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Osvrt na prirodne insekticide na bazi dijatomejske zemlje

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Poljoprivreda/Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

http://dx.doi.org/10.18047/poljo.22.1.2

Poljoprivredni fakultet u Osijeku, Poljoprivredni institut Osijek
Faculty of Agriculture in Osijek, Agricultural Institute Osijek

ISSN 1330-7142
UDK: 632.937
DOI: 10.18047/poljo.22.1.2

A REVIEW OF NATURAL INSECTICIDES BASED ON DIATOMACEOUS EARTHS

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Scientific review
Pregledni znanstveni članak

SUMMARY

The efficacy of diatomaceous earth (DE) formulations against insect pests is greatly influenced by environmental conditions. It is effective only under dry conditions and becomes wholly ineffective if it is wet. High air relative humidity and especially rain greatly reduce or completely destroy the efficacy of DE when used outdoors. Furthermore, depending on the insect species and commodity treated, DE takes from one to several days to kill insects, even in a dry environment. Because of these factors, DE is in limited use outdoors, especially in regions with a high precipitation. The main uses of DE are for the protection of stored agricultural products against insect infestation, in food industry for structural treatment and for indoor use against some household insects living in a dry environment. The mode of DEs action against insects, the effect of humidity and moisture on DE efficacy, brief overview of the research of DE use in stored grain protection and attempts to overcome DE limitations with combined use of DE and other reduced-risk methods with the insecticide activity for direct mixing with grains are discussed in this article.

Key-words: diatomaceous earth, insect pests, effectiveness, advantages, disadvantages

INTRODUCTION

Diatomaceous earth (DE) is a dust composed of unicellular algae fossilized bodies called diatoms. DE exists in a natural state as soft chalky rock deposits. The majority of these deposits were formed in Eocene/ Miocene Epochs about 30 to 80 million years ago (Round et al*.* 1990).

DE is prepared for commercial use by quarrying, drying and milling. The only changes to DE during this process are the reduction in moisture content and the reduction in mean aggregate particle size. The result of this process is a fine, talc-like dust with mean particle size distribution of 0.5 microns to more than 100 microns (the majority are between 10 and 50 microns) (Quarles, 1992; Subramanyam and Roesli, 2000).

DE usually contains between 80 and 95 percent amorphous silicon dioxide which is a generic term for various silicas (WHO, International Agency for Research on Cancer. IARC 1986).

THE MODE OF DE ACTION

For centuries, grain was protected from insect infestation by adding some form of powder or dust directly to the grain whereas, very often DE was applied. People live, raise animals and farm on DE deposits, and many species of wildlife live on DE deposits, all without detrimental effects (GRAS, 2006).

DE is probably one of the safest and most effective naturally occurring insecticides (Ebeling, 1971; Fields and Muir, 1996). It has a physical mode of action since it kills insects by desiccation. The theory of the DE the insecticidal action mechanism was developed as early as 1931 when Zacher and Kunike described the "Zacher effect". They discovered that the DE action mode is mainly by dehydration or desiccation.

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An insect's body is covered with a layer of wax made up of cuticular lipids. These lipids are present on the outermost layer of an insect's exoskeleton, called its epicuticle, and serve the critical function of restricting water loss from the body and preventing desiccation. DE particles stick (adsorb) to the lipids on insect's epicuticle and prevent the lipids from restricting water loss (Ebeling, 1971; Golob, 1997). As a result, insect loses body moisture through the damaged spots on its epicuticle and dies, after a period of time, of desiccation.

The outdoors efficacy of DE depends on the environmental conditions present when it is applied, especially the air relative humidity and the water presence. It is well known that DE is not normally fast acting. Depending on the ambient conditions and the pest species, it will take several days to control most insect species.

