

POSSIBLE NEW INSECTICIDES IN THE PROTECTION OF STORED WHEAT

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Abstract

ALMASI, R., D. POSLONCEC and Z. KORUNIC, 2013. Possible new insecticides in the protection of stored wheat. *Bulg. J. Agric. Sci.*, 19: 1076-1084

With the aim of the efficient control of pests and significant decrease in insecticide residues in stored goods, some new formulations of powder insecticides have been developed by combining natural and synthetic substances. The developed formulations are as follows: the mixture of diatomaceous earth (DE) and deltamethrin (DM) (DE/DM) containing 900g/kg of DE and 1g/kg of DM active substance (a.s.), DE and pyrethrin (Py) (DE/Py) containing 900g/kg of DE and Py 10g/kg a.s. and DE and spinosad (Spin) (DE/Spin) containing 900g/kg of DE and 10g/kg a.s. of spinosad. The goal of this research work was the evaluation of the efficiency of formulations DE/DM, DE/Py and DE/Spin in the control of *Sitophilus oryzae* L. and *Rhizopertha dominica* F. on wheat. In the experiments, the wheat variety Pobeda grown in Vojvodina (Serbia) in 2011, containing 12.12% moisture content was used. The applied doses (ppm or grams per 1000 kg of grain) were: DE/DM and DE/Spin - 50, 100 and 150, DE/Py - 100, 200 and 300. The experiments were carried out at the temperature from 25 to 28°C and air humidity (r.h.) of 65 to 80%. The results of the efficiency were evaluated after 7, 14 and 21 days of the exposure of insects to the treated wheat and the effects on the offspring after 66 days (*S. oryzae*) and after 70 days (*R. dominica*). The doses of DE/DM (150 ppm) and DE/Py (200 ppm) caused 100% mortality of *S. oryzae* during 14 days of the exposure to treated wheat and completely suppressed its offspring. However, the highest researched dose of DE/Spin of 150 ppm during 21 days controlled *S. oryzae* by 98.5% and reduced its offspring by 92%. The lowest doses of all the three formulations (50 ppm of DE/DM and DE/Spin and 100 ppm of DE/Py) controlled the imagoes and offspring of *R. dominica* by 100%. In the conditions under which the experiment was carried out the recommended doses for *S. oryzae* control on wheat can be 150 ppm of DE/DM, 200 ppm of DE/Py and probably 200 ppm of DE/Spin, and for the control of *R. dominica* the lowest doses of all the three formulations.

Key words: stored wheat, *Sitophilus oryzae*, *Rhizopertha dominica*, diatomaceous earth, pyrethrin, deltamethrin, spinosad, insecticide mixture

Abbreviations: DE - Diatomaceous earth; a.s. - Active substance; Py - Pyrethrin; DM - Deltamethrin; Spin - Spinosad; PBO - Piperonyl butoxide

Introduction

The insects, which occur in stored agricultural goods, cause significant economic losses in grain and food industry. In the past several decades, the application of synthetic, conventional insecticides in the protection of stored agricultural products has become a regular practice. However, with the latest knowledge and evidence of the harmful effect of synthetic insecticides on people's health and the environment, the occurrence of insect populations resistant to insecticides and a great concern of grains and grain products consum-

ers about the pesticide residues in the processed goods, numerous regulations and recommendations have been passed aimed at the decrease in the use of synthetic insecticides by grain industry. Therefore, there are a great need and demand for the production and introduction of new, safer, less dangerous and less toxic insecticides and other measures of protection of the stored agricultural products (Fields, 1999). Among others, particular researches by a great number of authors have been directed towards the study of less dangerous and less toxic natural insecticides or the combination of two or more insecticides from already existent groups (Daglish,

1994; Lord, 2001; Korunić, 2007; Athanassiou and Korunić, 2007; Korunić and Rozman, 2010).

