

## NEW INSIGHTS INTO THE MULTIPLE PROTECTIVE FUNCTIONS OF DIATOMACEOUS EARTH DURING STORAGE OF AGRICULTURAL PRODUCTS

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### Abstract

*Testing and recommending means of fertilization as well as protective measures against the action of abiotic and biotic stressors during plant vegetation and post-harvesting, in a sustainable and environmentally friendly way, is one of the major challenges of researchers in the field, in close interdependence with the expectations of practitioners and last but not least of consumers. From this point of view, considering the chemical composition of diatomite, the use of diatomaceous earth may be a non-chemical alternative and / or complementary measure to the application of chemical fertilizers or plant-health treatments. The purpose of this review is to bring together recent, relevant data on the beneficial effects of diatomaceous earth use, in terms of insecticidal and fungistatic effects, also as mycotoxins adsorbent during storage of agricultural products.*

**Key words:** diatomite, insects, fungi, mycotoxins, storage, agricultural products.

### INTRODUCTION

During the storage of agricultural products, one of the main means of integrated pest management (IPM) is the reduction of chemical residues in food, by decreasing the doses used and the application of less toxic compounds. So, selected control strategies must be integrated for effective management of stored grain insects (Upadhyay and Ahmad, 2011).

Consequently, postharvest researches should be focused on reduction and elimination of synthetic pesticides, in close interdependence with the expectations of practitioners and last but not least of consumers. Accordingly, testing and recommending of protective measures against the action of biotic and abiotic stressors, during vegetation period, also post-harvesting, in a sustainable and environmentally friendly way, is one of the major challenges of researchers in the field (Rozman, 2015).

One of the very promising alternatives to traditional residual grain protectants (Kavallieratos et al., 2015) or chemical control of urban pests (Hosseini et al., 2014) is the use

of diatomaceous earth (DE) or diatomite (Golob, 1997), a natural inert compound from geological deposits. It consists mainly of the fossilized skeletons of diatoms, which contain in principal silicon dioxide (87-94%) important amounts of alumina (Al<sub>2</sub>O<sub>3</sub>), also ferric oxide (Fe<sub>2</sub>O<sub>3</sub>) (Tsai et al., 2006; Kaufhold et al., 2008). The diatomaceous deposits result from an accumulation in oceans or fresh waters of the amorphous silica (opal, SiO<sub>2</sub>C<sub>n</sub>H<sub>2</sub>O) cell walls of dead diatoms, which are microscopic single-cell aquatic plants (algae).

It has been widely reported that silicon (Si), a second most abundant element in soil, could reduce drought stress in plants (Rizwan et al., 2015). At the same time, it is known that silicon mediates the defense response to pests (see reviewed by Bakhat et al., 2018). In addition to improving soil characteristics (e.g. air penetration, water retention capacity, infiltration and so on), thanks to its physical properties (e.g. small particle size, high surface and high porosity) ([http:// www.mineralszone.com/minerals/diatomite.html](http://www.mineralszone.com/minerals/diatomite.html)), DE has proven to be the efficacy of other uses as well, such as protection against stress agents during the storage of agricultural products. As recently it

was also mentioned by Lupu and Manole (2015), DE is one of the most effective mechanical insecticides, for controlling insects damaging cereal seeds.

The safety of such treatments for humans and animals health is fully justified, due to the fact that DE has no toxic effects on mammals (rat oral LD 50>5000 mg/kg of body weight) and do not leave toxic residues in the product. Otherwise, diatomaceous earths are classified by EPA (Environmental Protection Agency) in the category of GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA, 1995).

In view of these considerations, this compound was studied by many researchers: applied alone (Sahaf et al., 2007; Hosseini et al., 2014), in combination with different additives (Korunic and Riozman, 2010) or other treatments types (Korunic, 1997) such as essential oils (Yang et al., 2010; Popescu et al., 2016; Lupu et al. 2017), aromatic plants (Pacheco et al., 2016), microorganisms, such as entomopathogenic fungi (Lord, 2001; Michalaki et al., 2007) and last but not least, it was registered as a grains protectant in different countries (Korunic, 1997; Pinto Júnior et al., 2010). Between its successful characteristics there can be mentioned: its unique way of action, low insect resistance development, high persistence on grain, high level of adherence on grain, easy removal from the grain and low mammalian toxicity (Korunic, 2013).

In this context, the purpose of this review is to bring together recent, relevant data on the beneficial effects of diatomaceous earth use, in terms of insecticidal and fungistatic effects, also as mycotoxins adsorbent during storage of agricultural products.

## **INSECTICIDAL EFFECT OF DIATOMACEOUS EARTH**

The insecticidal effects of DE depends on DE origin, commercial formulation, rates of application, exposure period, and last but not list, to the plant species treated, the insect species etc. (Wakil and Schmitt, 2015; Machekano et al., 2017; Perišić et al., 2018).

As Perišić et al. (2018) noticed, in the case of two DE originated from Serbia and one commercial formulation (Protect-It, Hedley

Technologies Ltd. Canada) applied against *Rhyzopertha dominica* (F). (*Coleoptera, Bostrichidae*) which produces the lesser grain borer, it was noticed an adults increase mortality, when the exposure period was longer and the application rates were increased. Of course, the DE effects were somehow different, in relation with the plants species (wheat, barley, rye, oats and triticale grains) and not negative influences were registered as regard as some grains features (moisture, proteins and ashes content).