DE's adsorptive capacity for lipids, and its insecticidal efficacy, is also affected by the size, shape and a surface topography of diatom species, uniformity of particles and the purity of the formulation (Korunić, 1998, 2013). Depending on the species, size and shape of the diatoms, there are numerous pores and holes in the surface of the diatom particles that make up its active surface. Particles that have a larger available active surface have a higher sorption capacity for the lipids and are more effective desiccants. Particles of high purity with empty holes being as dry as possible (natural DE after processing usually contain about 4-8% of free water, although 5% is preferable) have the highest sorption capacity and the highest efficacy against insects (Quarles, 1992; Subramanyam and Roesli, 2000; Korunić, 2013).

In addition to the adsorptive ability, DE also has abrasive property. The abrasive effects of DE may include abrasion or laceration of the digestive tract, resulting in internal desiccation (Jackson and Webley, 1994). Further, DE may also plug the insect's spiracle (or breathing organ), which results in suffocation (Jackson and Webley 1994). These two effects often work in combination. It is known that insect removes dust material from its legs and antennae by passing them between its mouth parts (Flanders, 1941). It is probably that this cleaning activity plays a significant role in increasing the DE efficacy through internal desiccation.

If insects are attracted to the presence of DE, they will stay longer and pick up more particles on their legs and antennae. As a result, they will bring more DE particles to their mouths. We believe that the food grade bait in some DE formulations (yeast and honey) attracts insects to the DE and keeps them in contact with DE for longer period of time. As a result, the insects will pick up more DE particles on their body, legs and antennae. This will lead to the faster desiccation (both internal and external) and faster and higher insect mortality.

THE EFFECT OF HUMIDITY AND MOISTURE ON DE EFFICACY

The presence of moisture significantly reduces the efficacy of DE in three ways: a) by reducing its sorption capacity for lipids on insect's epicuticle; b) by reducing the dispersion properties (as further explained) of DE that are characteristics of its talc-like form; and c) since an insect loses water from the body more slowly, it is able to replace the lost water more easily, under wet conditions.

With respect to DE's sorption capacity, it is well known that diatom particles absorb both surface water and, depending on the relative humidity, water that is present in the air. As relative humidity increases, the quantity of water absorbed by DE increases. The efficacy of DE is significantly reduced by increasing the moisture content of DE to above 8-10%. When particles are filled with a film of water and the holes and pores are completely saturated with water, DE loses its sorption ability for wax and its insecticidal efficacy is lost entirely (Ebeling, 1971; Le Patourel, 1986; Le Patourel and Zhou, 1990; Aldryhim, 1990, 1993; Quarles, 1992; Golob, 1997; Korunić, 1997; Fields and Korunić, 2000).

The presence of water also adversely affects DE's dispersion properties. As DE dries on various surfaces (after it becomes wet), it tends to agglomerate and stick to the surfaces on which it dries rather than stick to the insects that crawl over the surface. Once DE dries, the particles of DE stick together forming cohesive deposits making it difficult for separate particles to be picked up by passing insects. Although DE particles are again effective after drying, the reduced dispersion of individual particles reduces the extent to which they adhere to passing insects. As a result, the insect does not pick up the necessary quantity of particles to facilitate desiccation.

It will be recalled that separate, dry DE particles must adhere to the insect's body in order to be effective. DE will control insects as long as it is dry and in sufficient concentrations to ensure that the insects come in contact with enough particles. It follows that, DE is effective in controlling insects on primary under dry conditions.

BRIEF OVERVIEW OF THE RESEARCH OF DE USE IN STORED GRAIN PROTECTION

First publications about the DE use in stored grain protection had been published in the first half of the 20^{th} century (Calvert, 1930; Zacher and Kunike, 1931; Germar, 1936; Zacher, 1937a,b; Chiu, 1939a,b; Flanders, 1941; Alexander et al., 1944a,b,c). Since then hundreds of research papers have been published in many international journals.

Research has also been done on the effect of DE on numerous pests such as ants, bedbugs, textile pests, various caterpillars in agriculture, crickets, termites, earwigs, June beetles, potato beetles, silverfish, fleas, centipedes, pillbugs, bed bugs, poultry mites, ticks, snails etc. (Wilbur et al., 1971a,b; De Crosta, 1979; Snetsinger, 1982, 1988; Rambo, 1992).