Diatom Research and Consulting, a Canadian company, has developed the new low-toxic formulations of powder insecticides by combining natural/natural and natural/synthetic substances, with the aim of the efficient control of pests and the significant decrease in DE negative effect on grains as well as the decrease in insecticide residues in stored goods. The mixtures, which have been developed, are diatomaceous earth (DE) and deltamethrin (DE/DM) contain 900g/kg DE and 1 g/kg of DM active substance (a.s.), DE and pyrethrin (DE/Py) containing 900g/kg of DE and 10g/kg of a.s. Py, and DE and spinosad (DE/Spin) containing 900g/kg of DE and 10g/kg of a.s. spinosad. These mixtures, applied in efficient doses, contain very low concentrations of active substances so that the harmful effects are significantly decreased in comparison with the case when the formulations which contain a significantly higher content of a.s. are applied individually (Korunić, oral presentation).

Diatomaceous earth (DE) is a natural substance registered in many countries as an insecticide for direct mixing with the stored grain goods and for treating empty storehouses (Korunić, 1998, Subramanyam and Roesli, 2000). However, DE has certain drawbacks, which prevent it from being used more widely (Fields, 1999).

In the last few years, with the aim of decreasing the drawbacks in its use, DE has been investigated combined with other measures as well as in the mixtures of insecticides of natural/natural and natural/synthetic origin. The combinations of DE with higher temperature were studied (Fields et al., 1997; Dowdy, 1999) by cooling off the grain mass and DE (Nickson et al., 1994), and in the mixture of DE with phytogetic fungi (Lord, 2001; Akbar et al. 2004; Michalaki et al., 2006, 2007; Kavallieratos et al., 2006) in the mixture with synthetic insecticides (Korunić and Rozman, 2010), in the mixture of DE and plant insecticides (Korunić, 2007; Athanassiou and Korunić, 2007).

Pyrethrin is one of the oldest insecticides in use for pest control. Considering the fact, that natural pyrethrin has a minimal effect on human health and marked efficiency with a paralyzing effect on insects it is, in fact, the only natural insecticide, which is used in the protection of stored agricultural products. It is applied in the doses of 1.5 to 3 ppm of a.s. on grain goods. In order to prevent the recovery of insects after the treatment, the pyrethrin formulations contain a synergist, most frequently piperonyl butoxide (PBO) which enhances the efficiency of pyrethrin and prevents its decomposition in insect bodies (ETOXNET, 1994; NPTN, 1999).

With the purpose of finding a possibility of replacing PBO and lowering the concentrations in the use of pyrethrin and

DE, a formulation of the mixture of DE, pyrethrin and plant extract, which might replace PBO in the formulation, has been developed.

The pyrethroid deltamethrin is used in the protection of stored agricultural products, plant protection, domestic animals protection and in public health care. It has a wide range of impact and is efficient in the control of *Rhyzopertha dominica* (F.) resistant to organophosphoric insecticides but not efficient enough on *Sitophilus* spp. and *Tribolium* spp. (Daglish, 1998). However, the resistance of *R. dominica* to deltamethrin was established (Lorini and Galley, 1995). The recommended dose of deltamethrin for the application is 0.25 ppm for short-term and long-term protection of goods (Bayer Environmental Science, 2009). In order to decrease the negative effect of DE and to decrease the residues of the very stable and persistent deltamethrin in the processed goods the mixture of DE and deltamethrin was developed and, probably, due to the combination of two different ways of affecting insects, drying out and toxicity, the synergism between the two substances was established which made the application of significantly decreased doses of DE and deltamethrin in their mixture possible (Korunić and Rozman, 2010).

Spinosad is a metabolite of soil borne bacterium *Saccharopolyspora spinosa* Mertz and Yao of a very low toxicity to mammals. The lethal dose, which kills 50% of laboratory, rat's populations (LD_{50}) through mouth and skin amounts to > 5000 mg/kg. Spinosad is applied in the protection of more than 100 of various plant species in the USA and has been registered in more than 25 countries in the world. In the USA the dose of 1 ppm or 1 mg of active substance per 1 kg of goods was registered for the protection of stored agricultural products. Based on the results by a large number of authors and published papers, in the registered dose, spinosad is the most efficient against *R. dominica*, significantly less efficient against *S. oryzae* and the least efficient against *T. castaneum*. The significantly higher doses than 1 ppm are necessary for the successful control of *T. castaneum* and *Oryzaephilus surinamensis* L. (Mertz and Yao 1990; Thompson et al., 1997; Thompson et al., 2000; Fang et al., 2002; Sparks et al., 2001; Subramanyam et al., 2003; Chintzoglou et al., 2008; Athanassiou et al., 2008).