Several DEs based on natural deposits are now commercially available, and have proved very effective against stored grain pests. The area of Eastern Europe is considered "rich" in diatomaceous earth deposits, due to the existence of large areas with siliceous-based deposition. The DEs of these areas have been used in the past for several applications, including their use as insecticides, and some of them are now the main ingredients in commercially available formulations. For instance, an amorphous silica DE from the Former Yugoslav Republic of Macedonia (FUROM) is the main ingredient for the DE formulation Protect-It (Hedley Technologies, Canada), which is one of the most commonly used DEs as insecticides worldwide (Athanassiou et al., 2011).

As Çetin and Taş (2012) states that Turkey is also considered to be a country where there are rich deposits of natural diatomite, with a reserve of about 125 million tons and the largest one (106 million tons) is at Hırka (Kayseri). Even under these circumstances DE-based products are not commercially available locally, to combat insects during grain storage, but Scanning Electronic Microscopy (SEM) preliminary studies on different DE proveniences have been carried out by Sağlam et al. (2017). They emphasized the existence of differences between Turkish DEs and commercial DEs, as regard as shape and size of DE bodies. The authors' conclusion was that there are necessary further studies with a view to better understand the interrelationship between the physical characteristics (e.g. shape and size) and the biological efficacy of DEs against stored product insect pests.

DE from six areas in Romania were analyzed from chemical and mineralogical point of view,

in order to select the most effective one for development of ecological products intended to control insect pests in grain storehouses. The characterization was done using X-ray fluorescence, wet-chemical analysis, Fourier - Transform Infrared Spectroscopy (FTIR) and X-ray powder diffraction. The target occurrences were those at Adamclisi, Borodu Mare, Chiuzbaia, Padina Mare, Tășad and Pătărlagele, ranging in age from Oligocene to Upper Pliocene. The mineralogy of all deposits was predominated by the presence of quartz, amorphous silica, feldspars and clay minerals, while the mineralogy of the diatomite rock from Tășad was characterized by the presence of aragonite (up to 55 wt.%), due to contamination by late sediments, issued from a hot-spring activity. Romania samples of DE had satisfactory results as regard as the insecticidal effects; beginning with 100 ppm and good economically results starting with 300 ppm (Dumitraș et al., 2015; Dumitraș et al., 2017).

The insecticidal efficacy of the DE (obtained from three Romanian sources: Pătărlagele, Urloaia and Adamclisi), applied at four doses (100, 300, 500 and 900 ppm), against granary weevil, *Sitophilus granaries* L. (*Coleoptera: Curculionidae*), with two essential oils was evaluated in an experimental model, in laboratory tests (Manole et al., 2015). The results showed that mortality induced by DE was at the levels between 83.33% and 100% in all variants, after 21 d, compared with untreated control and a standard product Silicosec®. It has also been demonstrated that the insecticidal efficacy was highly influenced by exposure time, dose and essential oil type.

According to Pacheco et al. (2016), as compared with some potential repellent action of aromatic plants or control variants, DE application emphasized statistical difference as regard as the number of dead insects of *Zabrotes subfasciatus* (Boheman) (*Coleoptera: Chrysomelidae: Bruchinae*) in common beans, in 210 and 240 storage days. On the other hand, it is important to mention that during 180 storage days, the aromatic tested plants did not affected the physiological quality of seeds, while after 90 days, DE increased abnormality symptoms in common-bean seedlings.

Using of some combined treatments, such as a mixture between a biological agent (*Beauveria bassiana*) ( $3 \times 10^{10}$  conidia per kg), DE (150 ppm) and a neonicotinoid (Imidacloprid) (5 ppm) have been efficient against three beetle species (*R. dominica*), *Tribolium castaneum* Herbst. (*Coleoptera: Tenebrionidae*), *Cryptolestes ferrugineus* Stephens (*Coleoptera: Laemophloeidae*), and the *Psocoptera* species (*Liposcelis paeta* Pearman) (*Psocoptera: Liposcelididae*), the major stored wheat insect pest species under farm conditions of Pakistan (Wakil and Schmitt, 2015). The authors suggested that the obtained results have a practical importance, particularly for local farmers.

DE applied with a naturally derived insecticide (Spinosad), as DE Insecto had effective protective influence to the larvae the Indian meal moth, *Plodia interpunctella* (Hübner), assuring a mortality up to 86-97% of first instars of the insect, at 500 and 1000 ppm (Subramanyam et al., 1998). When Spinosad was applied alone at 1 ppm, the larvae mortality registered was 97.6-99.6% (Fang et al., 2002). In the case of maize grain, Machekano et al. (2017) noticed that a combination of DE with Spinosad and DE with deltamethrin emphasized the same efficacy against *Prostephanus truncates* (Horn), *Sitophilus zeamays* (Motschulsky) and *T. castaneum*, as the commercial combinations of organophosphates and synthetic pyrethroids used in current commercial grain protectants: fenitrothion 1.0% w/w (10000 ppm) + deltamethrin 0.13% w/w (130 ppm). The authors also mentioned that such treatments are safer and possibly cheaper effective alternative, to protect seeds against pests attack.

