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ALUMINA POWDERS AS NOVEL NON-TOXIC INSECTICIDE AGAINST BEAN WEEVIL (Acanthoscelides obtectus Say)

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Abstract

Alumina powders were obtained by auto combustion method and calcined for 1 h at temperatures ranging from 1000ºC to 1200ºC. X-ray powder diffraction analysis revealed an overwhelming presence of γ–Al_2O_3 for the powder calcined at 1000ºC, while at 1200ºC only the presence of α–Al_2O_3 was detected. Insecticide properties of these powders were investigated on bean weevil (*Acanthoscelides obtectus* Say). Mortality of treated and untreated male and female adults was checked in a period of 7 days. The powder calcined at 1200ºC was the most efficient, and 100% mortality of both male and female insects were reached after 7 days of exposure. It was also observed that insecticide efficiency increased with the increase of α–Al_2O_3 phase present in the powders and the female insects were less susceptible to the powders' toxicity in general. Scanning electron microscope analysis of the treated adults with Al_2O_3 indicated that mortality of bean weevils can be related to adsorption of the powders onto their exoskeleton. Development of F_1 progeny was similar for all the powders and the number of hatched larvae was significantly reduced in comparison with the untreated seeds, while the percentage of damaged seeds dropped from 70% to 15%. These results suggest that relatively cheap and non-toxic α–Al_2O_3 powders can be considered for use in pest control, especially in organic seed production.

Keywords: alumina; pest control; bean weevil, *Acanthoscelides obtectus*.

Introduction

The bean weevil (*Acanthoscelides obtectus* Say) is considered as one of the most serious stored bean pests worldwide (Jovanović et al., 2007). Its oviposition and growth are continuous, and the larvae feed on the seeds. After emergence from the seeds, the adults reproduce either in the field or in the stored seeds in a continuous cycle (Ayvaz et al., 2010). Since bean is one of the most important nutritive sources in developing countries, its protection in stored conditions has been attracting much attention in past decades. Protection of agricultural stored products against insect pests is of utmost importance to secure a continuous and safe food supply all over the world (Pemonfe et al., 1997). Conventional treatments have been used for this purpose, but for the last decades there have been numerous reports of environmental contamination by use of toxic pesticides, mostly organophosphorous and pyrethroid compounds (Nikpay, 2006). Their use has been constantly challenged by resistance of insects, consumer's demand for pesticide-free products and restrictive governmental regulations (Subramanyam et al., 1994). This led to replacement of the existing chemical insecticides by a new class of non-toxic, nanostructured inorganic compounds, such as silica, alumina, diatomaceous earth and inert dusts (Barik et al., 2012; Reynolds et al., 2009; Stadler et al., 2010; Stadler et al., 2012). Especially these compounds have been reported to be effective in control of stored product insects (Nikpay et al., 2006; Stadler et al., 2010; Stadler et al., 2012). This approach was successfully demonstrated in pest control of

rice weevil (*Sitophilus oryzae* L.) and lesser grain borer (*Rhyzopertha dominica* F.) by nanostructured alumina (Stadler et al., 2010; Stadler et al., 2012).
The cause of mortality of insects by applying these inert inorganic powders has not yet been fully clarified. However, this approach being cost effective and environmentally friendly is worth further investigations and optimization of the existing formulations. Thus, in this study, Al_2O_3 powders obtained after calcination at different temperatures were tested as potential insecticides against *A. obtectus*. The comparison of insecticidal properties was observed for male and female adults and F_1 progeny.