The use of DE for structural treatment in stored product facilities was studied by Desmarchelier et al. (1987, 1993), Wright (1990), McLaughlin (1994), Bridgeman (1994), Korunić and Fields (1995), Korunić et al. (1996).

During the last 20 years DE has been the subject of several review papers (Quarles, 1992; Golob, 1997; Korunić, 1998; Subramanyam and Roesli, 2000; Fields and Korunić, 2002; Quarles and Winn, 1996; Nikpay, 2006; Quarles, 2007: Korunić, 2013; Shah and Khan, 2014) with the numerous references cited within each of review. Mostly, the advantages of DE were described as natural inert material, safe and effective insecticide, with the physical mode of action and long persistence not leaving hazardous residues and very often with conclusions that DE is a promising insecticide.

However, still today DE is not commercially in wider use for direct mixing with grains to be placed on the market because of the great obstacles and disadvantages described by Korunić (2016 in press Pesticides & Phytomedicine). The main limitations in wider use of DEs when directly mixed with grains are the effect of grain moisture and temperature on the effectiveness reduction (Table 1); the various effectiveness of different diatomaceous earths on wheat against the same insect species (Table 2); significant difference in the effectiveness of the same formulation of DE against different insect species on the same commodity (Table 3); significant effect of different type of commodity on the effectiveness of the same formulation of DE against the same insect species (Table 4); significant effects on grain bulk density (test weight) (Table 5) and grain flowability reduction. The dusty appearance of treated grain, safety reasons due to very small DEs particles present in the air for hours after the treatment and smaller than 5 microns that may enter into the lung causing inflammation are also limitations.

Table 1. The mortality of *C. ferrugineus* (Stephens) held at various temperature and moisture conditions on wheat treated with DE, (Protect-It®) (Korunić and Fields, 1998)

Tablica 1. Mortalitet C. ferrugineus (Stephens) na pšenici tretiranoj s DZ (Protect-It®) pri različitoj temperaturi i vlazi zrna (Korunić i Fields, 1998.)

Exposure (days) *Izloženost(dana)*	Dosage (ppm) *Doza (ppm)*	15 °C			20 °C		
		Moisture content/*Sadržaj vlage zrna**					
		12%	15%	17%	12%	15%	17%
3	0	0±0 a	0±0 a	3±3 a	0±0 a	0±0 a	0±0 a
	100	15±3 b	5±1 b	1±1 a	41±4 b	29±3 b	3±3 a
	200	89±1 c	43±1 c	47±3 b	95±1 c	80±5 c	89±1 b
	300	99±1 d	87±1 d	57±1 c	100±0 c	95±1 d	100±0 c

*For a given duration, moisture and temperature combination, means followed by different letters are significantly different, >0.05 Student-Newman-Keuls test

Table 2. The efficacy of different diatomaceous earths applied on Canadian Western hard red spring wheat against the rice weevil *Sitophilus oryzae* L. (RW) and red flour beetle *Tribolium castaneum* Herbst (RFB) (Korunić, 1998)

Tablica 2. Učinkovitost različitih vrsta dijatomejske zemlje primijenjenih na kanadsku tvrdu jaru pšenicu u suzbijanju rižinoga žiška Sitophilus oryzae L. (RŽ) i kestenjastoga brašnara Tribolium castaneum Herbst (KB) (Korunić, 1998.)

Diatomaceous earth *Dijatomejska zemlja*	LC_{50} (95% CI) – ppm	
	RW after 5 days *RŽ nakon 5 dana*	RFB after 14 days *KB nakon 14 dana*
Celite 209	270 (213-340)	417 (328 - 529)
DE Australia	438 (346 - 553)	494 (383 - 637)
Perma Guard	680 (555 - 832)	1211 (728 - 2014)
DiaFil 610	829 (561 - 1223)	1477 (447 - 4883)
Melocide DE 100	1137 (546 - 3734)	2047 (1178 - 3556)

Probit analysis. CI = confidence intervals. Concentrations from 100 ppm to 1700 ppm;

25 adults of the rice weevil and 20 adults of the red flour beetle, unknown ages, mixed sex in each replicate of 5. Temperature: 25°C; RH: 55%; moisture content of wheat: 14%

Table 3. The mortality of four insect species held at 25°C at various moisture content on wheat treated with DE (Protect-It®.) at 300 ppm (Korunić et al., 1997)

Tablica 3. Mortalitet četiri vrste kukaca pri temperaturi 25°C i pri različitoj vlazi zrna pšenice tretiranoj s 300 ppm DZ (Protect-It®) (Korunić et al., 1997.)