Besides DE protective effects, it is very important to use lower effective doses (Kavallieratos et al., 2015). So, combining DE with low levels of other insecticidal compounds such as: DEBBM (a mixture of two natural compounds: bitterbarkomycin (BBM) and DE), DEA (a mixture of abamectin and DE) and DESgBAIT (a mixture of DE, silica gel Sipernat 50 S and food grade bait) was interesting and promising in the case of maize and wheat grains protection against *R. dominica* and *Sitophilus oryzae* (L.) (*Coleoptera: Curculionidae*) and one external

feeder, *Tribolium confusum* Jacquelin du Val (*Coleoptera: Tenebrionidae*).

Successful results were also obtained for wheat, by Athanassiou et al. (2009), in laboratory conditions, when DE was applied in combination with bitterbarkomycin (BBM), an extract obtained from the roots of *Celastrus angulatus*, against adults of the maize weevil, *S. zeamais* the red flour beetle, *T. castaneum* and the rusty grain beetle, *Cryptolestes ferrugineus* (Steph.) (*Coleoptera: Cucujidae*). Application of BBM or DEBBM assured a higher mortality of *S. zeamais*, as compared with DE alone. Moreover, for *T. castaneum*, with few exceptions, by DEBBM application the mortality was significantly higher, as compared with that caused by DE or BBM. As for instance, after 14 days of exposure, mortality was 90% on wheat treated with 100 ppm of DEBBM. *C. ferrugineus* has been shown to be the most susceptible species, in which case, only after 5 days of exposure even at the lowest DEBBM dose, the recorded mortality was 90%.

In addition, so many botanical insecticides such as different plant materials, plant extracts, essential oils (Yang et al., 2010) or some natural products of plants (Isman et al., 2010) proved to be effective when are alone applied (Sahaf et al., 2007) or when added to other treatments combination, effects can be synergistic or antagonistic (Ziaee et al., 2014). Often, applying only essential oils can result in very weak insecticide efficacy, because these are volatile rapidly, but associated with DE, even when *Carum copticum* (L.) was assured in a sub-lethal concentration, there was an increase of insects' locomotor activity. This higher insects' mobility results in more pronounced contact of the insect exoskeleton (cuticle) with DE, what it means implicitly a more lipids absorption (Lord, 2001), pronounced dehydration, more abrasion and ultimately death of the insect (Ebeling, 1971). Moreover, as it was mention above, the DE particle size is defining for treatment to be successful as Ziaee et al. (2014) demonstrated. The synergistic effect of essential oil and DE on *T. confusum* was noticed when DE particle size was less than 37  $\mu\text{m}$ , while in the case of larger particles (149  $\mu\text{m}$ ) there was not registered such effect. The author's explanation

was that smaller particles assure a larger surface area relating to volume. In such conditions, DE has an improving capacity to maintain oils in their small pores, and their release will be very slow. A strong additive efficacy of garlic (*Allium sativum* L.) essential oil applied with DE against adult rice weevils *S. oryzae* and redflour beetles, *T. castaneum* was obtained by laboratory bioassays (Yang et al., 2010). In such combination, there was registered a longer lasting period of essential oil, but only a small increase in persistence. Thus, authors recommended extensive research to optimize the application rates of the mixture treatment, to improve toxicity and persistence and to evaluate the economics of any formulation of garlic essential oil plus DE, too. A synergistic effect on the mortality of *R. dominica* was noticed for *C. sinensis* essential oil, when it was combined with kaolin, an antagonistic one, when the DE was added to this (Campolo et al., 2014).

Another promising IPM strategy proposed to additional research was that indicated by Michalaki et al. (2007), based on application of DE associated with the entomopathogenic fungi (EF) such as *Paecilomyces fumosoroseus*, (Wise) Brown, taking into consideration that both substances can be applied by the same technology, as traditional grain protectants. In this context, results obtained by Lord (2001) with *Beauveria bassiana* (Balsamo) Vuillemin, (the most important and common EF) at rates of 11, 33, 100, and 300 mg of conidia per kilogram of grain with and without single rates of DE that killed 10% or less of the target beetles, emphasized the synergism against adult of *R. dominica*, at all applied doses. The author states the followings: taking into account that the two types of treatments are complementary in their optimal environmental action, the formulation of myco insecticides -lipophilic materials is of interest, both for the control of adults and of the immature stages, of different stored - products insect pests.

Through a combined treatment of entomopathogenic fungi, *Metarhizium anisopliae* (Metschinkoff) Sorokin and *B. bassiana*, with DE SilicoSec (DE commercial formulation) applied to wheat at 200 mg/kg and 400 mg/kg against *T. castaneum* and *Oryzaephilus surinamensis* L. showed a significantly reduced

pathogenicity after 7 days of exposure, excepting *R. dominica*. It was demonstrated that the isolates were virulent to the beetles and the efficacy increased in combination with DE, especially in the case of longer exposure time. At the same time, it was noted that the most resistant species was *T. castaneum*, followed by *R. dominica* (Shafiqhi et al., 2014).

As excellently reviewed by Batta and Kavallieratos (2018), there are many research reports which present different effects of combinations between EF and other components, including DEs against stored-products insects. In the case of EF and DE a synergistic effect was noticed, but not with natural enemies belonging to arthropods. It was mentioned a true thing, that the action of EF against insects pests, especially for stored grain is compatible with the food safety and environmental regulations, and implicitly maybe a success alternative in the future, to implement the use of these biocontrol agents instead synthetic insecticides.