With intent to increase the efficiency of spinosad and reduce the differences in the sensitivity between some insect species, the mixture of DE with spinosad which proved to be efficient in the doses lower than 100 ppm of DE and 0.6 ppm of a.s. spinosad was developed (Korunić, oral presentation).

The aim of our research work was to establish the efficiency of more recent developed formulations of insecticides, the mixtures of DE with deltamethrin, DE with spinosad and DE with pyrethrin, against store grain insects *Rhyzopertha dom-*

inica (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in the wheat grains.

Material and Method

Test insects

The testing was carried out on the laboratory population of *Sitophilus oryzae* L. and *Rhyzopertha dominica* F. Both species were bred in glass jars of 1.0 l overall grains of wheat with moisture content of about 12%. The temperature in the insectarium was $27 \pm 1^\circ\text{C}$ and relative air humidity was 60-70%. In the experiment, the *S. oryzae* adults aged 7 days and *R. dominica* adults aged 18 days were used. Each insect species was tested individually.

Insecticide formulations

The following insecticide formulations were investigated in the experiment: the mixture of diatomaceous earth (DE) and deltamethrin (DM) which contains 900g/kg of DE and 1g/kg of DM active substances (a.s.) marked as DE/DM, the mixture of DE and pyrethrin (Py) which contains 900g/kg of DE and 10g/kg of a.s. Py marked as DE/Py, and the mixture of DE and spinosad which contains 900 of DE and 10g/kg of a.s. spinosad marked as DE/Spin. The formulations were developed in the laboratory of Diatom Research and Consulting Inc., Toronto, Canada.

Each formulation was investigated in 3 concentrations: DE/DM and DE/Spin in 50, 100 and 150 grams per 1000 kg of wheat grains or 50, 100 and 150 ppm (parts per million) and DE/Py in 100, 200 and 300 ppm.

Experimental protocol

The experiments were carried out at the entomology laboratory of the Faculty of Agriculture in Novi Sad, Vojvodina after the harvest of wheat in 2011 was over. In the experiments the wheat variety Pobeda, which is sown on large areas in Vojvodina, was used. The whole, undamaged grains of pure wheat with the moisture of 12.12% were used.

The testing was done under the laboratory conditions according to the modified method EPP0 (PP 1/203 and 1/204) (OEPP/EPP0, 2004 a and b) and the procedure described by Collins (1990) and Chanbang et al. (2007).

The insecticide mixtures were applied to the whole grains of wheat in the following way. The amount of 1000 g of whole-wheat grains was poured into 3-liter glass jars each. Based on the concentrations described in 2.2 (the recommendation of Diatom Research and Consulting Inc., Canada) a mixture of insecticides of particular concentration was added into each jar, and then the jar was closed with a lid and set on the shaker for 1 min (Heidolph Promax 2020). The treated 1000 g was

divided into 4 equal replicates containing 250 g each. The replicates were poured into plastic dishes of 1.5 l. In each replicate 50 insects (mixture of both sexes) of *S. oryzae* and *R. dominica* were placed. Four replicates of untreated wheat were used as control groups.

The mortality of both insect species (from all the treatments) was determined after 7, 14 and 21 days after the introduction of insects into jars. The jars were closed with linen and left in the room at the temperature of 25-28°C and air humidity of 65-80% in the shift day/night 12/12 hours. After the examination, the dead insects were removed 7 and 14 days later, and after 21 days, all the insects were removed from jars. The number of the offspring of *S. oryzae* was counted after 56 days and of *R. dominica* after 70 days, before another generation of insects was developed.

Data analysis

The mortality of insects was calculated by the formula of Abbott (Abbott, 1925). The significance of the differences between the treatments and concentrations as well as the obtained offspring was compared with the untreated control group for each species separately by the variance analysis (ANOVA) at the level of 5% probability. The analyses were done in the program Statistica 9.1.

