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The three-year development of new natural insecticide formulations based on inert dusts and botanicals in Croatia

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Abstract: The authors describe a three-year research project financed by the Croatian Science Foundation, DIACROMIXPEST IP -11-2013-5570 on the development of natural insecticide formulation in Croatia in order to apply in the stored products protection. The significance of these formulations is the technology of mixing and various combinations of inert dusts based on Croatian diatomite with several Croatian plant substances. The synergy of physical and chemical mode of action of these formulations obtained better performance on insects compared with the same substances applied alone, but also slowed down the possible emergence of resistance. Given that all of the substances in the formulations of natural origin, there should be no concern about harmful residues of the insecticide in processed goods, on surfaces and in space.

Key words: natural insecticides, inert dusts, diatomite, plant substances, formulations

Introduction

The research project of the Croatian Science Foundation under the title "Development of new natural insecticide formulations based on inert dusts and botanicals to replace synthetic, conventional insecticides" – DIACROMIXPEST IP –11-2013-5570 is carried out for a period of three years. It started on July 1, 2014 and lasted until June 30, 2017. During the three years research, natural insecticide formulations have been developed using substances of Croatian origin that are in the group of "food grade" and/or "organic" substances. The main feature of these formulations is in the new mixing technology and the combinations of substances of a different mode of action on insects, such as physical (inert dusts) and chemical (plant extracts). Combined physical and chemical (toxic) effects have increased efficacy on insects when the same substances are applied alone, and such a complex mode of action significantly slows down the possible occurrence of resistance. The basic application of these new formulations should be in the protection of stored agricultural products, and with minor modifications may be used in other areas of pest control e.g. public health and communal hygiene, plants protection in vegetation, veterinary medicine and food industry. There should not be any concern about the harmful insecticide residues in processed commodities, on surfaces and in the area. In this project, the research team consists of experts from Croatian Geological Survey (Josip Halamić and Ines Galović), Diatom Research and Consulting Inc.

from Canada (Zlatko Korunić) and the Faculty of Agriculture in Osijek (Vlatka Rozman – project leader, Anita Liška, Renata Baličević and Pavo Lucić).

Briefly about project work

Geological field studies of all potential diatomite deposits in Croatia were carried out. Physical, chemical and paleontological analyses of collected diatomite samples were performed. All the diatomite samples were tested and selected in terms of efficacy on test insects (*Sitophilus oryzae* L., *Rhyzopertha dominica* Fab., *Tribolium castaneum* Herbst.) and compared to the standard DE. Laboratory tested Croatian aromatic and food plants with a potential insecticidal activity: laurel (*Laurus nobilis* L.), lavender (*Lavandula x intermedia*), lemon balm (*Melissa officinalis* L.), mint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), marjoram (*Origanum marjorana* L.), oregano (*Origanum vulgare* L.), woodland sage (*Salvia nemorosa* L.), sage (*Salvia officinalis* L.), clary sage (*Salvia sclarea* L.), savory (*Satureja hortensis* L.), wild thyme (*Thymus serpyllum* L.), garden thyme (*Thymus vulgaris* L.), sunflower (*Helianthus annuus* L.), rapeseed (*Brassica napus* L.). Selected plants laboratory tests were performed in combination with selected effective diatomite samples. It was made a full range of research based on the development of potential formulations, as well as insecticidal effectiveness tests of formulations selected on the basis of the long-term protection on treated wheat. At present, the stability of new Croatian formulations is being investigated under regular storage conditions.

The project activity

From the beginning until the end of the three-year project, the following activities were carried out:

- Every year, the training activities on Faculty of Agriculture in Osijek were held by Dr. Zlatko Korunić from Diatom Research and Consulting Inc., to present laboratory methods of obtaining plant extracts with water and alcohol, mixing plant powder with DE, mixing DE with plant extracts, obtaining formulations and methods for laboratory and insect breeding. In addition, he held the invited lectures for students with themes of inert dusts as insecticides, DE mode of action and review of recent research of DE.
- Field prospecting of 26 potential diatomite sources in Croatia (Banovina – 11 localities; Slavonia (Psunj – Požeška gora) – 12 localities; Medvednica –- Žumberak – Hrvatsko Zagorje – 3 localities) was made.
- Geographic position was determined and geological columns of 14 sampled diatomite sites were created: (Slavonija: Banićevac, Opatovac; Banovina: Bačuga, Martinovići, Prnjavor Čuntićki, Vukičevići, Šušnjar, Mali Deanovići; Medvednica-Žumberak-Hrvatsko Zagorje: Markuševec, Podsusedsko Dolje, Jurjevčani, Jurjevčani-1, Crkovec).
- 56 different diatomite samples were collected for further laboratory testing.
- All 56 diatomite samples were preliminarily selected for efficacy on insect test and comparison with the standard DE (Celatom® – Mn51) in Diatom Research and Consulting Inc.
- 9 potentially effective diatomite samples were selected for further experiments.
- Physical and chemical analysis of 9 potential diatomite samples was made: moisture, pH value, calcimetry, main elements, trace elements, x-rays analysis.
- 91 diatomaceous species with 8 varieties and 1 form in selected diatomite samples under LM (light microscope) and SEM (electron microscopy) were determined.

- Plant material with potential insecticidal activity was collected and selected: laurel (*Laurus nobilis* L.), lavender (*Lavandula x intermedia*), lemon balm (*Melissa officinalis* L.), mint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), marjoram (*Origanum marjorana* L.), oregano (*Origanum vulgare* L.), woodland sage (*Salvia nemorosa* L.), sage (*Salvia officinalis* L.), clary sage (*Salvia sclarea* L.), savory (*Satureja hortensis* L.), wild thyme (*Thymus serpyllum* L.), garden thyme (*Thymus vulgaris* L.), sunflower (*Helianthus annuus* L.), rapeseed (*Brassica napus* L.).

- Selected methods and mode of mixing plant material were tested in powder form, alcohol and water extracts, essential oils, plant extracts formulation with DE and application on different surfaces: stack, ceramics, wood.

- Combinations of 5 selected formulations of inert dusts with diatoms and plant powders and plant extracts were preliminarily tested.

- Additional field geological sampling of thicker layers of diatomite deposits in Croatia was done. At the Podusedsko Dolje (PD) site, horizons from PD-1 to PD-5 for composite sample production (PDK) were sampled.

- The geographic position and geological characteristics of the Podusedsko Dolje site were determined.

- Complete physical-chemical analyses of PDK composite sample were done: moisture, pH value, calcimetry, main elements, trace elements, x-rays analysis.

- Paleontological analyses of PDK composites were performed with the determination of 90 diatomaceous species under the electron (SEM) and light microscope (LM), and the morphometry and taxonomy of diatomaceous species were performed, as well as the proportion of diatoms per sample.

- 3 powder formulations and 2 liquid formulations were developed. They were obtained by mixing plant extracts, essential oils and inert dusts.

- Formulation lethal doses (LD50 and LD90) and effective dose (ED50) for progeny as well as their influence on test weight were determined.

- The efficacy of components and formulations in different doses applied to different surfaces was analyzed: stack, ceramics and wood.

- Two formulations of F1H and F2H prepared for field tests in normal storage conditions were also developed.

- Currently, there are still ongoing long term efficacy field tests (6 months) with selected formulations F1H; F2H and compared with DE Celatom® MN 51 applied on mercantile wheat and corn infested with test insects.

Project dissemination

Dissemination of results arising from the three-year project can be seen through the following publications:

Rozman, V., Korunić, Z., Halamić, J., Liška, A., Baličević, R., Galović, I. & Lucić, P. 2015: Development of new natural insecticide formulations based on inert dusts and botanicals to replace synthetic, conventional insecticides – presentation of the research project of Croatian Science Foundation (in Croatian). In: Proceedings of the 27th Scientific and Educational Seminar DDD and ZUPP 2015 Disinfection, Disinfestation and Deratization and Protection of Stored Agricultural Products (ed. Korunić, J.): 197-201. 24-27 March 2015, Mošćenička Draga, Croatia, Korunić d.o.o., Zagreb. ISSN 1849-6741.