The utility of DE has also been proven against house pests, in dwellings in urban areas. Hosseini et al. (2014) noticed that DE treatment is a promising procedure against German cockroaches, *Blattella germanica* (L.), the most common roaches in houses and restaurants. Increase of DE doses from 2.5 to 25 g/m<sup>2</sup>, respectively for 24, 48 and 72 h exposure period determined after 24 h a proportionally increasing of adults and nymphs mortality rate. After specimens transfer to the beakers contained food and water, results obtained after 1 week were not significant from the statistical point of view, as regard as the lethality of 50% of DE plus water, on the German cockroach nymphs.

Also, the bed bugs (*Cimex lectularius* L.) still creates major problems in the built environment, as Romero et al. (2017) mentioned, in the recent bibliographic synthesis.

Therefore, even if proactive bed bug management programs prove to be effective, sustainable and economically viable in the long term than reactive and insecticide - only programs, the adoption of good practices can be limited by the budget limitation and interest of affordable multiunit housing providers.

Nowadays, increased attention needs to be paid to the problem of the phenomenon of resistance

to insecticides in this case (Dang et al., 2017), or other cases, too. Careful monitoring of the resistance to insecticide is required, also an understanding of the resistance mechanisms can determine a proper use of insecticides and / or timely modification of chemical control strategies can be recommended. Thus, thanks to previous bioassay, genetic, morphological, biochemical and behavioral studies and due to the evolution of investigation approaches, such detailed studies are accessible.

One of the most plausible explanations of DE efficacy is the epicuticular effect for insect dehydration. When the insect moves through DE, the particles are picked up by the insect's body and absorb wax from the insect's cuticle, due to DE adsorptive property. Thanks to its high amounts of amorphous silica particles and as a consequence in water loss, the insect dies by dehydration (Ebeling, 1971; Lupu et al., 2016) and partially through abrasion (Subramanyam and Roesli, 2000).

The toxicological successfully effect on insects is related to amorphous silica content, with uniform particle size (<10 µm), with a high capacity to oil sorption, an extensive and active surface area, next to a lower content of clay and other metal oxides (Korunic, 1997).

Nevertheless, the use of DE involves certain disadvantages, such as those regarding health concerns, the reduction in bulk density, differences in insect species tolerance to the same DE formulation, the effects of grain moisture and temperature on the effectiveness against insects, the influence of various commodities on DE efficacy or the use of DEs in some other fields (Koruniæ, 2016). That is why, to be a wider acceptance of DE as a means to control stored-product insect pests, the studies must be continued, with a view to develop a safe enhanced formulation, with a low DE concentration and as a consequence a minimal adverse effect on bulk density and grain flowability.

## ANTIFUNGAL EFFECT OF DIATOMACEOUS EARTH

The use of kaolin and DE based treatments and/or combined sweet orange [*Citrus sinensis* (L.) Osbeck] peel essential oil has led to the detection of toxic effects on *R. dominica* and

the damage wheat microbial populations. The efficacy of different formulations, application rates and exposure times for insect mortality and progeny production were different. Thus, a synergistic effect on the mortality of *R. dominica* was noticed for *C. sinensis* essential oil, when it was combined with kaolin, and an antagonistic one, when the DE was added to this. As regard as yeasts, moulds, as well as total mesophilic aerobic bacteria growths were reduced when the *C. sinensis* essential oil was applied alone, as compared with the other dusts and dust-essential oil-based treatments (Campolo et al., 2014).

Recent research carried out by Fernández and Bellotti (2017) on the modification of DE on the basis of quaternary ammonium groups has led to obtain hybrid materials, a more effective form of DE, which can be used as antimicrobial filter material.

#### **MYCOTOXIN ADSORBENT PROPERTIES OF DIATOMACEOUS EARTH**

The quality of the products is subject to the influence of many abiotic and biotic stress factors (Delian, 2006), so knowing the critical control points during harvesting, drying and storage stages of the grain is essential for the development of effective post-harvest prevention strategies, including those related to mycotoxin contamination (Magan and Aldred, 2007). Mycotoxins are secondary metabolites produced by pathogenic fungi, accumulated in the field conditions, such as pre-harvest deoxynivalenol produced by *Fusarium graminearum* or post-harvest zearalenone (ZEA) due to *F. poae*; also ochratoxin due to *Penicillium verrucosum* (Magan et al., 2010). There is the risk that a good quality raw material may be contaminated if it is not stored in an appropriate manner, too (Kabak et al., 2006).

In both cases, the presence of mycotoxins causes contamination across the whole food chain and shows toxicity to animals and humans (He and Zhou, 2010; Raiola et al., 2015; Lupu et al., 2017), having a significant economic impact on animal agriculture, also (Smith and Girish, 2012).

To overcome this inconvenient, expertly performed crop protection practices are

absolutely necessary (reviewed by Raiola and Ritieni, 2014) and adequate procedures are need to be apply, with a view to reduce the possible mycotoxins incidence (He and Zhou, Besides, because the preventive methods applied during the growing, harvesting and storage periods are only meant to reduce the risk of contamination, post-harvest detoxification procedures are required (He and Zhou, 2010). The results of the recent research carried out in our country (Popescu et al., 2016) led to the production of an eco-friendly bioproduct, through the use of clean technology of green chemistry, based on the well-known DE features and the antimicrobial characteristics of essential oils extracted from medicinal plants. The product has been shown to have a significant repellent potential against adults of *Sitophilus granaries*, as well as an antifungal action of thyme volatile oils coupled with diatomite as insecticide and mycotoxin absorbing agent has been highlighted.