Results

The efficiency of the studied formulations of insecticides against *S. oryzae* and *R. dominica* is shown in Tables 1-3.

Formulation DE/Py

The efficiency of the formulation DE/Py against *S. oryzae* and *R. dominica* is shown in Table 1.

The dose of DE/Py formulation of 100 ppm did not completely control the adult insects of *S. oryzae* rice even after 21 days of the exposure to the treated wheat (mortality 86.5%) and it reduced the offspring by 97%. However, the dose DE/Py of 200 ppm was highly efficient against adults of *S. oryzae* (mortality 96.5% after 7 days and 100% after 14 days). That dose also completely suppressed the offspring of *S. oryzae*.

However, the dose of DE/Py of 100 ppm was highly efficient against the adults and offspring of *R. dominica*. After 14 days of the exposure to the wheat treated with 100 ppm the mortality of *R. dominica* was 100%. The offspring of *R. dominica* was totally suppressed by the use of 100 ppm of DE/Py formulation.

Formulation DE/DM

The efficiency of the formulation DE/DM against *S. oryzae* and *R. dominica* is shown in Table 2.

The dose of 50 ppm of the formulation DE/DM was not high enough to control the adults and offspring of *S. oryzae* successfully. Although highly efficient, the dose of 100 ppm did not completely control the adults of *S. oryzae* (98.5% mortality after 21 days of the exposure to the treated wheat) and it reduced the offspring by about 99%. However, the dose

Table 1
The effectiveness of formulation DE/Py against *S. oryzae* and *R. dominica* on treated wheat

Species	Concentration (ppm)	Average mortality (%) after days			Number of adults (progeny); after 56 and 70 days X±SE**
		7 X±SE*	14 X±SE*	21 X±SE*	
<i>S. oryzae</i>	Untreated	0.0±0.00 ^a	2.0±0.82 ^a	3.0±1.29 ^a	396.5±21.4 ^c
	100	72.5±3.09 ^c	82.0±3.16 ^b	86.5±2.63 ^b	11.0±6.38 ^a
	200	96.5±2.06 ^e	100.0±0.00 ^c	100.0±0.00 ^c	0.0±0.00 ^a
	300	100.0±0.00 ^e	100.0±0.00 ^c	100.0±0.00 ^c	0.0±0.00 ^a
<i>R. dominica</i>	Untreated	0.0±0.00 ^a	2.0±0.82 ^a	2.5±0.96 ^a	80.0±8.19 ^b
	100	63.5±3.86 ^b	100.0±0.00 ^c	100.0±0.00 ^c	0.0±0.00 ^a
	200	90.5±1.50 ^d	100.0±0.00 ^c	100.0±0.00 ^c	0.0±0.00 ^a
	300	100.0±0.00 ^e	100.0±0.00 ^c	100.0±0.00 ^c	0.0±0.00 ^a

*means in the same column followed by the same letters are not significantly ($P>0,05$) different as determined by the LSD-test

**number of live insects (adults progeny)

Table 2
The effectiveness of formulation DZ/DM against *S. oryzae* and *R. dominica* on treated wheat

Species	Concentration (ppm)	Average mortality (%) after days			Number of adults (progeny); after 56 and 70 days X±SE**
		7 X±SE*	14 X±SE*	21 X±SE*	
<i>S. oryzae</i>	Untreated	0.0±0.00 ^a	2.0±0.82 ^a	3.0±1.29 ^a	396.5±21.4 ^d
	50	26.0±3.16 ^b	33.5±3.40 ^b	45.5±2.22 ^b	138.5±19.26 ^c
	100	92.0±2.16 ^e	96.0±1.41 ^c	98.5±0.96 ^c	5.75±3.47 ^a
	150	99.5±0.50 ^e	100.0±0.00 ^d	100.0±0.00 ^c	0.0±0.00 ^a
<i>R. dominica</i>	Untreated	0.0±0.00 ^a	2.0±0.82 ^a	2.5±0.96 ^a	80.0±8.19 ^b
	50	56.0±3.56 ^c	97.5±0.96 ^{cd}	100.0±0.00 ^c	0.0±0.00 ^a
	100	63.5±6.24 ^{cd}	100.0±0.00 ^d	100.0±0.00 ^c	0.0±0.00 ^a
	150	68.5±1.89 ^d	100.0±0.00 ^d	100.0±0.00 ^c	0.0±0.00 ^a

