NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 39, 2022
First Online: December, 2021. DII 2067-5720 RAR 2022-113

INSECTICIDAL EFFECT OF PĂTÂRLAGELE DIATOMACEOUS EARTH AGAINST *Acanthoscelides obtectus* ADULTS

Constantina Chireceanu[1], Petru Cardei[2], Alina Geicu[1], Ioana Florescu[1], Floarea Burnichi[3], Maria Călin[4]

[1]Research and Development Institute for Plant Protection Bucharest, 8 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

[2]National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry Bucharest, 6 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

[3]Research and Development Station for Vegetables Buzău, 23 Mesteacănului str., Buzău, Buzău County, Romania

[4]Research and Development Station for Vegetables Bacău, 220 Calea Bârladului, Bacău, Bacău County, Romania

[*]Corresponding author. E-mail: cchireceanu@yahoo.com

ABSTRACT

In the present study there are presented the results obtained in laboratory tests to evaluate the efficacy of the Romanian diatomaceous earth from Pătârlagele deposit in Buzău County (DE PatRom) against adults of three population of the bean weevil *Acanthoscelides obtectus* L. (one maintained in laboratory conditions at the Plant Protection Institute Bucharest and the others two came from fields bean from Buzău and Bacău) compared with two commercial formulations (SilicoSec® and PyriSec®). The DE PatRom was applied at four doses 100, 300, 500 and 900 ppm while SilicoSec® and PyriSec® at the doses registered in common practice. Tests were carried out at 25±2°C and at 70± 5% relative humidity (RH). Adult mortality was assessed 3, 7 and 14 days after treatment. The PyriSec DE was the most effective reaching a total mortality of adults for all three insect populations tested even after 3 days, followed by SilicoSec DE which caused mortality between 74 and 99% after 3 days and 100% after 7 days. The mortality levels of *A. obtectus* adults reached by Romanian DE PatRom varied depending on dose, period after treatment and insect population. The mortality of bean weevil increased with increasing dose and period after treatment. The highest mortality (of 93, 96 and 98.5% to adults in Buzău, Bucharest and Bacău population, respectively) was recorded in bean treated with 900 ppm after 7 days. The results in experiments showed that the *A. obtectus* adults of Bacău population were more susceptible and those of Buzău population were more tolerant to all DEs tested.

Keywords: Pătârlagele diatomaceous earth, bean weevil, Romania.

INTRODUCTION

The bean weevil *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchidae) is the most dangerous pest that decimates the production of common bean (*Phaseolus vulgaris* L.) during the storage period (Săpunaru et al., 2006; Ayvaz et al., 2010; Karabörklü et al., 2010). It is widely distributed throughout the world causing significant damage to stored dried beans, leading to weight loss and nutritional value of beans, decreased germination and makes the seeds vulnerable to attack of other insects and fungal contamination. Therefore, the beans quality is depreciated and can no longer be used for consumption or for sowing new crops in the field.

The common bean is a field crop of great economic importance worldwide and an important source of protein (Myers and Kmiecik, 2017). At the 2020 level, the common bean was cultivated in Romania on 11100 ha with a total production of 11400 tons (INS, 2021). The control of *A. obtectus* in Romania is mainly based on treatments with chemical insecticides and fumigations (Porca et al., 2003; Săpunaru et al., 2006). In the context of increasing demand for healthy food and environmental protection, it is becoming increasingly clear that the use of chemical insecticides needs to be reconsidered by reducing or eliminating from pest control programs. Despite the rapid effect and high effectiveness, the chemical insecticides are often associated with many negative side

Received 1 October 2021; accepted 7 December 2021.

effects for both human security and the environment (ex. presence of residues in seeds, emergence of pest resistance to pesticides). In this context there is an increasing interest in finding new and successful methods to protect the harvested products from insect pests maintaining in the same time a good level of food safety. The diatomaceous earth (DE) is one of the products the most studied to control insects in storages. It is considered to be the most effective natural powder with insecticide properties (Shah and Khan, 2014). The open literature referring to the DE based treatments for bean weevil is very rich (Baier and Webster, 1992; Bohinic et al., 2013; de Oliveira et al., 2018; Jumbo et al., 2019; Wille et al., 2019). DE applied alone (Lorini and Beckel, 2006; Jumbo et al., 2019; Wille et al., 2019) or in combination with various plant products (extracts, essential oils) (Bohinc et al., 2013; Manole et al., 2016; Adarkwah et al., 2017) or microorganisms (*Beauveria*, *Trichoderma*, *Metarhizium*) (Dal Bello et al., 2006; Shafighi et al., 2014; Gad et al., 2020), has been shown to have a high insecticidal effect against storage pests including the bean weevil, offering an important and promising alternative to synthetic insecticides.