Materials and methods

Synthesis and characterization of alumina powders: 0.03 moles of aluminium-nitrate nonahydrate ($Al(NO_3)_3 \cdot 9H_2O$, “Centrochem”, Serbia) was dissolved with citric acid monhydrate ($C_6H_8O_7 \cdot H_2O$, „Lachner“, Czech Republic) in minimal amount of distilled water, whereas the citrate to nitrate molar ratio was set at 0.8. pH value was kept at 8 by adding ammonium hydroxide (NH_4OH, „Lachner“, Czech Republic) into the solution. First, the solution was heated at 80 ºC with constant stirring until a viscous gel was formed. Then, the temperature was raised up to 200ºC to start the autocombustion reaction. The resulting black precursor powder was collected and calcined at 1000ºC, 1100ºC and 1200ºC for 1 h in a chamber oven. Phase content of the powders was determined by X-ray diffraction (XRD, RIGAKU® RINT 2000) analysis using a CuK_α radiation ($\lambda_{CuK\alpha} = 1.54178 \times 10^{-10}$ m) from 20º to 80º 2θ angle.
Test insects: Male and female insects of *A. obtectus* were reared separately under controlled conditions (27ºC±1ºC, relative humidity 65%±5% and photoperiod L16:D8) and were 48 h old prior to testing. The insects were provided from the Institute for Biological Research "Siniša Stanković" (Belgrade, Serbia).
Lethal effect of the alumina powders: Al_2O_3 powders were mixed with 40 g bean grains by rotary shaker (Multifix GmbH, Germany) for 10 min, to form 1% mass concentration. The mixtures were further divided into four smaller portions of 10 treated grains with 10 male, or 10 female insects placed separately in a 90/14 mm Petri dish. Additional four control portions contained only untreated grains with insects. All tests were carried out simultaneously under controlled conditions and mortality rate was tracked after each day, during 7 days.
Effectiveness of powders on reduction of number of individuals in F1 progeny: Al_2O_3 powders were mixed with 40 g bean seeds in 200 ml glass jars by rotary shaker (Multifix GmbH, Germany) for 10 min, to form 1% mass concentration. Each batch was divided into four smaller samples with 10 g treated seeds, which were placed in 90/14 mm Petri dishes together with 10 female and 10 male insects. Control sample was prepared in the same way, but contained only untreated seeds. Each testing was repeated four times. After 15 days dead insects were removed from the Petri dishes and the development of F_1 progeny was investigated. The number of F1 progeny produced was recorded after 30 days. The experiments were carried out under controlled laboratory conditions (T=27ºC ± 0.5ºC, RH= 65%, L16:D8).
SEM analysis: In order to determine any changes of the exoskeleton, the tested insects were analyzed by scanning electron microscopy (SEM, TESCAN Vega TS5130MM).

Results and discussion

XRD analysis of the powders calcined at different temperatures was presented in Figure 1. The changes in diffraction patterns indicated a gradual transformation from γ–Al_2O_3 to α–Al_2O_3 as the calcination temperature increased from 1000ºC to 1200ºC.

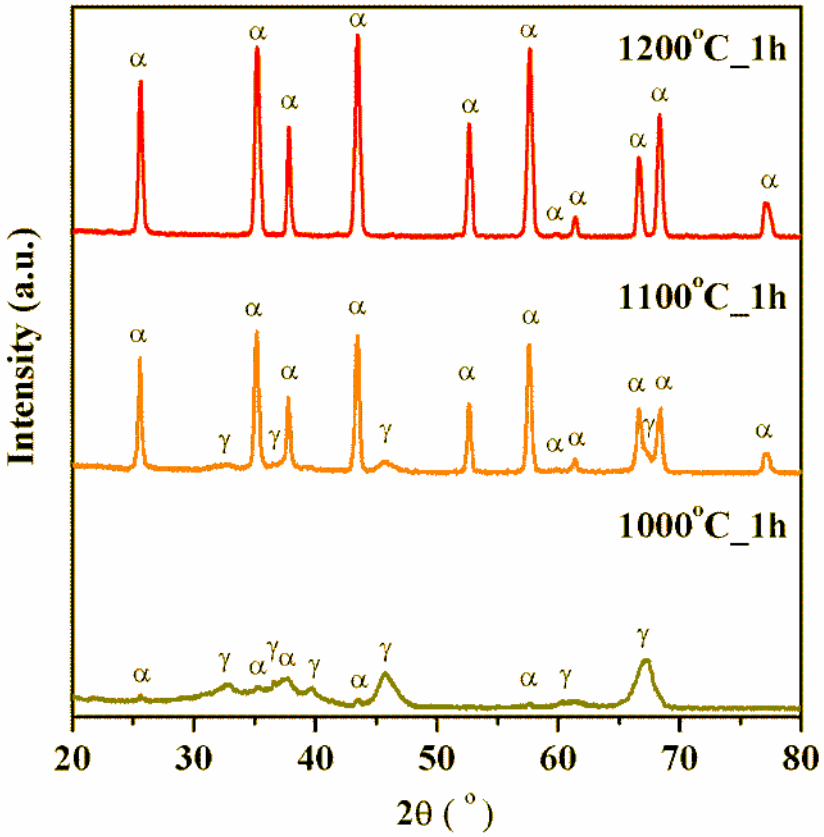

Figure 1. XRD patterns of alumina powders calcined at 1000–1200ºC for 1h.

