2013. Insecticidal effects of Morocan plant extracts on development, energy reserves and enzymatic activities of *Plodia interpunctella*. Span J Agric Res 11: 189-198. https://doi.org/10.5424/sjar/2013111-692-11

Brais S, Belanger N, Guillemette T, 2015. Wood ash and N fertilization in the Canadian boreal forest: soil properties and response of jack pine and black spruce. Forest Ecol Manage 348: 1-14. https://doi.org/10.1016/j.foreco.2015.03.021

Campolo O, Romeo FV, Malacrino A, Laudani F, Carpinteri G, Fabroni S, Rapisarda P, Palmeri V, 2014. Effects of inert dusts applied alone and in combination with sweet orange essential oil against *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and wheat microbial population. Ind Crop Prod 61: 361-369. https://doi.org/10.1016/j.indcrop.2014.07.028

Demissie G, Tefera T, Tadesse A, 2008. Efficacy of Silicosec, filter cake and wood ash against the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on three maize genotypes. J Stored Prod Res 44: 227-231. https://doi.org/10.1016/j.jspr.2008.01.001

Fields P, Korunić Z, Fleurat-Lessard F, 2001. Control of insects in post-harvest: inert dusts and mechanical means. In: Physical control methods in plant protection; Vincent

C, Panneton B & Fleurat-Lessard F (eds.), pp. 248-260. Springer-Verlag, Heidelberg. https://doi.org/10.1007/978-3-662-04584-8_17

Gahukar RT, 2014. Factors affecting content and bioefficacy of neem (*Azadirachta indica* A. Juss.) phytochemicals used in agricultural pest control: A review. Crop Prot 62: 93-99. https://doi.org/10.1016/j.cropro.2014.04.014

Golob P, 1997. Current status and future perspectives for inert dusts for control of stored product insects. J Stored Prod Res 33: 69-79. https://doi.org/10.1016/S0022-474X(96)00031-8

Hakbijl T, 2002. The traditional, historical and prehistoric use of ashes as insecticide, with an experimental study on the insecticidal efficacy of washed ash. Environ Archaeol 7: 13-22. https://doi.org/10.1179/env.2002.7.1.13

Hamza AF, El-Orabi MN, Gharieb, OH, El-Saeady AHA, Hussein ARE, 2016. Response of *Sitophilus granarius* L. to fumigant toxicity of some plant volatile oils. J Rad Res App Sci 9: 3-14. https://doi.org/10.1016/j.jrras.2015.05.005

Jean WG, Nchiwan NE, Dieudonne N, Christoper S, Adler C, 2015. Efficacy of diatomeacous earth and wood ash for the control of *Sitophilus zeamais* in stored maize. J Entomol Zool Stud 3: 390-397.

Kavallieratos NG, Athanassiou CG, Korunić Z, Mikeli NH, 2015. Evaluation of three novel diatomaceous earths against three stored-grain beetle species on wheat and maize. Crop Prot 75 132-138. https://doi.org/10.1016/j.cropro.2015.05.004

Korunic Z, 1998. Diatomaceous earths, a group of natural insecticides. J Stored Prod Res 34: 87-97. https://doi.org/10.1016/S0022-474X(97)00039-8

Korunić Z, 2016. Overview of undiserable effects of using diatomaceous earths for direct mixing with grains. Pesticides & Phytomedicine (Belgrade) 31: 9-18. http://www.doiserbia.nb.rs/img/doi/1820-3949/2016/1820-39491602009K.pdf.

Kranjc N, Piškur M, Klun J, Premrl T, Piškur B, Robek R, Mihelič M, Sinjur I, 2009. Lesna goriva-drva in lesni sekanci. Proizvodnja, standardi kakovosti in trgovanje. Gozdarski Inštitut Slovenije. Založba Silva Slovenica, p. 81. [in Slovenian].

Maltas A, Sinaj S, 2014. Wood ashes: a new fertilizer to Swiss agriculture. Agrarforshung Schweiz. 5: 232-239.

Mohapatra D, Kar A, Giri SK, 2015. Insect pest management in stored pulses: an overview. Food Bioprocess Technol 8: 239-265. https://doi.org/10.1007/s11947-014-1399-2

Mkenda PA, Stevenson PC, Ndakidemi P, Farman DI, Belmain SR, 2015. Contact and fumigant toxity of five pesticidal plants against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in stored cowpea (*Vigna unguiculata*). Int J Trop Insect Sci 35: 172-184. https://doi.org/10.1017/S174275841500017X

NRC, 1992. Neem. A tree for solving global problems. National Research Council, Nat Acad Press, Washington DC, USA.

Rojht H, Horvat A, Athanassiou CG, Vayias BJ, Tomanović Ž, Trdan S, 2010a. Impact of geochemical composition of diatomaceous earth on its insecticidal activity against adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J Pest Sci 83: 429-436. https://doi.org/10.1007/s10340-010-0313-6

Rojht H, Horvat A, Trdan S, 2010b. Local Slovenian quartz sands have low insecticidal activity against rice weevil (*Sitophilus oryzae* [L.], Coleoptera, Curculionidae) adults. J Food Agric Environ 8: 500-505.

Rojht H, Horvat A, Trdan S, 2012. Characteristics of diatomaceous earth as biopesticide for control of stored pests. Acta Agric Slovenica 99: 99-105. [in Slovenian]. http://aas.bf.uni-lj.si/marec2012/13rojht.pdf.

Statgraphics Centurion XVI, 2009. Statpoint technologies Inc. Warrenton, VA, USA.

Subramanyam B, Roesli R, 2000. Inert dusts. In: Alternatives to pesticides in stored-product IPM; Subramanyam B & Hagstrum DW (ed.), pp: 321-380. Kluwer Acad Publ, Dordrecht. https://doi.org/10.1007/978-1-4615-4353-4_12

Tadese A, Basedow T, 2005. Laboratory and field studies on the effect of the natural control measures against pests in stored maize in Ethiopia. Zeitschrift für Pflanzenkrankenheiten und Pflanzenshutz/J Plant Dis Prot 112: 156-172. http://www.ulmer.de/Laboratory-and-field-studies-on-the-effect-of-natural-control-measures-against-insect-pests-in-stored-maize-in-Et,QUlEPTM1NjMxJk1JRD01MTgwMiZBUk9PVD00OTEwJlRFTVBfTUFJTj1TY2llbnRpZmljc19QJ0cmFpdC5odG0.html

Throne JE, Weaver DK, 2013. Impact of temperature and relative humidity on the life history parameters of adult *Sitotroga cerealella* (Lepidoptera: Gelechiidae). J Stored Product Res 55: 128-133. https://doi.org/10.1016/j.jspr.2013.10.003

Trdan S, Bohinc T, 2013. Research on insecticidal efficacy of single and combined use of different natural substances against granary weevil (*Sitophilus granarius* L.). Zbornik predavanj in referatov 11. Slovenskega posvetovanja o varstvu rastlin z mednarodno udeležbo, Bled, 5.-6. Marec 2013:160-167. [in Slovenian].

Vayias BJ, Athanassiou CG, 2004. Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec® against adults and larvae of the confused flour beetle, *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae). Crop Prot 23: 565-573. https://doi.org/10.1016/j.cropro.2003.11.006

Vayias BJ, Athanassiou CG, Korunić Z, Rozman V, 2009. Evaluation of natural diatomaceous earth deposits from south-eastern Europe for stored-grain protection: the effect of particle size. Pest Manag Sci 65: 1118-1123. https://doi.org/10.1002/ps.1801