Diatomaceous earth (DE) or diatomite (D), also known as *diahid, kieselguhr, kieselgur,* is a natural fossil rock with a high content (60 to about 93%) of siliceous fragments of diatoms (Korunić, 2013) accumulated over a long period of time in geological deposits worldwide (Adarkwah et al., 2017). Diatoms are single-celled seaweed that secretes a siliceous skeleton called frustula (Juravle, 2015). The mechanism of action of DE against insects results from its direct contact with the body of insects which causes death by desiccation and sorption, damaging the wax layer that protects the cuticle of insects (Korunić, 2013).

In this work, we evaluated in laboratory conditions the insecticidal effect of the Romanian diatomaceous earth from the Pătârlagele deposit against adults of three populations of *A. obtectus* in three bean varieties compared to two formulated DE products, SilicoSec® and PyriSec®, which are registered as phytosanitary products in the European Union for protection of stored cereals and are available on the commercial market.

To complete the results of DE treatments, the progeny production of the bean weevil was evaluated after treatment.

MATERIAL AND METHODS

Study site

The study was carried out in the Entomology laboratory of the R&D Institute for Plant Protection (RDIPP) Bucharest in 2019.

Insect's origin

The storage insect tested in the study was the bean weevil *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchidae). Adults of three populations of *A. obtectus* were used in distinct experiments: one population from the stock culture of the RDIPP (B), and two populations obtained from infested common bean from fields that belong to Vegetable Research and Development Station Buzău (Bz) and Vegetable Research and Development Station Bacău (Bc), respectively, the 2018 bean production. The population at RDIPP is maintained on common bean in laboratory conditions at 25±2°C, 70±5% RH and total darkness.

Diatomaceous earth

Diatomaceous earths used for each insect population were: one Romanian DE (PatRom) obtained from the Pătârlagele deposit (Buzău County, eastern Romania) and two commercial formulations, SilicoSec® (Biofa AG, Münsingen, Germany) and PyriSec® (Agrinova GmbH, Obrigheim/ Mühleim, Germany) which are already registered for pest control in stored grains and are now commercially available.

The PatRom DE contains 28% SiO_2, 21% opal-Ct, 25% clay minerals and 26% minor feldspar (Sebe-Radoi, 2017) while SilicoSec® contains 96% SiO_2 (Erb-Brinkmann, 2000) and PyriSec® contains Silicosec with 1.2% natural pyrethrum, 3.1% piperonyl butoxide (Vayias et al., 2006).

The crude PatRom DE was mechanically processed before its use in the experiments to result an amorphous powder with particles of sub-micron size with properties specific to nanomaterials. This DE was selected for these tests due to its evident insecticidal effects against some of stored cereals pests in Romania, as shown in the publications of Chiriloaie et al. (2013), Lupu et al. (2016), Manole et al. (2015) and Athanassiou et al. (2016). Four doses (100, 300, 500 and 900 ppm) of PatRom DE were used in each experiment. PyriSec® and SilicoSec® were used at the recommended doses of 0.2 g and 0.05 g, respectively. A control lot without treatment was added for each experiment. Each treatment was organized in 4 replicates.

Each sample contained 100 g beans placed in 480 ml glass jar was treated with diatomaceous earths and homogenised manually for five minute for a uniform distribution of the product throughout the seed mass. Fifty adults about five days old were added into treated and control jars and then covered with a perforated mesh lid to prevent insects from escaping. The samples were maintained in a room with temperature of 25±2ºC and relative humidity of 70±5%, 8:16 h light:dark (Figure 1).