Insects *Kukci*	Exposure (days) *Izloženost (dana)*	Mortality (%)* *Mortalitet (%)*		
Cryptolestes ferrugineus	1	92 ± 3 b	100 ± 0 a	75 ± 5 a
	2	100 ± 0 a	100 ± 0 a	100 ± 0 b
Oryzaephilus surinamensis	5	100 ± 0 a	92 ± 2 a	71 ± 3 a
	9	98 ± 2 a	99 ± 1 b	95 ± 3 b
Sitophilus Oryzae	5	95 ± 3 a	74 ± 3 b	53 ± 2 a
	9	97 ± 2 a	99 ± 1 a	99 ± 2 b
Rhyzopertha Dominica	5	72 ± 3 a	39 ± 7 a	17 ± 2 a
	14	90 ± 3 b	68 ± 5 b	58 ± 5 b

*For a given species, moisture and application combination, means followed by different letters are significantly different P>0.05 Student-Newman-Keuls test; Insects held on untreated wheat had less than 2% mortality

Table 4. The influence of various commodities on the efficacy of DE (Protect-It®) against *Sitophilus oryzae* L. (RW) and *Tribolium castaneum* Herbst (RFB), at dose of 300 and 500 ppm, respectively (Korunić, 2007b)

Tablica 4. Utjecaj različite vrste robe na učinkovitost DZ (Protect-It®) u suzbijanju Sitophilus oryzae L. (RŽ) i Tribolium castaneum Herbst (KB), pri dozama 300 i 500 ppm (Korunić, 2007.b)

Grains *Žitarice*	Class *Sorta*	Grade *Klasa*	MC (%) *Vlaga zrna (%)*	Mean±Std. dev./ *Prosjek+Std. dev.* * Mortality (%)/*Mortalitet (%)* Adult RW after 21 days *RŽ nakon 21 dan*	Mean±Std. dev./ *Prosjek+Std. dev.* * Mortality (%)/*Mortalitet (%)* Adult RFB after 6 days *KB nakon 6 dana*
Wheat *Pšenica*	Ontario soft feed	Feed	14.3	46±13 b,c,d	23±20 a,b
	Canada Prairie Spring Red	1	14.3	51±30 c,d,e	95±4 d
	Hard Red Spring	1	14.6	N/A	91±4 d
	Hard Red Spring	2	14.0	85±17 g,h,i	82±8 c,d
	Hard Red Spring	3	13.8	84±10 g,h,i	91±14 d
	Extra Strong Red Spring	1	14.5	86±14 g,h,i	84±21 c,d
	Extra Strong Red Spring	2	14.5	71±15 e,f,g,i	84±10 c,d
	White Prairie Spring	1	14.6	70±5 d,e,f,g	58±31 c
	Amber Durum	1	14.2	54±8 c,d,e,f	91±4 d
	Amber Durum	2	13.6	53±8 c,d,e,f	53±18 b,c
Rye *Raž*	N/A	N/A	14.3	78±12 f,g,h,i	84±21 c,d
Maize *Kukuruz*	With 7.8% oil	1	13.3	23±22 a,b	N/A
	With 4.4% oil	1	13.3	10± 5 a	N/A
Rice *Riža*	Milled	N/A	13.0	2±3 a	N/A
	Paddy	N/A	13.0	100± 0 i	N/A
Sorghum *Sirak*	N/A	N/A	13.0	N/A	4±8 a

