Session 7

Contact Pesticides, Residual Products, and Plant Extracts

Laboratory Evaluation of Turkish Diatomaceous Earths as Potential Stored Grain Protectants

Sezgin Akçali[1], Ali Arda Işikber [1*], Özgür Sağlam[2], Hasan Tunaz[1], Mehmet Kubilay Er[1]

[1]Kahramanmaraş Sütçü İmam University, Agriculture Faculty, Plant Protection Department, Avşar Campus, 46100, Kahramanmaraş, TURKEY

[2]Namık Kemal University, Agriculture Faculty, Plant Protection Department, Tekirdağ, TURKEY

* Corresponding Author: isikber@ksu.edu.tr

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In this study, efficacy of local diatomaceous earths (DE) collected from different regions of Turkey against stored grain insects, *Sitophilus oryzae* (L.), *Tribolium confusum* du Val. and *Rhyzopertha dominica* (F.), was investigated. For this purpose, biological tests were carried out at concentrations of 500 and 1000 ppm (mg DE / kg wheat) of 9 local diatomaceous earths and one commercial diatomaceous earth, namely Silicosec® as positive control at 25 ± 1 ° C temperature and 65 ± 5% relative humidity in wheat. In addition, the studies on some of the chemical and physical analysis of the tested diatomaceous earths (silicon dioxide (SiO_2) ratio, particle size and adhesion rate on commodity) were also conducted. In biological tests conducted at 500 ppm concentration for 14 days of exposure in wheat the highest mortality rates (97 to 98%) of *S. oryzae* adults were recorded in CB2N-1, AGN-1 and BGN-1 diatomaceous earths, while the highest mortality rates of *T. onfusum* adults were obtained from only AGN-1 and BGN-1 diatomaceous earths. In the case of *R. dominica*, the highest mortality rate (64.4%) was recorded only in CB2N-1 diatomaceous earth. At concentration of 1000 ppm for 14 days of exposure in wheat, 100% mortality of *S. oryzae* adults was observed in all tested local diatomaceous earths except FB2N-1 and Silicosec® while mortality rates of *T. confusum* adults ranging from % 95 to %100 were obtained in all tested local diatomaceous earths except FB2N-1, FBN-1 and Silicosec®. In the case of *R. dominica* adults, mortality rates ranging from 80% to 93% were recorded in CB2N-1, CCN-1 and AG2N-1 diatomaceous earths. In conclusion, laboratory bioassays indicated that CB2N-1 and BGN-1 local diatomaceous earths had high efficacy against *S. oryzae*, *T. confusum* and *R. dominica* adults and thus could be potential to be successfully used for controlling stored grain insect pests as a grain protectant.

Key Words: Turkish diatomaceous earth, wheat, Sitophilus oryzae, Tribolium confusum, Rhyzopertha dominica

Introduction

Stored products and especially grains and their by-products are the most important durable food category for human nutrition. During storage, these commodities are attacked by a numerous pests, particularly insect species, known as stored product pests, which cause very serious quantitative losses and qualitative degradations. Apart from the direct infestation *per se*, the presence of these pests and the substances that they produce may seriously endanger human health. In these commodities, which are also known as durable stored products, residual contact insecticides and fumigants are currently used as the main way to avoid insect infestation. Nevertheless, most of these pesticides are very toxic to mammals, and some of them leave dangerous residues on the product which may accumulate in the human body through the food chain. Moreover, most major insect species are now resistant to many of these pesticides, while some of these substances, such as methyl bromide, are extremely dangerous for both human health and the environment. Methyl bromide was thus banned by 2005 in industrialized countries according to the Montreal protocol, and it is expected to be completely withdrawn from the developing countries until 2015 (Bell 2000). Therefore, there is an urgent need to evaluate alternatives to traditional pesticides, which will have low mammalian toxicity, will be cost-effective and will be environmentally-compatible.