Figure 1. (A) *Acanthoscelides obtectus* adult; (B) glass jar with beans treated with diatomaceous earths and bean weevils; (C) view of DE products used in experiments: PyriSec® (1), SilicoSec® (2), PatRom (3)

The adult mortality was evaluated 3, 7 and 14 days after infestation by counting the dead insects that were removed at each assessment. After 14 days of exposure all insects were eliminated and the bean samples were maintained in the same conditions as in the experiment to monitor the emerging adults of *A. obtectus* from the seeds both in the treated and the control samples. The dead and live weevils were counted and eliminated from bean samples at each time intervals of assessment.

Some characteristics of bean seeds used in the experiments are presented in Table 1.

Table 1. Main characteristics of bean seeds used in the experiments

Origin place of *A. obtectus* population	Beans size and colour	Number of variants
Buzău	White, Large (228 seeds/100 g)	7 (+1 control)
Bacău	White Large (228 seeds/100 g)	7 (+1 control)
Bucharest	White, rounded, small (648 seeds/100 g)	7 (+1 control)

Descriptive statistical analysis of experimental data

Descriptive statistics represent the quantitative, numerical description of a statistical population. The description can be made graphically or by indicators. The main statistical indicators used were mean median, variant, mean deviation. A synthetic descriptive statistical indicator is the box-plot graphical representation.

Calculating the effectiveness of treatments

For a variant of rigorous calculation of the treatments effectiveness, Abbot's formula can be used (Fleming and Retnakaran 1985; Abbot, 1987):

$$e = 100 \cdot \frac{\mu - \mu_w}{100 - \mu_w} \qquad (1)$$

Where: μ is the percent of dead insects in treated sample, and μ_w is the percent of dead insects in control sample.

As the mortality process described in these experiments is dynamic, a time-dependent efficacy can be defined, because the action of DEs treatment on the pest populations is not instantaneous, it takes place in time. Therefore, formula (1) is extended to dynamic efficacy (2):

$$e(t) = 100 \cdot \frac{\mu(t) - \mu_w(t)}{100 - \mu_w(t)}, t > 0 \qquad (2)$$

Correlation analysis of adult mortality with DE product dose

In order to observe numerically but also to estimate a dependence of the efficacy on product dose, the correlation between the efficacy values [adult mortality and efficacy defined as in (1)] and the product dose was calculated.

RESULTS AND DISCUSSION

The experimental results are presented in table and figures. The results on the mean percent mortality (%) of the *A. obtectus* adults are given in Figure 2. These were obtained by analyzing the four repetitions for each experience, the main target being evaluation of adult mortality of three populations of *A. obtectus* treated with three diatomaceous heart based products, one of Romanian origin, PatRom, and two commercially available formulations, PyriSec® and SilicoSec®. Adult mortality levels of the three populations were different depending on DE type, dosage and exposure period. The most effective DE product was PyriSec® that caused 100% mortality of the *A. obtectus* adults even 3 days after treatment to all three tested populations. A high mortality was also evaluated in the bean treated with SilicoSec®, of 74, 81.5 and 99% for Bz, B and Bc populations, respectively, 3 days after treatment, and 100% for all populations, one week after treatment. Both products were used at the dose recommended in common practice.

Regarding the PatRom diatomaceous earth, the results in our experiments show that the mortality of bean weevils increased with increasing dose and period after treatment. These results confirm data from previous studies reported for several coleopterans pest to stored grain cereals in Romania, the rice weevil, *Sitophilus oryzae* (Chiriloaie et al., 2013), the granary weevil, *Sitophilus granaries* L. (Curculionidae) (Manole et al., 2015; Lupu et al., 2016), the red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae) and the saw toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Silvanidae) (Athanassiou et al., 2016).

There was also variation in the toxicity of PatRom DE to the three populations of *A. obtectus*. Among the three populations tested, the Bc population was most affected by PatRom treatments irrespective of dose applied, while the Bz population was less affected. The highest mortality reached by PatRom was recorded in bean treated with 900 ppm in all weevil bean populations tested after 7 days (93, 96 and 98.5% mortality to adults belonging to Bz, B and Bc population, respectively). The doses of 100, 300 and 500 ppm were less effective than the highest dose of 900 ppm, the adult mortality being lower (between 16.5 and 57%) compared with the 900 ppm treatments.