*means in the same column followed by the same letters are not significantly ($P>0,05$) different as determined by the LSD-test

**number of live insects (adults progeny)

Table 3
The effectiveness of formulation DE/Spin against *S. oryzae* and *R. dominica* on treated wheat

Species	Concentration (ppm)	Average mortality (%) after days			Number of adults (progeny); after 56 and 70 days X±SE**
		7 X±SE*	14 X±SE*	21 X±SE*	
<i>S. oryzae</i>	Untreated	0.0±0.00 ^a	2.0±0.82 ^a	3.0±1.29 ^a	396.5±21.4 ^d
	50	7.0±1.29 ^b	22.0±2.45 ^b	34.5±3.30 ^b	395.5±9.07 ^d
	100	59.5±6.39 ^c	91.0±3.11 ^c	94.0±1.15 ^c	52.5±3.57 ^b
	150	85.0±2.65 ^d	97.0±1.29 ^d	98.5±0.96 ^d	24.75±5.12 ^a
<i>R. dominica</i>	Untreated	0.0±0.00 ^a	2.0±0.82 ^a	2.5±0.96 ^a	80.0±8.19 ^c
	50	100.0±0.00 ^e	100.0±0.00 ^d	100.0±0.00 ^d	0.0±0.00 ^a
	100	100.0±0.00 ^e	100.0±0.00 ^d	100.0±0.00 ^d	0.0±0.00 ^a
	150	100.0±0.00 ^e	100.0±0.00 ^d	100.0±0.00 ^d	0.0±0.00 ^a

*means in the same column followed by the same letters are not significantly ($P>0,05$) different as determined by the LSD-test

**number of live insects (adults progeny)

of 150 ppm totally controlled the adults and offspring of *S. oryzae*.

The lowest dose of 50 ppm of DE/DM totally controlled the adults (100% mortality after 21 days) and the offspring of *R. dominica*.

Formulation DE/Spin

The efficiency of the formulation DE/Spin against *S. oryzae* and *R. dominica* is shown in Table 3.

The application of the formulation DE/Spin of 50 ppm was not high enough for the control of adults of *S. oryzae* (34.5% efficiency only after 21 days) and its offspring. The population of offspring was similar to the population in the control group. Likewise, the doses of 100 and 150 ppm, although highly efficient, did not manage to control the adults and offspring of *S. oryzae* very even after 21 days of the exposure to the treated wheat. Considering a very high efficiency of the doses of 100 and 150 ppm of DE/Spin formulation after 14 and 21 days (91 and 97% mortality) and the reduction of offspring with the dose of 150 ppm (87%) one can suppose that the dose of 200 ppm might cause 100% mortality of adult insects and suppress the rice weevil offspring.

However, the dose of DE/Spin formulation of 50 ppm completely controlled adults and offspring of *R. dominica*.

Discussion and Conclusions

The primary advantages of the use of DE are low toxicity to mammals and stability i.e. inertness. The basic component of DE is amorphous silicon dioxide, broadly known as safe, harmless substance, which is used as food additive (USA EPA, 21 CFR 182.90, 182.1711). A number of papers show that it hardly has any significant effect on the quality of products, baking, pasta production and barley malt (Aldryhim, 1990; Korunić et al., 1996).