One of the most promising alternatives over the use of traditional pesticides in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplanktons, also known as diatoms, which occur in fresh and salt water since the Eocene period and produce a soft sedimentary rock, which is composed mainly by amorphous silica ($SiO_2 + H_2O$). The DEs currently mined vary remarkably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO_2 content, pH and tapped density (Korunic 1997). DEs are probably the most efficacious natural resource-based dry materials that can be used as insecticides (Korunic 1998). DEs act in the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss. They are non-toxic to mammals (rat oral LD_{50}>5000 mg/kg of body weight), leave no toxic residues on the product and according to the US EPA they are classified in the category of GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA 1995). Moreover, they are used as insulating materials against both heat and sound, as explosive additive, as well as in filters for beverages such as beer or fruit juice and abrasives in tooth paste (Korunic 1998). Finally, DEs are completely compatible with organic food production (Subramanyam and Roesli 2000). Regarding their insecticidal use, DEs can be applied with the same application technology with traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al. 2005). Moreover, since they are inert (silicaceous) materials, no interaction with the environment occurs. Thus, DEs persist in the treated substrate, providing a long-term protection against pests, which is currently a ''red flag'' for the use of conventional pesticides.

Several DEs, based on natural deposits, are now commercially available, and have proved very effective against stored grain pests (Subramanyam and Roesli 2000, Athanassiou et al. 2011). However, the investigation for newer, naturally-occurring DEs that are more effective in insect control is still in progress, especially in areas rich to silicaceous rocks. Korunic (1997, 1998) in an extensive screening of DEs from several parts of the world, found that local DEs from the Former Yugoslavia were very effective, and could be used with success against stored-grain pests. Similar results have been reported by Indic et al. (1998) for certain DEs from Serbia. An extensive screening by Athanassiou et al. (2011) also illustrated the presence of several DE deposits that have certain insecticidal properties from Croatia, Serbia and Slovenia. The DEs of this area 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 Yugoslavia 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 DE-based insecticides worldwide (Fields and Korunic 2000).

Based on the first evidence and preliminary samplings, it seems that Turkey is considered to have rich natural DE deposits, and there is clear evidence for the existence of large DE deposits at some areas in Turkey (Özbey and Atamer 1987, Mete 1988, Sıvacı and Dere, 2006, Çetin and Taş 2012). The European continent has the richest reserves in terms of diatomite reserve in the world and America has been following it. Diatomite reserve of Turkey is about 125 million tons. Hırka (Kayseri) known in Turkey has the largest diatomite reserve (106 million tons) (Çetin and Taş 2012). However, there is no information on the efficiency of local DEs from these areas in Turkey against stored grain insects. The objective of this study was to determine the physiochemical properties of the local DE deposits in Turkey and their efficacy against several stored grain insects.

Materials and methods

Test insects:

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), adults used in the bioassays were taken from a culture that was kept in the laboratory on whole wheat at 26 ± 1ºC, 65 ± 5 % relative humidity (RH) whereas the lesser grain borer*, Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), adults were taken from a culture kept on whole wheat 29 ± 1ºC, 65 ± 5 % RH. The confused flour beetle*, Tribolium confusum* du Val (Coleoptera: Tenebrionidae), adults were obtained from cultures reared in 1 l glass jars at 25 ± 1°C and 65± 5 % relative humidity (RH) on a diet of wheat flour mixed with dry brewer's yeast (17:1, wt:wt) using standard culture techniques. All individuals used in the tests were < 2 week old.

Wheat variety

Untreated, clean, with very little dockage (0.8%) and infestation-free wheat (*Triticum aestivum* L., variety of Elbistan Yazlığı) was used for experimentation. One gram of wheat variety tested corresponds to 21.3 seeds. The moisture content of the three products, as determined by a Dickey-John moisture meter (Dickey-John Multigrain CACII, DICKEY-John Co., Lawrence, KS), ranged between 11.0 and 11.4%.

Sampling, collection and preparation of local diatomaceous earths

In biological tests, diatomite earths with code of CCNA-1, CB2N-1 (Çankırı province), AGN-1, ACN-1, AG2N-1 (Ankara province), BGN-1, BHN-1 (Kayseri province), FB2N- 1 and FBN-1 (Aydın province) collected from different regions of Turkey and commercial DE, namely Silicosec® (Biofa Company-Germany) were used in the biological tests. In each DE reserve, 10 DE samples with 500 gr were randomly taken from different points of DE reserve. At least totally 5 kg DE sample was taken from each DE reserve. The DE samples were cut into small pieces with the aid of a hammer and placed in metal trays and then dried at a temperature of 100 ± 10ºC for 24 hours in a ventilated oven (MEMMERT UN75, Germany) to a moisture content of 3-5%. After drying, small pieces of DE samples were ground in a laboratory mill at full speed for 10 sec. All samples were then shifted through a standard sieve of 149 µm. After these DE process, final DE product called as natural DE deposit was used in the biological tests.