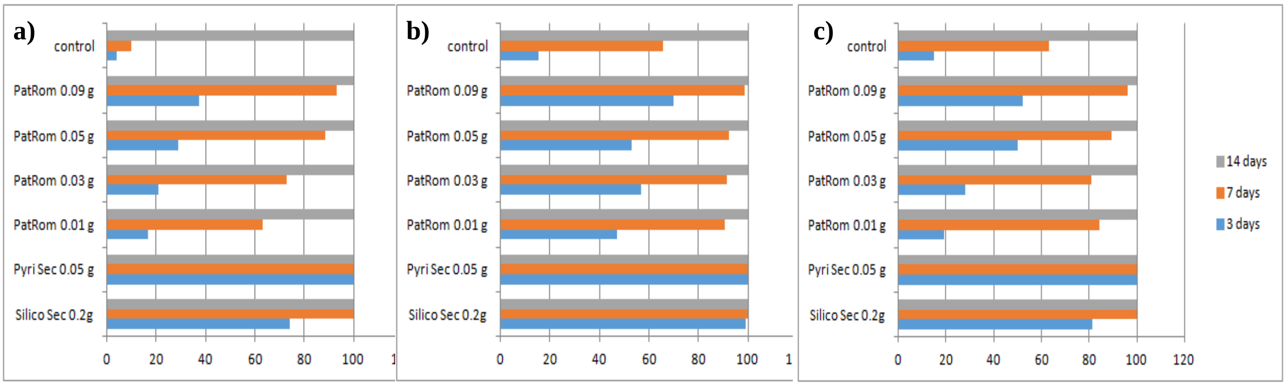

Figure 2. Mean percent mortality (%) of the *A. obtectus* adults belonging to three populations, Buzău (a), Bacău (b) and Bucharest (c), after 3, 7 and 14 days exposure to common bean treated with three diatomites, the Romanian PatRom to four doses and the commercial formulations PyriSec, SilicoSec

Graphs from figure 2 can give a first hierarchy of the effectiveness of the DEs treatments. The first criterion proposed is the speed of treatment to kill the insects. Using this criterion, the ranking for the beans type depending on insect population is shown in Table 2.

Table 2. Hierarchy of the DEs treatment according to effectiveness criterion of the shortest time to kill the bean weevil adults (3 days after treatments)

Ranking	Buzău *A. obtectus* and bean	Bacău *A. obtectus* and bean	Bucharest *A. obtectus*, commercial bean
1	Pyri Sec	Pyri Sec	Pyri Sec
2	Silico Sec	Silico Sec	Silico Sec
3	PatRom 0.09 g	PatRom 0.09 g	PatRom 0.09 g
4	PatRom 0.05 g	PatRom 0.05 g	PatRom 0.05 g
5	PatRom 0.03 g	PatRom 0.03 g	PatRom 0.03 g
6	PatRom 0.01 g	PatRom 0.01 g	PatRom 0.01 g
7	Untreated sample	Untreated sample	Untreated control

The analysis of the repetition series was also done using the boxplot graphs from figure 3. The analysis of the boxplot diagrams for each partial experiment shows that the series of repetitions of the mortality values of insect do not show aberrant values to be eliminated from the calculation. It is also observed that, in most cases (over 70%), the read values are concentrated around the median and the arithmetic mean of the readings is a suitable value for calculations and interpretations. In the descriptive statistical analysis is obtained a graphical representation of the dependence of the average mortality of the bean weevil population on the concentration of diatomite and time, for each of the three populations according to the place of origin.