Many formulations of DE are available at present and are commercially produced for pest control in storehouses in many countries (Fields and Korunić, 2000; Subramanyam and Roesli, 2000; Arthur, 2003; Athanassiou et al., 2004). The efficiency of DE depends on a large number of factors: a dose, formulation, species and development stage of insects, application method, environment and the sort of product to which it is applied (Arthur, 2003). The formulations of DE are applied in the doses of 500 to 1000 g/ton of goods and even in higher doses. Even very low doses and especially 300 g/t or more significantly decrease the hectoliter weight of grain goods thus decreasing the market value of goods (Korunić et al., 1998). In addition, DE significantly affects the looseness of grain mass. There is a great difference in the resistance to DE of particular insect species. The higher humidity of grain

goods as well as higher relative air humidity significantly decreases the efficiency of DE (Fields, 1999). High doses of DE can contain an increased percentage of crystalline silicate and it can bring about respiratory problems in workers with a possibility of the silicosis development (Subramanyam and Roesli, 2000). Some formulations of DE also contain other substances like silica gel (synthetic, amorphous silicon dioxide), honey in powder, non-activated yeast and sugar (Korunić and Fields 1995; Subramanyam and Roesli 2000; Quarles and Winn, 1996). However, even these formulations should be used in rather high doses, which have negative effects on the decrease in hectoliter mass (Jackson and Webley, 1994; Korunić et al., 1996).

Therefore, due to the mentioned drawbacks, it is particularly important to study the new formulations of DE or the combinations of DE with other substances aimed at significant decrease in the doses, which are used. There have been researches into a possibility of the use of DE in the combination with extreme temperatures (Fields et al., 1997; Dowdy, 1999) by cooling off the grain goods with surface application of DE (Nickson et al., 1994), or the combination with phyto-genic fungi (Lord, 2001; Akbar et al., 2004; Michalaki et al., 2006 and 2007; Kavallieratos et al., 2006) in mixture with synthetic insecticides (Korunić, 2001; Stathers, 2003; Arthur, 2004a, 2004b; Athanassiou, 2006; Athanassiou and Korunić, 2007; Chanbang et al., 2007), in the mixture with plant extracts (Korunić, 2007, Athanassiou and Korunić, 2007). The experiments carried out with these combinations frequently pointed out the increased and even synergistic effect of the substances in the mixtures (Korunić, 2001; Lord, 2001; Stathers, 2003; Korunić, 2007; Athanassiou and Korunić, 2007, Korunić and Rozman, 2010).

Nowdays, in use there are formulations, which contain DE and pyrethrin. The following formulations of the mixture of DE and pyrethrin are known: Diacide Home Guard, Diatect V, Diatect II, Bug-B-Gon and Perma-Guard D-20, Perma Guard 21 (Kalinovic et al., 2011). The formulations usually contain 0.2% of active substance pyrethrin and 1% of the synergist PBO. The only exception is Diatect V which contains 0.5% of a.s. pyrethrin but no PBO. Kalinovic et al. (2011) established the significant difference in the effect of formulations Diatect V and Diatect II, which contain the same DE but different amounts of active substance pyrethrin (Diatect V with 5% of a.s. pyrethrin and without PBO and Diatect II with 0.2% of a.s. pyrethrin and 1% of PBO). The results confirm a significant role of PBO in enhancing the efficiency of pyrethrin and point at the already known fact that the formulations of pyrethrin insecticides have to contain the synergist PBO or a substance with a similar effect. The formulation Diatect V that does not contain PBO, applied in the concentration of 1.5

ppm of active substance pyrethrin was significantly less efficient than pyrethrin formulations, which contain PBO and only 0.6 ppm of active substance pyrethrin (Kalinovic et al., 2011). However, besides its rather high price, the future of the application of pyrethrin in storehouses is uncertain as the USA EPA has made a decision to ban the use of PBO. Without PBO pyrethrin in the recommended doses can not successfully control store pests, so that it is necessary to increase the application doses significantly and the recovery of insects is quite possible (ETOXNET, 1994; NPTN, 1999).