*ANOVA (Tukey). P>0.05. Means in each column followed by the same letter are not significantly different. Control mortality RW (%): paddy rice10±2; Ontario soft feed wheat 5±2; all other commodities 0.0±0.0. Control mortality RFB (%): all grains 0.0±0.0

Table 5. The reduction of bulk density by DE (Protect-It®) on various grains as compared to untreated grain, measured by the Canadian Grain Commission

Tablica 5. Utjecaj DZ (Protect-It®) na smanjenje hektolitarske mase različitih vrsta žitarica uspoređujući s netretiranim žitaricama, prema "Canadian Grain Commission"

Grains *Žitarice*	Application method *Metoda aplikacije*		Bulk density (kg/hl, ±SEM) *Hektolitarska masa (kg/hl, ±SEM)*					
			Dosage (ppm) * *Doza (ppm)*					
			0	100	200	300	400	500
Oats *Zob*	dry *suho*	lab *laboratorij*	62.7±0.4	1.0	1.5	1.6	2.3	3.2
Barley *Ječam*	dry *suho*	lab *laboratorij*	64.7±0.3	2.3	4.3	4.3	4.6	4.7
Rye *Raž*	dry *suho*	lab *laboratorij*	75.4±0.5	2.3	3.0	3.2	3.4	4.3
Maize *Kukuruz*	dry *suho*	lab *laboratorij*	64.3±0.2	1.4	2.0	2.4	2.5	2.0
Durum wheat *Durum pšenica*	dry *suho*	lab *laboratorij*	78.5±0.5	4.2	-	5.5	-	5.8
HRS** *Tvrda crvena jara pšenica*	dry *suho*	lab *laboratorij*	81.0±0.3	4.0	5.6	5.8	6.2	6.8

* All concentrations were significantly different from 0 ppm within a given grain, Dunnett's test $P<0.05$; **HRS - hard red spring wheat

Due to all these significant and unacceptable disadvantages in direct mixing with the grains, it is quite clear that nowadays use of DE as a grain protectant has minimal or small chances to be accepted by a grain industry. However, DEs may have a wider application on farms to protect grains for own use, for feed protection, in veterinary field and for structural treatment in grain and the food industry.

ATTEMPTS TO OVERCOME DE LIMITATIONS FOR DIRECT MIXING WITH GRAINS

In order to overcome the limitations of DEs use several studies are conducted to discover new ways of DE use and thus to continue the use of this safe dust. In order to reduce DE dosages, it is often mixed with other compounds such as silica gel, dry honey, not activated yeast and sugar to increase the efficacy. However, these mixtures still have a significant negative effect on grain bulk density and flowability (Jackson and Webley, 1994; Korunić and Fields, 1995; Quarles and Winn, 1996; Korunić et al., 1997 Subramanyam and Roesli, 2000; Korunić et al., 2015).

One of the possible solutions to the implications that are caused by high doses of DEs is a combined use of DE with other reduced-risk methods with the insecticide activity, such as extreme temperatures (Fields et al., 1997; Dowdy, 1999), grain cooling with surface treatment with DE (Nickson et al., 1994) or in a mixture with entomopathogenic fungi (Lord, 2001; Akbar et al., 2004; Kavallieratos et al., 2006; Vasilakos et al., 2006; Michalaki et al., 2007), in a mixture with reduced concentrations of synthetic insecticides (Korunić, 2001; Stathers, 2003; Arthur 2004a,b; Athanassiou, 2006; Chanbang et al., 2007; Korunić and Rozman, 2010) or in a mixture with plant extracts (Korunić, 2007a; Athanassiou and Korunić, 2007) or in a mixture with bacterial metabolite (Vayias et al., 2009). Experimentation with other components often revealed enhanced and sometimes synergistic effectiveness (Korunić, 2001; Lord, 2001; Stathers, 2003; Korunić, 2007a; Athanassiou and Korunić, 2007; Athanassiou et al., 2009; Korunić and Rozman, 2010). However, these mixtures still contained quite high concentrations of DE with significant effects on the reduction of bulk density and flowability.