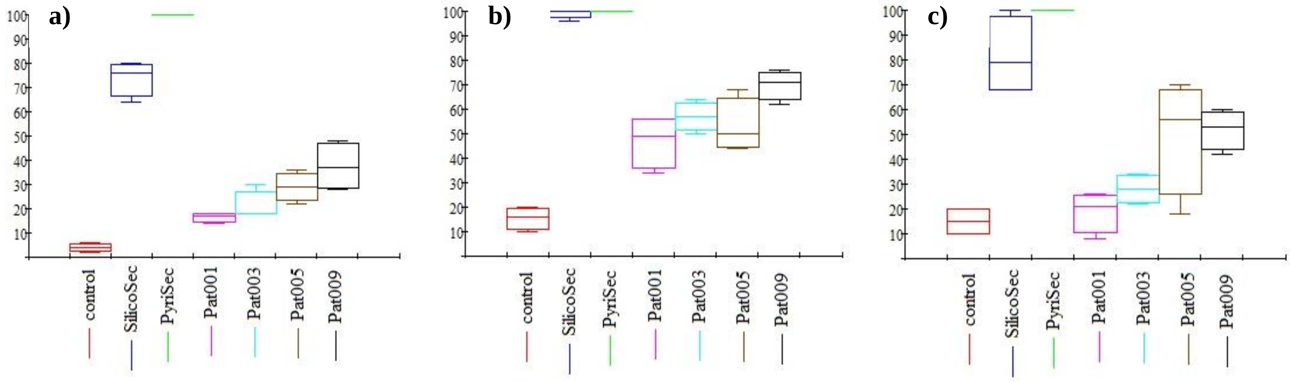

Figure 3. Boxplot diagrams of adult mortality of *A. obtectus* from Buzău (a), Bacău (b) and Bucharest (c) populations 3 days after DEs application

Although the boxplot diagrams are especially useful for characterizing and sorting experimental data or series of repetitions, for example, however in some cases they can also give a conclusion on the effectiveness of each treatment at the time to which these diagrams refer. Thus, a hierarchy of treatment (Table 3) can be obtained from the point of view set out above, similar to that given in Table 2. The meanings of the parameters in formula (2), with the same symbols as in (1) are similar, with difference that here the parameters can change in time, t being the temporal parameter. Since the experiments were performed during a period of 14 days, more or less equivalent to the adult longevity of *A. obtectus* (Ahmed et al., 2019), it is obvious that the final efficacy may be $e(kT)$, where T is the adults longevity duration, and k is a positive subunit number, the fraction of the duration of adult longevity. The constant k can be chosen by the operator or can be standardized. In this case, the function (2) is defined on the time interval $(0, T)$.

Table 3. Values of dynamic efficacies 3 and 7 days after DEs treatment for three *A. obtectus* populations

DEs Treatment	Buzău		Bacău		Bucharest	
	3 days	7 days	3 days	7 days	3 days	7 days
SilicoSec 0.2 g	72.917	100.000	98.817	100.000	78.235	100.000
PyriSec 0.05 g	100.000	100.000	100.000	100.000	100.000	100.000
PatRom 0.01 g	13.021	58.889	37.278	72.303	4.706	57.880
PatRom 0.03 g	17.708	70.000	49.112	75.219	15.294	48.370
PatRom 0.05 g	26.042	87.222	44.379	78.134	41.176	71.467
PatRom 0.09 g	34.896	92.222	64.497	95.627	43.529	89.130

According to formula (2), because for all variants, at $t = T = 14$, the mortality was 100%, the efficacy becomes infinite. Thus, it is observed that the efficacy is recommended to be appreciated at intermediate times, preferably up to $T/2$, because it can overlap with natural mortality. The values obtained for the dynamic efficiency (2) at the observation times 3 and 7 days are given in Table 4. It is observed from Table 4, the significant high values of the correlations, which means that the dependence between the two measures of efficacy and dose of the diatomaceous earths is very strong, almost linear.

Table 4. Correlations between adult mortality and efficacy and diatomite dose for data obtained on the third and seventh day after treatments

Correlation type	*A. obtectus* population					
	Buzău		Bacău		Bucharest	
	3 days	7 days	3 days	7 days	3 days	7 days
Adult mortality - diatomite dose	0.992	0.933	0.916	0.964	0.900	0.891
Product efficacy - diatomite dose	0.992	0.934	0.917	0.64	0.900	0.892

Analysis of progeny production

The production of progeny resulting in the bean samples treated with DEs is also a criterion for evaluating their effectiveness as it is confirmed in literature (Mikami et al., 2010; Adarkwah et al., 2017; Jumbo et al., 2019; Kabir and Bukar, 2019; Gad et al., 2020). According to these studies, the insecticidal activity of DE formulations reduced and supressed the insect pest progeny after treatments.

Data on the progeny production of bean weevil days after treatment (DAT) and its dynamics are displayed on the graphs in Figure 4.