One of the strategies for reducing the exposure to mycotoxins is to decrease their bioavailability, by including various mycotoxin-adsorbing agents in the compound feed, which leads to a reduction of mycotoxin uptake, as well as distribution to the blood and target organs. Mycotoxin binders can work in three ways: by physically binding toxins and metal ions by adhesion; by binding toxins through electrostatic charge or cation exchange capacity. They can eliminate the source of the toxins, by increasing cell membrane permeability of the fungi, which are the cause of mycotoxin production. There are a range of commercially available mycotoxin binders and antifungal agents, which have been shown to have varying potency effects at reducing the presence or eliminating the toxicity of mycotoxins. The development of a successful commercial mycotoxin binder incorporates the best of each of these active ingredients at the required concentration, to ensure that the overall function acts to reduce the harmful effects of mycotoxins in animal nutrition (Pearce et al., 2010). There are a number of approaches that can be taken to minimise mycotoxin contamination in animal feeds and these involve prevention of fungal growth and therefore mycotoxin formation, and strategies to reduce or eliminate mycotoxins from

contaminated commodities, especially feed additives. It seems impossible to completely remove such mycotoxins from the animal and human food chain, given that feed and its precursors can be stored and transported throughout a range of time intervals, atmospheric humidity and temperatures. OcroTox (an activated DE) has been tested as a detoxifying agent of ochratoxin A (OTA), a secondary metabolite produced by some strains of *Aspergillus ochraceus* and *Penicillium verrucosum*, in laying hens (Denli et al., 2008). Addition of OcroTox to the contaminated diet alleviated the negative effects resulting from OTA, reaching values not significantly different from the control diet for most of the parameters, except the relative weight of the liver, showing that OcroTox counteracted the deleterious effects caused by OTA (Boudergue et al., 2009). Another important way to improve characteristics of the silica based materials is to combine with other components, organic or inorganic, resulting composite materials or materials with core-shell structure (Florea et al., 2012).

The mode of action of the feed supplements is different: mould inhibitor for animal feeds with a preventive action, mycotoxin binder, or absorption of moisture (<http://orbitec.es/feed-additives-feed-ingredients/mold-inhibitor/>).

Naturally-balanced feed supplements help to amend the existing deficiencies in major feed elements, with no harmful effect on humans, animal health or the environment. There are such supplement, commercially available (Diatomaceous earth\*), based on ground fossil shells from the remains of single-cell algae called diatoms. The product is untreated, unheated, food grade, fresh water variety. The fine drying powder absorbs moisture and dehydrates on contact. It can be used as an animal feed additive, up to 2% of feed ration (<http://www.ohioearthfood.com/animal-feed-supplements.html>) as FDA recommends (Food and Drug Administration, USA, 1995).

In consequence, prevention of mycotoxicoses in livestock and poultry by different dietary strategies such as inorganic and organic adsorbents, mycotoxin inactivators and nutritional supplements, and knowing their detoxifying, decontaminating mechanism and relative efficacy have been considered. The use

of mineral additives, as for instance DE (as a mycotoxin-binding agent) added to animal feed has been shown to reduce the toxic effects of mycotoxins, due to the sequestering (absorbing) in the digestive tract of the animals (Smith and Girish, 2012). Moreover, the use of diatomite along with other based on natural silica materials has proven effective in biomedical applications, such as use in drug delivery as carrier for Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), given its physicochemical and functional characteristics and its good biocompatibility (Krajišnik et al., 2017).

Studies carried out by Sprynskyy et al. (2012) have shown that talc had stronger adsorption properties for ZEA at its hydrophobic surface, than diatomites, that have a more hydrophilic surface, in the case of synthetic gastric fluids. The authors mentioned that the driving force for the physical adsorption process that link ZEA and DE is probably due to the hydrophobic bonding between the surface siloxane groups of DE and the partially positive electrical charges of ZEA molecules. In the same time, the ZEA adsorption on DE can be limited by competition of less polar toxin molecules, with the stronger polar water molecules.

There are many concerns to study the DE efficacy. The study of the bioactive properties of DE and essential oils found an innovative approach of feed treatment in the sequence of food chain from storehouse to animal body grown in farm, with ecological products obtained from cheap minerals and renewable vegetal sources, such as DE and essential oils, with insecticidal-fungicidal and adsorbent properties (Lupu et al., 2017).

## CONCLUSIONS

Using the diatomaceous earth as protectant for stored products against pest infestations constitutes one of the most efficient naturally occurring dust. Among the main advantages of this treatment are: it is safe, has low toxicity to mammals, does not affect the final quality of the product, provides long-term protection, is registered as a food additive and is an alternative to the application of synthetic pesticides (Shafiqhi et al., 2014).

The combination of diatomaceous earth with plants oils provides two important advantages. Firstly, it is more cost-effective as it decreases both the amount of oil and DE needed, and it reduces the adverse effects of the oil, i.e. its strong odor and volatility. Secondly, essential oil and diatomaceous earth are environmentally compatible, an essential consideration in the development of insecticidal treatments for grain (Yang et al., 2010).