The results of the lethal effect of the alumina powders on treated and untreated male and female adults in a period of 7 days were presented in Figure 2. After 24 hours all insects were alive, only after 48 h the first mortality cases were observed. Al_2O_3 powder calcined at 1200ºC began to be lethal against male adults after 48 h. After 72 h, mortality was 40%, after 96 h, more than 60%, after 120 h, 70% and after 144 h, mortality reached 100%. In the case of females Al_2O_3 powder calcined at 1200ºC began to be lethal after 72 h, with mortality of 20%. The maximal lethal effect of almost 100% was reached after 168 h. Al_2O_3 powders calcined at 1100ºC and 1000ºC showed their lethal effect on male adults after 72 h and 96 h. They showed maximal lethal effect after 168 h, 100% and 97.5%, respectively. These powders exhibited lethal effect on female adults first after 120 h and maximal lethal effect they showed was after 168 h, 92,5% and 62,5%.

It was clear that pure α–Al_2O_3 powder obtained after calcination at 1200ºC was the most lethal against both male and female adult insects. Taking into account untreated adult insects, it is clear that female insects live a bit longer, probably due to their final role in laying eggs that were previously fertilized. This trend is also observed for treated insects, leading to a conclusion that females are less overall susceptible to alumina powders. Furthermore, lethal effect decreased almost monotonously with the increase of γ–Al_2O_3 content in the powders for female insects, while the powders calcined at 1000ºC and 1100ºC showed no significant difference in their insecticidal activity against male insects.

a b

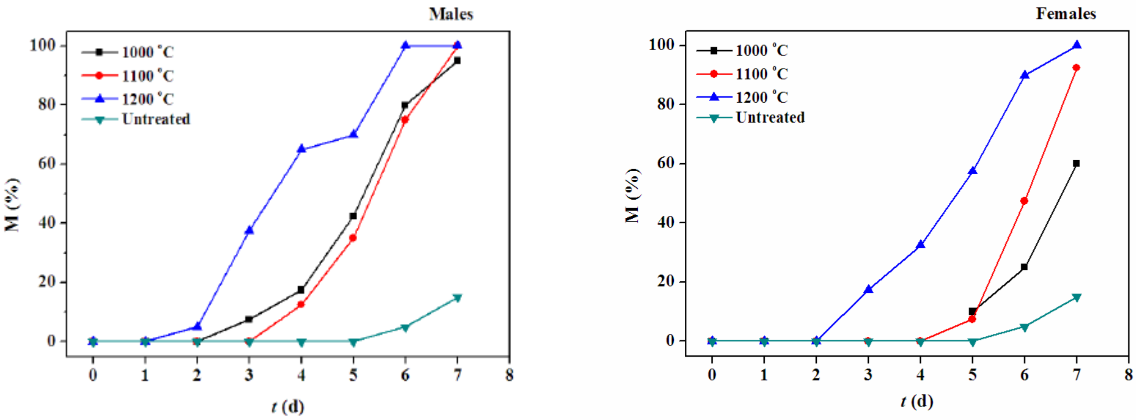

Figure 2. Lethal effect of powders against male (a) and female insects (b) in a period of 7 days.

Compounds like α–Al_2O_3 powder, if attached to an exoskeleton of insects, are able to adsorb cuticular lipids, which can cause dehydration of insects and their death, consequently (Stadler et al., 2012). SEM analysis of untreated and insect treated with Al_2O_3 was presented in Figure 3 and it is easy to notice how Al_2O_3 particles adhere to the exoskeleton of the treated imago. Thus, mortality rate is certainly related to a degree of adhesion of these particles onto the insect exoskeleton, keeping in mind that α–Al_2O_3 are inert and non-toxic. Degree of powder adhesion depends on factors such asspecific surface area and concentration of powders, humidity, temperature and insect pubescence (Fields et al., 2000; Stadler et al., 2012). On the other hand, by optimizing these parameters it is possible to raise efficiency of alumina powders, and accordingly, even lower concentrations could provide satisfying efficiency. Thus, these results suggest that α–Al_2O_3 powders, being the most effictive against *A. obtectus*, can be considered as an alternative in pest control of the stored products and in organic seed production, as well (Krnjajić, 2003; Obradović and Krnjajić, 2003).