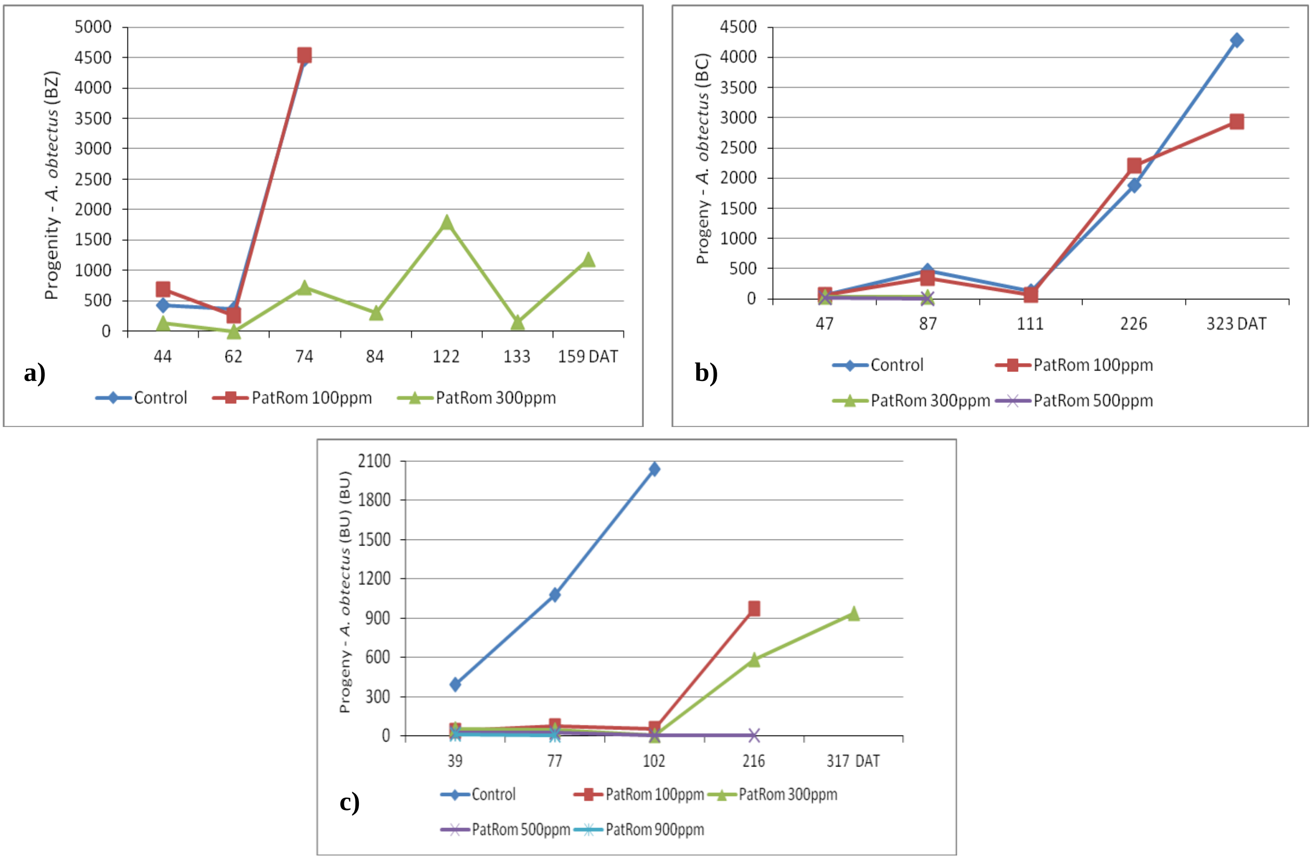

Figure 4. Progeny production of *A. obtectus* belonging to three populations, Buzău (a), Bacău (b) and Bucharest (c), evaluated different days after treatment

The number of adult progeny was recorded at different DATs for tested populations. Maximum DAT was 159, 316 and 323 days for Bz, B and Bc population, respectively. The progeny production of *A. obtectus* was recorded in the control bean and treated with PatRom DE. No adults emerged from SilicoSec® and PyriSec® bean. Most of the progeny was found in the bean in control and treated with 100 and 300 ppm

PatRom in all populations. In the beans were PatRom was applied at their highest doses (500 and 900 ppm) the adult emergence of *A. obtectus* was reduced. The situation was different among the weevil populations. The highest number of emerged adults was found in samples with the Bz population, that were ended earlier because of the rapid and excessive multiplication of the weevils that completely consumed the beans.