Lucić, P., Liška, A., Rozman, V., Baličević, R. & Đumlić, M. 2015: The potential use of lavender (*Lavandula x intermedia*) in protection of stored wheat against storage insects. (in Croatian). In: Proceedings & Abstracts 8th international scientific/professional conference Agriculture in Nature and Environment Protection (ed. Mirjana Baban and Sanda Rašić): 160-165. 1-3 June 2015, Vukovar, Croatia, Glas Slavonije d.d., Osijek. ISSN: 1848-5456.

Rozman, V. 2015: Control of stored products pests by natural products. In: 10th Conference on Integrated Protection of Stored Products IPSP 2015 IOBC-WPRS Book of Abstracts (eds. Darka Hamel and Pasquale Trematerra): 50. 28 June-1. July 2015, Zagreb, Croatia, Croatian Center for Agriculture, Food and Rural Affairs, Zagreb. ISBN: 978-953-7867-18-8.

Rozman, V. 2015: Control of stored products pests by natural products. IOBC-WPRS Bulletin 111: 295-299.

Liška, A., Rozman, V., Korunić, Z., Halamić, J., Galović, I., Lucić, P. & Baličević, R. 2015: The potential of Croatian diatomaceous earths as grain protectant against three stored-product insects. In: 10th Conference on Integrated Protection of Stored Products IPSP 2015 IOBC-WPRS Book of Abstracts (eds. Darka Hamel and Pasquale Trematerra): 35. 28 June-1. July 2015, Zagreb, Croatia, Croatian Center for Agriculture, Food and Rural Affairs, Zagreb. ISBN: 978-953-7867-18-8.

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Rozman, V., Korunić, Z., Halamić, J., Liška, A., Baličević, R., Galović, I. & Lucić, P. 2016: Second year of research project of Croatian Science Foundation on development of new formulations of natural insecticides – DIACROMIXPEST (in Croatian). In: Proceedings of the 28th Scientific and Educational Seminar DDD and ZUPP 2016 Disinfection, Disinfestation and Deratization and Protection of Stored Agricultural Products (ed. Korunić, J.): 265-269. 5-8 April 2016, Mošćenička Draga, Croatia, Korunić d.o.o., Zagreb. ISSN 1849-6741.

Liška, A., Korunić, Z., Rozman, V., Lucić, P., Baličević, R., Halamić, J. & Galović, I. 2016: Laboratory assessment of the plant formulation insecticidal efficacy on Rice weevil (*Sitophilus oryzae* L.) and Lesser grain borer (*Rhyzopertha dominica* Fab.) by spraying on different structural surfaces (in Croatian). In: Proceedings of the 28th Scientific and Educational Seminar DDD and ZUPP 2016 Disinfection, Disinfestation and Deratization and Protection of Stored Agricultural Products (ed. Korunić, J.): 271-280. 5-8 April 2016, Mošćenička Draga, Croatia, Korunić d.o.o., Zagreb. ISSN 1849-6741.

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Since 1 June 2015 all the information on the DIACROMIXPEST project has been available (in Croatian and English) on the following link www.diacromixpest.eu including presentations of the project work in public together with the presentations sets, complete published scientific papers and summaries of the meetings, photo gallery from the fields, and links to the institutions involved in the project.

The current status of the research

Generally, we are satisfied with what we have done so far. However, we hoped that Croatia has a thicker deposits of diatomaceous earth that could be the basis for their exploitation and commercialization. However, significant results have been obtained in each case from the geological and paleontological aspect. We explored all potential diatomite locations in Croatia, sampled through 56 samples, out of which 9 potential samples were completely physically and chemically analyzed. The 91 diatomaceous species with 8 varieties and 1 form under electron (SEM) and light microscope (LM) were determined, and morphometry and taxonomy of all were performed, as well as the proportion of diatomaceous particles per sample.

Therefore, in terms of our research of natural formulations, our field samples cannot be characterized as diatomaceous earth, but as inert dust with a specific proportion of diatomaceous earth having the potential for the development of natural formulations in combination with plant substances. In general, this research proves the possibility of making and producing Croatian formulations in the near future on the basis of inert dusts and plant substances.

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