Another possibility to overcome the obstacles in DEs use for grain protection is combining the nowadays discovered most effective DEs with another natural insecticides having different mode of action against insects; desiccation of insects caused with DE with toxicity against insects caused with another substances (Korunić, 2001; Korunić, 2007a, Athanassiou and Korunić, 2007). In many cases the existing synergy between DE and another substance(s) greatly enhanced the effectiveness of a mixture and therefore, the needed effective dosages of DE had been greatly reduced for approximately 4 to 10 times in the comparison with dosages of DE when used alone (Korunić, 2007; Athanassiou and Korunić, 2007; Almaši et al., 2013; Rozman et al., 2015).

If this newer enhanced formulation can respond to the limitations of DE use for direct mixing with grains, there will be a wider adoption of DE to control stored-product insect pests.

CONCLUSION

There has been an interest in using diatomaceous earth on stored grain as an alternative to synthetic residual insecticides because of concerns with insecticide residues in grain, worker safety and resistant insect populations. DE is now registered as a grain protectant or for structural treatment in several countries (Australia, Canada, China, Croatia, Germany, USA and other Asian countries). The mode of action of DE is different from the synthetic insecticides used in grain. DE absorbs the insect's cuticular waxes, and insects die from desiccation. The main advantages of using DE are its low mammalian toxicity and its stability. The main limitations are: reduction of the bulk density and flowablity of grain, dusty to apply, low efficacy against some insects and reduction in efficacy at high moisture contents. There can be a 20-fold difference in insecticidal activity among DEs of different geological sources. The commodity treated can also be a factor in determining the concentrations of DE required to obtain control. DE has been combined with other treatments: heat, cold and phosphine fumigation to control stored product insect infestations in bulk grain and in food processing facilities. Attempts are made to overcome DE limitations for direct mixing with grains. Several studies are conducted to discover new ways of DE use in grain protection. DE is mixed with other safe, natural substances in order to continue the use of this safe dust.

ACKNOWLEDGEMENTS

Financial support for this manuscript was provided by the Croatian Science Foundation (Scientific research project IP-11-2013-5570: "Development of new natural insecticide formulations based on inert dusts and botanicals to replace synthetic, conventional insecticides").

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OSVRT NA PRIRODNE INSEKTICIDE NA BAZI DIJATOMEJSKE ZEMLJE

SAŽETAK

Klimatski uvjeti imaju značajan utjecaj na djelotvornost dijatomejske zemlje (DZ). DZ je djelotvorna u suhim uvjetima i postaje praktično nedjelotvorna na štetne kukce u vlažnoj okolini. Visoka relativna vlaga zraka i oborine na otvorenome, kao i visoka vlaga zrnate poljoprivredne robe, smanjuju ili u potpunosti eliminiraju djelotvornost DZ-a na štetne kukce. Također, ovisno o vrsti kukaca, kao i o vrsti tretirane zrnate robe, početna je djelotvornost spora i niska te, da bi se ostvarila zadovoljavajuća djelotvornost, izloženost štetnih kukaca na tretiranoj robi trebala bi biti tijekom više dana. Uporaba DZ-a u vanjskome prostoru, naročito u područjima s obilnim oborinama, ne daje zadovoljavajuće rezultate. Stoga, glavna je uporaba DZ-a u zaštiti uskladištenih poljoprivrednih proizvoda, u industriji hrane, za obradu raznih površina i za suzbijanje štetnika u zatvorenome prostoru. Način djelovanja DZ-a na štetne kukce, utjecaj relativne vlage zraka i vlage robe na djelotvornost, sažeti pregled istraživanja djelovanja DZ-a u zaštiti uskladištenih žitarica, kao i istraživanja o mogućnosti smanjenja negativnog utjecaja na tretiranu robu, s kombiniranom primjenom DZ-a i drugih insekticidnih metoda smanjenoga rizika razmatraju se u ovome članku.

Ključne riječi: dijatomejska zemlja, štetni kukci, učinkovitost, prednosti, nedostaci

(Received on 1 April 2016; accepted on 3 May 2016 – *Primljeno 1. travnja 2016.; prihvaćeno 03. svibnja 2016.*)