CONCLUSIONS

The results in our study showed that the Romanian diatomaceous earth PatRom tested in laboratory conditions was effective against adults of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchidae) at 900 pp after seven days (between 93 and 98.5% adult mortality) and protected the beans from the emergence of the pest progeny. However, further studies are required in order to propose that this DE be included in the management programs of stored product pests on a large scale as an alternative way for the chemical control of these pests.

ACKNOWLEDGEMENTS

The study was performed in pre-implementation stage of the project CCCDI-UEFISCDI, number PN-III-P1-1.2-PCCDI-2017-0659 PCCDI, within PNCDI III, financed by the Romanian Ministry of Research and Innovation.

REFERENCES

Abbot, W.S., 1987. *A method of computing the effectiveness of an insecticide*. Journal of the American Mosquito Control Association, 3(2): 302-303.

Adarkwah, C., Obeng-Ofori, D., Hormann, V., Ulrichs, C., Scholler, M., 2017. *Bioefficacy of enhanced diatomaceous earth and botanical powders on the mortality and progeny production of Acanthoscelides obtectus (Coleoptera: Chrysomelidae), Sitophilus granarius (Coleoptera: Dryophthoridae) and Tribolium castaneum (Coleoptera: Tenebrionidae) in stored grain cereals*. International Journal of Tropical Insect Science, 1: 16.

Ahmed, S.S., Naroz, M.H., Abdel-Aziz, S.Y., Awad, M.A.R., Abdel-Shafy, S., 2019. *Morphological, molecular and biological studies on common bean weevil Acanthoscelides obtectus (Say) in Egypt.* Journal of Entomology, 16(1): 30-38.

Athanassiou, C.G., Kavallieratos, N.G., Chiriloaie, A., Vassilakos, T.N., Fatu, 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, 69(1): 25-34.

Ayvaz, A., Sagdic, O., Karaborklu, S., Ozturk, I., 2010. *Insecticidal activity of the essential oils from different plants against three stored-product insects*. Journal of Insect Science, 10:1. 10.1673/031.010.2101.

Baier, A.H., and Webster, B.D., 1992. *Control of Acanthoscelides obtectus Say (Coleoptera: Bruchidae) in Phaseolus vulgaris L. seed stored on small farms - I. Evaluation of damage*. Journal of Stored Products Research, 28(4): 289-293.

Bohinic, T., Vayias, B., Bartol, T., Trdan, S., 2013. *Assessment of insecticidal efficacy of diatomaceous earth and powders of common lavender and field horsetail against bean weevil adults*. Neotrop Entomology, 42(6): 642-648.

Chiriloaie, A., Ciobanu, M., Drosu, S., 2013. *Preliminary studies regarding insecticidal effect of diatomaceous earth against the rice weevil, Sitophilus oryzae L. (Coleoptera: Curculionidae) on stored wheat.* Romanian Journal for Plant Protection, 6: 27-30.

Dal Bello, G., Padín, S., Juárez, P., Pedrini, N., De Giusto, M., 2006. *Biocontrol of Acanthoscelides obtectus and Sitophilus oryzae with diatomaceous earth and Beauveria bassiana on stored grains*. Biocontrol Science and Technology, 16(2): 215-220.

de Oliveira, M.R., da Silva Bonome, L.T., von Hertwig Bittencourt, H., Zarowni, E., da Silva Lefchak, L., 2018. *Alternative treatments in bean seeds for repelling Acanthoscelides obtectus (Say)*. Journal of Seed Science, 40(4): 362-369.

Erb-Brinkmann, M., 2000. *Application of silica dust (SilicoSec®) in Germany - Practical Experiences*. In: Adler, C., and Scholler, M. (eds.), Integrated Protection of Stored Products. IOBC Bulletin, 23: 239-242.

Fleming, R.A., and Retnakaran, A., 1985. *Treatment Data Using Abbott's Formula*. Journal of Economic Entomology, 78: 1179-1181.

Gad, H.A., Al-Anany, M.S., Sameer, W.M., Al-Anany, F.S., 2020. *Control of Acanthoscelides obtectus with Trichoderma harzianum applied alone or in combination with diatomaceous earth on a stored common bean*. Plant Protection Science. 56(2): 107-115.