The innovative product developed in Romania (Popescu et al., 2017) consists of 15-25% potassium salts of fatty acids from vegetable oils, 3-5% potassium acetate, 1.5-3% glycerin, 1-5% essential oils extracted from aromatic herbs selected from spontaneous or cultivated flora, due their recognized insecticidal and fungicidal properties, a natural insect attractant, 40-65% bioactive mineral vehicle such as insecticidal diatomaceous earth, a biopolymeric adhesive if necessary, 1.5-2% unsaponifiable substances and water. The novel granulated eco-friendly product, based on natural non-toxic and accessible raw materials ensures attracting insects to stored grains, due to ecological attractants including volatile esters, followed by dehydration insects attracted into contact with diatomaceous earth.

Another Romanian invention (Lupu et al., 2017) relates to a method of protecting stored cereals against insects and toxigenic fungi. The method consists in placing, prior to the introduction of the cereals into the storage place, on the basis of the cereal volume, some pellets containing plant debris and diatomite, soaked with volatile oil of thyme, and after the filling of the deposit, dried diatomaceous earth is administered on the cereals surface, in thin layer, as a contact insecticide.

Considering some undesirable effects, to be a wider acceptance of diatomaceous earth as a means to control stored-product insect pests, the studies must be continued, with a view to develop a safe enhanced formulation, with a low DE concentration and as a consequence with a minimal adverse effect on grain (Koruniæ, 2016).

The general conclusion may be that the ecological products are one of the most effective and valuable tools for good practice of risk management in the grain storage and agro

veterinary sector, as eco-effective alternatives to chemical treatments (Lupu et al., 2017).

## REFERENCES

- Athanassiou C.G., Korunic Z., Vayias B.J., 2009. Diatomaceous earths enhance the insecticidal effect of bitterbarkomycin against stored-grain insects. *Crop Protection*, Vol. 28, 123-127.
- Athanassiou C.G., Kavallieratos N.G., Vayias B.J., Tomanovi Z., Petrovi A., Rozman V., Adler C., Korunic Z., Milovanovi D., 2011. Laboratory evaluation of diatomaceous earth deposits mined from several locations in central and southeastern Europe as potential protectants against coleopteran grain pests. *Crop Protection*, Vol. 30, 329-339.
- Athanassiou C.G., Kavallieratos N.G., Chiriloaie A., Vassilakos T.N., Fătu V., Drosu S., Ciobanu M., Dudoiu R., 2016. Insecticidal efficacy of natural diatomaceous earth deposits from Greece and Romania against four stored grain beetles: the effect of temperature and relative humidity. *Bulletin of Insectology*, Vol. 69 (1), 25-34.
- Bakhat H.F., Bibi N., Zia Z., Abbas S., Hammad H.M., Fahad S., Ashraf M.R., Shah G.M., Rabbani F., Saeed S., 2018. Silicon mitigates biotic stresses in crop plants: A review. *Crop Protection*, Vol. 104, 21-34.
- Batta Y.A., Kavallieratos N.G., 2018. The use of entomopathogenic fungi for the control of stored - grain insects. *International Journal of Pest Management*, Vol.64 (1), 77-87.
- Boudergue C., Burel C., Dragacci S., Fravot M.C., Fremy J.M., Massini C., Prigent P., Debongnie P., Pussemier L., Boudra H., Morgavi D., Oswald I., Perez A., Avantaggiato G., 2009. Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety (scientific report submitted to EFSA). 10.2903/sp.efsa.2009.EN-22.
- Campolo O., Romeo F.V., 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. *Industrial Crop and Products*, Vol. 61, 361-369.
- Çetin M., Taş M., 2012. Biyolojik orjinli tek mineral: Diatomit. *Türk Bilim Araştırma Vakfı (TÜBAV) Bilim Dergisi*, Vol. 5 (2), 28-46.
- Dang K., Doggett S.L., Veera Singham G., Lee C.Y., 2017. Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae). *Parasites & Vectors*, Vol. 10, 318.
- Delian E., 2006. Fiziologia stresului biotic la plante. Editura Cartea Universitară. București.
- Denli M., Blandon J.C., Guynot M.E., Salado S., Perez J.F., 2008. Efficacy of a New Ochratoxin-Binding Agent (Ocratox) to Counteract the Deleterious Effects of Ochratoxin A in Laying Hens. *Poultry Science*, Vol. 87, 2266-2272.
- Dumitraş D.G., Marincea Ş., Lupu C., 2015.