Physical and chemical determination of local diatomaceous earths:

The adherence percentage of DEs on wheat kernels was determined. The cleaned 500 g sample of wheat grain was then mixed by hand (by shaking) with 1000 ppm of DE (1 g/kg, or 0.5 g/500 g) in a tightly closed glass jar for 1 min. Treated grain was then sieved thoroughly using laboratory sieve No. 10 (2 mm openings) with a lid and bottom, for 1 min by hand to separate dust from the grain. The sieve, with closed lid and bottom, was left undisturbed after sieving for at least 1 min. The dust collected in the bottom of the sieve was then measured with a precision balance (mg). The weight was subtracted from 500 mg, and the value was expressed as a percentage of adherence of DE on the wheat kernel. Particle size distribution of the diatom earths were determined using the Laser Diffraction Method Silicon dioxide (SiO_2) ratio was measured by dissolution in acid and Atomic Absorption Spectroscopy (AAS) method. These analyzes were carried out in the accredited Analysis Laboratory of the General Directorate of Mineral Research and Exploration (MTA).

Bioassays procedures:

The tested insect species were the adults of three major stored grain pests, *S. oryzae*, *R. dominica* and *T. confusum*. The bioassay protocol was 3 X 3 replicates (each series of tests was repeated three times, by preparing new lots each time). In this series of tests, the DEs was applied in 1-kg lots of soft bread wheat (*Triticum aestivum* L., variety of Elbistan Yazlığı) with 11% moisture content (m.c) at the DE dose rates of 500 and 1000 ppm (one lot per dose). The grain was left previously for 7 d at the appropriate conditions to equilibrate with the desired relative humidity levels. Based on the standard procedure, the lots were placed in glass jars, and shaken manually for approx. 3 min., to achieve equal distribution of the DE dust to the entire grain mass. There was an additional series of lots with untreated grain which serve as a control. Then, 3 samples, of 50 g each, were taken from each lot, and these samples were placed in small glass vials, which were closed, apart from a 1.5 cm hole at the top, covered with fine mesh for ventilation. After this procedure, 30 mixed sex adults, <15 d old were placed in each lot. The lots were placed in incubators set at 25±1 °C temperature and 65±5% RH. The mortality was assessed after 7 and 14 d of exposure in the treated substrate.

Data processing and analysis:

For tested three species, the control mortality was very low, but where it was considered necessary the mortality counts were corrected by using the formula of Abbot (1925). The data were analyzed, separately for each species, by using the GLM Procedure of SAS (SAS Institute 1995), with insect mortality as the response variable and type of DE formulation and dose rate, as the main effects. Means were separated by using the Least Significant Difference (LSD) test at $P<0.05$.

Results and Discussion

Laboratory analysis results showed that there were significant differences in the SiO_2 ratio of the local diatomaceous earths collected from different regions of Turkey. The highest SiO_2 ratio was obtained from the local diatomaceous earths collected from Aydın (FB2N-1) and Kayseri (BHN-1) province. The particle size of all local diatomaceous earths except FBN-1 diatomaceous earths ranged from 12.31 to 20.05 µm, while the particle size of the commercial formulation of Silicosec® was 12.51 µm. Adherence rates of FB2N-1, ACN-1, BHN-1, CCN-1 and Silicosec® diatomaceous earths on wheat kernel were found to be 89% or <89% while adherence rates of AGCN-1 and FBN-1 diatomaceous earth ranged from 80% to 89%. In the adherence test no diatomaceous earths had an adherence rate below 75%.