INS, 2021. *Producţia vegetală la principalele culturi*. Anul 2020.

Jumbo, L.O.V., Pimentel, M., Oliveira, E., Toledo, P.F.S., Faroni, L.R.A., 2019. *Potential of diatomaceous earth as a management tool against Acanthoscelides obtectus infestations.* Revista de Ciencias Agricolas, 36: 37-46.

Juravle, D.T., 2015. *Geologie generală*. Editura STEF, Iași.

Kabir, B.G.J., and Bukar, A., 2019. *Efficacy of different diatomaceous earth formulations in three wheat varieties against the lesser grain borer Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae).* Acta Entomologica Serbica, 24(1): 7-17.

Karabörklü, S., Ayvaz, A., Yilmaz, S., 2010. *Bioactivities of different essential oils against the adults of two stored product insects*. Pakistan Journal of Zoology, 42: 6.

Korunić, Z., 2013. *Diatomaceous earths - Natural Insecticides*. Pesticides and Phytomedicine (Belgrade), 28(2): 77-95.

Lorini, I., and Beckel, H., 2006. *Efficacy of "diatomaceous earth" to control the main stored grain pests*. Proceedings of the 9th International Working Conference on Stored Product Protection, 15-18 October 2006, Campinas, São Paulo, Brazil. Brazilian Post-harvest Association - ABRAPOS, Passo Fundo, RS, Brazil, 2006.

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, 8: 58-62.

Manole, T., Lupu, C., Fătu, V., Chiriloaie, A., Chițoran, F., 2015. *Experimental model for biological control of stored grain pests*. Journal of Agricultural Science and Technology, A, 5: 793-798.

Manole, T., Lupu, C., Fătu, V., Popescu, M., 2016. *New formulation of ecological products used for the biological control of cereals weevil, Sitophilus granarius L. (Coleoptera: Curculionidae) in laboratory conditions*. Romanian Journal of Plant Protection, 9: 27-34.

Mikami, A.Y., Pissinati, A., Fagotti, D., de Oliveira, Menenzes Junior, A., Ventura, M.U., 2010. *Control of the Meexican bean weevil Zabrotes subfascitus with kaolin*. Ciência Rural, Santa Maria, 40(7): 1497-1501.

Myers, J.R., and Kmiecik, K., 2017. *Common bean: Economic importance and relevance to biological science research*. The common bean genome. Springer, Cham: 1-20.

Porca, M., Oltean, I., Dobrin, I., 2003, *Chemical control of bean weevil, Acanthoscelides obtectus Say in storage condition*. Journal of Central European Agriculture, 4(3): 209-216.

Săpunaru, T., Filipescu, C., Georgescu, T., Bild, Y.C., 2006. *Bioecology and control of bean weevil (Acanthoscelides obtectus Say)*. Cercetări Agronomice în Moldova, 36: 5-12.

Sebe-Radoi, O.G., Dumitras, D.G., Marincea, S., Calin, N., Birgaoanu, D., Barbu, O.C., 2017. *Preliminary paleontological and mineralogical study of the diatomites from Patarlagele, Romania*. Goldschmidt Abstract, 2276.

Shafighi, Y., Ziaee, M., Ghosta, Y., 2014. *Diatomaceous earth used against insect pests, applied alone or in combination with Metarhizium anisopliae and Beauveria bassiana*. Journal of Plant Protection Research, 54(1): 62-66.

Shah, M.A., and Khan, A.A., 2014. *Use of diatomaceous earth for the management of stored-product pests.* International Journal of Pest Management, 60(2): 100-113.

Vayias, B.J., Athanassiou, C.G., Kavallieratos, N.G., Tsesmeli, C.D., Buchelos, C.T., 2006. *Persistence and efficacy of two diatomaceous earth formulations and a mixture of diatomaceous earth with natural pyrethrum against Tribolium confusum Jacquelin du Val (Coleoptera: Tenebrionidae) on wheat and maize*. Pest Management Science, 62: 456-464.

Wille, C.L., Wille, P.E., da Rosa, J.M., Boff, M.I.C., Franco, C.R., 2019. *Efficacy of recovered diatomaceous earth from brewery to control Sitophilus zeamais and Acanthoscelides obtectus*. Journal of Stored Products Research, 83: 254-260.