- A chemical and mineralogical survey of some diatomaceous deposits in Romania, Book of Abstracts of the 12th International Congress for Applied Mineralogy, Aug., 10-12, Istanbul, Turkey, pg. 25.
- Dumitraş D.G., Sebe-Rădoi O.G., Marinea Ş., Costea C., 2017. Preliminary mineralogical and paleoenvironmental study of the diatomites from Adamclisi, South Dobrogea, Romania. Geophysical Research Abstract, EGU 2017-6317, 19, EGU General Assembly, eISSN 1607- 7962.
- Ebeling W., 1971. Sorptive dust for pest control. Annu. Rev. Entomol., Vol. 161, 123-158.
- Fang L., Subramanyam B., Arthur F.H., 2002. Effectiveness of spinosad on four classes of wheat against five stored-product insects. Journal of Economic Entomology, Vol. 95, 640-650.
- FDA (Food and Drug Administration) USA, 1995. Specifications for diatomaceous earths as a maximum 2% animal feed additive. 21 CFR Section 573. 340.
- Fernández M.A., Bellotti N., 2017. Silica-based bioactive solids obtained from modified diatomaceous earth to be used as antimicrobial filler material. Materials Letter, Vol. 194, 130-134.
- Florea M.G., Ficai A., Oprea O., Guran C., Ficai D., Pall L., Andronescu E., 2012. Drug delivery systems based on silica with prolonged delivery of folic acid. Romanian Journal of Materials, Vol. 42 (3), 313 - 316.
- Golob P., 1997. Current status and future perspectives for inert dusts for control of stored-product insects. J Stored Prod Res., Vol. 33, 69-80.
- He J., Zhou T., 2010. Patented techniques for detoxification of mycotoxines in feeds and food matrices. Recent Pat Food Nutr Agric., Vol. 2 (2), 96-104.
- Hosseini A.A., Bazrafkan S., Vatandoost H., Abaei M.R., Ahmad M.S., Tavassoli M., Shayeghi M., 2014. The insecticidal effect of diatomaceous earth against adults and nymphs of *Blattella germanica*. Asian Pac J Trop Biomed, Vol. 4 (Suppl 1), S228-S232.
- Isman M., Miresmailli S., Machial C., 2010. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochem. Rev., Vol. 10, 197 -204.
- Kaufhold S., Dohrmann R.Ch., 2008. Ulrichs Shelf life stability of diatomites. Applied Clay Science, Vol. 41 (3-4), 158-164.
- Kavallieratos N.G., Athanassiou C.G., Korunic Z., Mikeli N.H., 2015. Evaluation of three novel diatomaceous earths against three stored grain beetle species on wheat and maize. Crop Protection, Vol. 75, 132-138.
- Korunić Z., 2016. Overview of undesirable effects of using diatomaceous earths for direct mixing with grains. Pestic. Phytomed. (Belgrade), Vol. 31 (1-2), 9-18.
- Krajišnik D., Daković A., Janičijević J., Milić J., 2017. Chapter 8 - Natural and Modified Silica-Based Materials as Carriers for NSAIDs. Microsized and Nanosized Carriers for Nonsteroidal Anti-Inflammatory Drugs, 219-258.
- Lord J.C., 2001. Desiccant Dusts Synergize the Effect of *Beauveria bassiana* (*Hyphomycetes: Moniliales*) on Stored-Grain Beetles. Journal of Economic Entomology, Vol. 94 (2), 367-372.
- Lupu C., Manole T., Chiriloaie A., 2016. Effectiveness of main sources of diatomaceous earth from Romania on populations of *Sitophilus granaries* L. (*Coleoptera: Curculionidae*) under controlled conditions. Romanian Journal of Plant Protection, Vol. VIII, ISSN 2248-129X; ISSN-L 2248-129X.
- Lupu C., Dudoiu R., Fatu V., Popescu M., Radu E., Oancea F., 2017. Management of mycotoxin contamination in stored grains by using ecological means. Abstract/Journal of Biotechnology, 256S, S44-S116.
- Lupu C., Fătu V., Manole T., 2017. Ecological treatment method for protecting stored grain against insects and toxigenic fungi. RO Patent application\_A2015-00918/2015, in: RO-BOPI 5, pp. 12.
- Machekano J., Mvumi B.M., Chinwada P., Richardson-Kageler S.J., Rwafa R., 2017. Efficacy of diatomaceous earths and their low-dose combinations with spinosad or deltamethrin against three beetle pests of stored-maize. Journal of Stored Products Research, Vol. 72, 128-137.
- Magan N., Aldred D., 2007. Post-harvest control strategies: minimizing mycotoxins in food chain. Int J Food Microbiol., Vol. 119, 131-139.
- Magan N., Aldred D., Mylona K., Lambert R.J., 2010. Limiting mycotoxins in stored wheat. Food Addit Cotam Part A Chem Anal Control Exp Risk Assess., Vol. 27 (5), 644-650.
- Manole T., Lupu C., Fătu V., Chiriloaie A., Chițoran F., 2015. Experimental Model for Biological Control of Stored Grain Pests, in: Journal of Agricultural Science and Technology A, Vol. 5, 793-798.
- Michalaki M.P., Athanassiou C.G., Steenberg T., Buchelos C.Th., 2007. Effect of *Paecilomyces fumosoroseus* (Wise) Brown and Smith (*Ascomycota: Hypocreales*) alone or in combination with diatomaceous earth against *Tribolium confusum* Jacquelin du Val (*Coleoptera: Tenebrionidae*) and *Ephestia kuehniella* Zeller (*Lepidoptera: Pyralidae*). Biological Control, Vol. 40, 280-286.
- Moore D., Lord J.C., Smith S.M., 2000. Pathogens. In: Subramanyam Bh., Hagstrum D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers., Dordrecht, pp. 193-227.
- Pacheco F.P., Nobrega L.H.P.N., Tonini M., Cruz-Silva C.T.A., 2016. Common bean seeds quality during storage under treatments with potential repellent of aromatic plants. Rev. Bras. Pl. Med., Campinas, Vol. 18 (2), 473-479.
- Pearce M., Shahin I., Palcu D., 2010. Available solutions for mycotoxin binding. Special Nutrients Inc.
- Perisic V., Vukovic S., Perisic V., Pesic S., Vukajilovic F., Andric G., Kljajic P., 2018. Insecticidal activity of three diatomaceous earths on lesser grain borer, *Rhyzopertha dominica* F., and their effects on wheat, barley, rye, oats and triticale grain properties. Journal of Sored products Research, Vol. 75, 38-46.