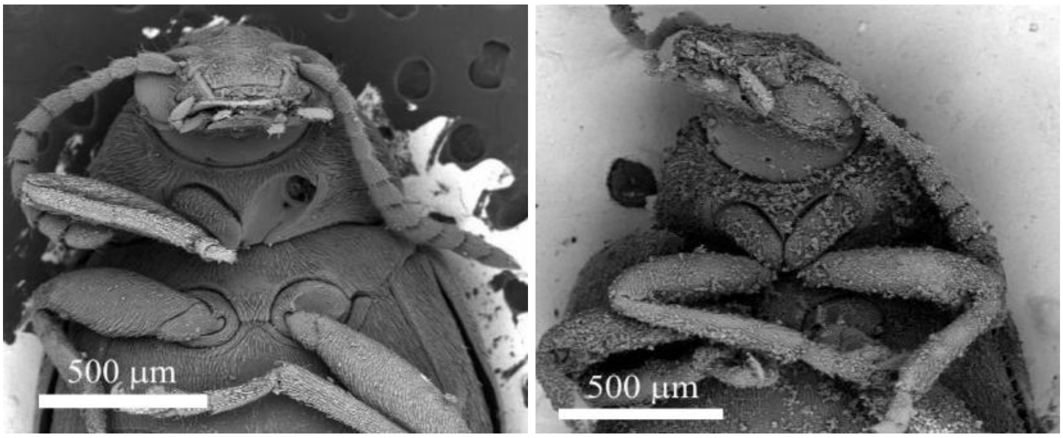

Figure 3. SEM images of untreated (left) and treated insect with Al_2O_3 (right).

When it comes to the lethal effect of alumina powders on F_1 progeny, again the powder calcined at 1200ºC exhibited the highest efficiency (Figure 4), but there is a slight difference in their efficiency. The number of larvae hatched from eggs under treated conditions was reduced about 75%. The percentage of damaged seeds as a consequence of larval activity is also significantly reduced (dropped from 70% to 15%), which confirmed the pesticide effect of alumina powders against *A. obtectus*. These results clearly serve as both qualitative and quantitative indicators of pest control efficiency of the stored products. This efficiency can be ehanced by increasing powder concentration, since 1% is probably too low for more robust *A. obtectus* compared with *Sitophilus oryzae* (L.), or *Rhyzopertha dominica* (F.), whereas this concentration was used as maximal (Stadler et al., 2010; Stadler et al., 2012).

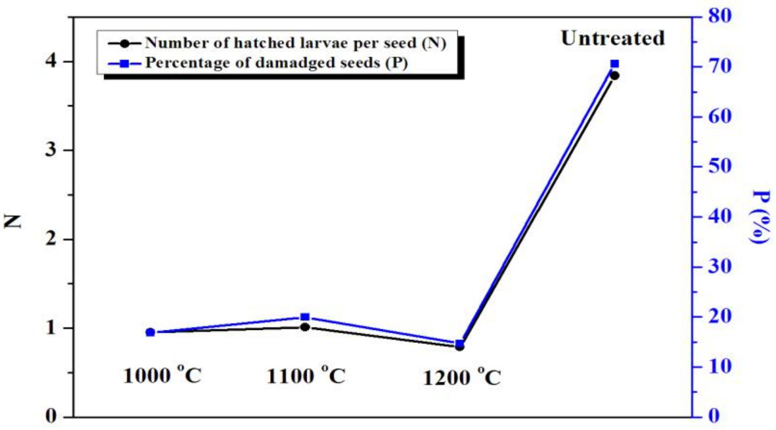

Figure 4. Development of F_1 progeny (a) and percentage of damaged seeds (b).

Conclusions

In this study comparative lethal effect of various Al_2O_3 powders calcined at different temperatures, 1000ºC, 1100ºC and 1200ºC for *Acanthoscelides obtectus* was successfully carried out. Al_2O_3 powder calcined at 1200ºC was the most efficient against adult insects, reaching 100% mortality after 6 days of exposure for males and 7 days for females, compared with 17,5% (males) and 12,5% (females) mortality of the untreated insects after 7 days. On the other hand, all the Al_2O_3 powders exhibit similar lethal effect level against larvae insects in F1 generation and percentage of the damaged seeds was significantly reduced. Al_2O_3 is also promising due to its cost-effectiveness and solid effeciency against *A. obtectus*. Their efficiency can be enhanced by increasing their concentration and/or by optimizing synthesis parameters with aim to obtain powders with appropriate particle size and increased specific surface area.

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