In biological tests conducted at 500 ppm concentration for 7 days of exposure in wheat the highest mortality rates of *S. oryzae* and *R. dominica* adults were obtained from CB2N-1, AGN-1 and CCN-1 diatomaceous earths while the highest mortality rates of *T. confusum* were recorded in CB2N-1, AGN-1, ACN-1 and CCN-1 diatomaceous earths. After 14 days of exposure at 500 ppm concentration, the highest mortality rates (97 to 98%) of *S. oryzae* adults were recorded in CB2N-1, AGN-1 and BGN-1 diatomaceous earths, while the highest mortality rates of *T. onfusum* adults were obtained from only AGN-1 and BGN-1 diatomaceous earths. In the case of *R. dominica*, the highest mortality rate (64.4%) was recorded only in CB2N-1 diatomaceous earth. At concentration of 1000 ppm for 7 days of exposure in wheat, mortality rates of *S. oryzae* adults ranging from 92% to 100% were found in CB2N-1, AGN-1, CCN-1, ACN-1, AG2N-1 and BGN-1 diatomaceous earths while mortality rates of *T. confusum* adults ranging from 86% to 98% were detected in AGN-1, CCN-1 and AG2N-1 diatomaceous earths. After 14 days of exposure at 1000 ppm concentration, 100% mortality of *S. oryzae* adults was observed in all tested local diatomaceous earths except FB2N-1 and Silicosec while mortality rates of *T. confusum* adults ranging from % 95 to %100 were obtained in all tested local diatomaceous earths except FB2N-1, FBN-1 and Silicosec. In the case of *R. dominica* adults, mortality rates ranging from 80% to 93% were recorded in CB2N-1, CCN-1 and AG2N-1 diatomaceous earths. In this study, it was determined that *S. oryzae* adults were more susceptible to tested diatomaceous earths than *T. confusum* and *R. dominica* adults. Whereas the susceptibility of *T. confusum* and *R. dominica* to tested diatomaceous earths was found to be mostly similar.

Conclusion

Laboratory bioassays indicated that CB2N-1 and BGN-1 local diatomaceous earths had high efficacy against *S. oryzae*, *T. confusum* and *R. dominica* adults and thus could be potential to be successfully used for controlling stored grain insect pests as a grain protectant.

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Lethal Effect of Turkısh Dıatomaceous Earth (Bgn-1) agaınst Adults of German Cockroaches (*Blatella Germanıca* L.)

Kadir Özcan[1], Hasan Tunaz[1], Ali Arda Işikber [1], * Mehmet Kubilay Er[1]

[1]Kahramanmaraş Sütçü İmam University, Agriculture Faculty, Plant Protection Department, Avşar Campus, 46100 Kahramanmaraş TURKEY

*Correspondence: htunaz@ksu.edu.tr

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In this study, mortality effects of BGN-1 which is local diatomaceous earths, were investigated against adults of German cockroach (*Blatella gemanica* (L.)) on concrete, ceramic floor tile and laminate flooring. On these three different surfaces, *B. germanica* adults were exposed to BGN-1 diatomaceous earth at the doses of 2.5, 5, 10, 20 g/m^2 along 6 days. In all surface applications of BGN-1 diatomaceous earth, exposure time and dose caused significant effect on mortality rates of *B. germanica* adults. It was determined that BGN-1 coded Turkish diatomaceous earth has the lowest mortality effect on all application surfaces at the dose of 2.5 g/m^2. 2.5 g/m^2 BGN-1 caused 100 % mortality after 6 days concrete surface and caused 100 % mortality at the end of the fourth day on ceramic floor tile and laminate flooring. On the other hand, doses of 5 and 10 g/m^2 of BGN-1 caused 100% *B. germanica* mortality on all surfaces at the end of the second day, while the highest dose of 20 g /m^2 of BGN-1 reached 100% *B. germanica* mortality at the end of the first day on all application surfaces. In general, the mortality activity of BGN-1 diatomites against *B. germanica* adults was found to be similar on all three surfaces. At the end of this study, local diatomaceous earth coded BGN-1 was found to be good alternatives for controlling *B. germanica* which is a medical pest insect.

Keywords: Turkish diatomaceous earth, *Blatella germanica*, surface application.

Introduction

The German cockroach is commonly found living area with people and scattered all over the world. It is also a major carriers of pathogens and main source of allergens. Therefore it is an important primary medical and economical insect pest. The cockroach is mainly controlled by synthetic insecticides (Rust et al., 1993). However, this cockroach widely developed resistance to these