- Popescu M., Oancea F., Desliu-Avram M., 2016. Vegetable oil conversion into core-shell bioproducts for stored grain protection. Scientific Bulletin. Series F. Biotechnologies, Vol. XX, 210-213.
- Popescu M., Oancea F., Deşliu-Avram M., 2017. Ecological product for stored grain protection and process for obtaining the same. RO Patent application\_A2015-00817/2015, in: RO-BOPI 5, pp. 13.
- Raiola A., Ritieni A., 2014. Good Food and Feed Processing Techniques. in Mycotoxin Reduction in Grain Chains.
- Raiola A., Tenore G.C., Manyes L., Meca G., Ritieni A., 2015. Risk analysis of main mycotoxins occurring in food for children: An overview. Food and Chemical Toxicology, Vol. 94, 169-180.
- Rizwan M., Ali S., Ibrahim M., Farid M., Adrees M., Bharwana S.A., Zia-Ur-Rehman M., Qayyum M.F., Abbas F., 2015. Mechanisms of silicon-mediated alleviation of drought and salts stress in plants: A review. Environ Sci Pollut Res Int., Vol. 20, 15416-31.
- Rizwan G., Muhammad E.T., 2017. Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review, in Environmental Science and Pollution Research, Vol. 24 (14). DOI: 10.1007/s11356-017-8904-x.
- Romero A., Sutherland A.M., Gouge D.G., Spafford H., Nair S., Lewis V., Choe D.H., Li S., Young D., 2017. Pest management strategies for bed bugs (*Hemiptera: Cimicidae*) in multiunit housing: a literature review on field studies. Journal of Integrated Pest Management, Vol. 8 (1).
- Rozman V., 2015. Control of stored products pests by natural products. Integrated Protection of Stored Products IOBC - WPRS Bulletin, Vol. 111, 295-299.
- Sağlam Ö., Işıkber A.A., Tunaz H., Kubilay E.R., Bahadır F., Şen R., 2017. Preliminary Checking of Some Turkish Diatomaceous Earth Similarities with Commercial Diatomaceous Earths under Scanning Electron Microscope (SEM). Tekirdağ Ziraat Fakültesi Dergisi. Journal of Tekirdag Agricultural Faculty, The Special Issue of 2nd International Balkan Agriculture Congress, May 16-18.
- Shafiqhi Y., Ziaee M., Ghosha Y., 2014. Diatomaceous earth used against insect pests, applied alone or in combination with *Metarhizium anisopliae* and *Beauveria bassiana*. Journal of Plant protection Research, Vol. 54 (1), 62-66.
- Sahaf B.Z., Moharrampour S., Meshkatsadat M.H., 2007. Chemical constituents and fumigant toxicity of essential oil from *Carum copticum* against two stored product beetles. Insect Sci., Vol. 14, 213-218.
- Smith T.K., Girish C.K., 2012. Animal Feed Contamination. Prevention and control of animal feed contamination by mycotoxins and reduction of their adverse effects in livestock. Effects on Livestock and Food Safety. A volume in Woodhead Publishing Series in Food Science, Technology and Nutrition, 326-351.
- Sprynskyy M., Gadza R., Nowak K.K., Buszewsk B., 2012. Removal of zearalenone toxin from synthetics gastric and body fluids using talc and diatomite: A batch kinetic study. Colloids and Surfaces B: Biointerfaces, Vol. 94, 7-14.
- Subramanyam B.H., Madamanchi N., Norwood S., 1998. Effectiveness of Insecto applied to shelled maize against stored-product insect larvae. Journal of Economic Entomology, Vol. 91, 280-286.
- Subramanyam B.H., Roesli R., 2000. Inert dusts. In: Subramanyam B.H., Hagstrum D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Massachusetts, pp. 321-379.
- Tsai W-T., Lai C-W., Hsien K-J., 2006. Characterization and adsorption properties of diatomaceous earth modified by hydrofluoric acid etching. Journal of Colloid and Interface Science, Vol. 127, 749-754.
- Upadhyay R.K., Ahmad S., 2011. Management Strategies for Control of Stored Grain Insect Pests in Farmer Stores and Public Ware Houses. World Journal of Agricultural Sciences, Vol. 7 (5), 527-549.
- Wakil W., Schmitt T., 2015. Field trials on the efficacy of *Beauveria bassiana*, diatomaceous earth and imidacloprid for the protection of wheat grains from four major stored grain insect pests. Journal of Stored Products Research, Vol. 65, 160-167.
- Yang F.L., Liang G.W., Xu Y-J., Lu Y.Y., Zeng L., 2010. Diatomaceous earth enhances the toxicity of garlic, *Allium sativum*, essential oil against stored-product pests. Journal of Stored Products Research, Vol. 46, 118-123.
- Ziaee M., Moharrampour S., Francikowski J., 2014. The synergistic effects of *Carum copticum* essential oil on diatomaceous earth against *Sitophilus granaries* and *Tribolium confusum*. Journal of Asia-Pacific Entomology, Vol. 17, 817-822.
- <http://www.ohioearthfood.com/animal-feed-supplements.html>
- <http://orbitec.es/feed-additives-feed-ingredients/mold-inhibitor/>
- <http://www.mineralszone.com/minerals/diatomite.html>