The pyrethroid deltamethrin has a wide range of effect on insects and is used in their control in different places including the protection of stored agricultural products. It is highly efficient on *R. dominica* and less efficient on *Sitophilus* spp. and *Tribolium* spp. (Daglish, 1998). Lorini and Galley (1995) established the significant resistance of *R. dominica* on deltamethrin. The combination of insecticides from different groups can postpone the occurrence of resistance and suppress the insects already resistant to pyrethroids. In order to suppress the insects already resistant to pyrethroids there have been researches into the combinations of pyrethroid and organophosphorous insecticides (Bengston et al, 1980, Gualiang et al., 1990, Duguet et al., 1990, Arthur, 1994, Daglish et al., 1996; Daglish, 1998, Wilkin et al., 1998). However, the concentrations of insecticides in those mixtures were rather high because, as a rule, when two insecticides were used, half a dose of one and half a dose of the other was mixed. Despite the higher efficiency, the residues of both insecticides in treated goods may be high and dangerous.

Korunić and Rozman (2010) established the existence of synergism in the formulation of insecticide, which contained very low doses of DE and deltamethrin. This formulation was developed with the aim of greatly reducing the drawbacks of the use of DE and decreasing deltamethrin residues in treated goods. In addition, due to the combined way of affecting insects, drying out of insects by DE and a toxic effect of deltamethrin it is possible that the development of insect resistance is significantly slowed down.

Numerous authors have studied the efficiency of spinosad on store pests and have found out that spinosad can be used as an alternative insecticide in storehouses but that its efficiency depends on the insect species and product types as well as on the dose and temperature. Spinosad significantly lowered the number of the imagos of *Rhizopertha dominica* (84-100%) and decreased the damage to grains (66-100%) when compared with the untreated wheat (Fang et al., 2002). The same authors point out the efficiency of spinosad on durum wheat for 1 mg/kg (1 ppm) against *S. oryzae*. Subramanyam et al. (2003) reported that spinosad in the amount of 1mg/kg kills adults *R. dominica* after 8 days. The mortality of all adults of

S. oryzae occurred after 8 days when 3 mg/kg was applied and after 14 days when 1 mg/kg was used. Likewise, Getchell (2006) also established that spinosad on the treated wheat was more efficient against *R. dominica* than against *S. oryzae*. Nayak et al. (2005), Athanassiou et al. (2008), obtained the similar results. Bonjour and Opit (2010) investigated two types of spinosad formulation and established the differences in their efficiency on insects.

Chintzoglou et al. (2008) investigated the possibility of combined application of spinosad and DE. The experiments comprised the formulation of spinosad powder with 0.125% of a.s. and DE, the formulation SilicoSec. Each powder was applied individually and in the mixture of both. For *S. oryzae* the following doses were studied: the dose of DE of 150 ppm, the spinosad doses of 0.0655%, 0.2875% and 0.625% and their mixtures. For *T. confusum* the dose of DE of 250 ppm and the doses of spinosad of 0.1875, 0.625% and 1.25% of a.s. spinosad were studied. The results of the experiment showed that by combining DE and spinosad a greater efficiency on insects could be achieved but that the efficiency depended on the insect species and the type of treated grain goods. The authors Bonjour and Opit (2010) proved that the efficiency of spinosad depended on the formulation type, as well.

In our study, the formulation DE/Py applied in the dose of 100 ppm totally suppressed the adults and offspring of *R. dominica* lesser whereas the dose of 200 ppm was necessary for the complete suppression of the adults and offspring of *S. oryzae*. In 100 ppm the formulation DE/Py contains 90 ppm of DE and 1 ppm of pyrethrin and in 200 ppm it contains 180 ppm of DE and 2 ppm of pyrethrin. When these two active substances are applied separately, DE is applied in doses of 500 to 1000 and more ppm (Subramanyam and Roesli, 2000) and the pyrethrin containing PBO in doses of about 3 ppm (NPTN, 1999).

The formulation DE/DM applied in dose of 50 ppm totally suppressed the adults and offspring of *R. dominica*. For the suppression of adults and offspring of *S. oryzae* a dose of 150 ppm was necessary. In 50 ppm the formulation DE/DM there is 45 ppm of DE and 0.05 ppm of a.s. DM and in 100 ppm it contains 0.1 ppm of DM and 90 ppm of DE. When the formulations of deltamethrin are applied, the recommended doses are 0.25 ppm for short-term protection and 0.50 ppm for long-term protection (Daglish, 1998) and doses of DE are from 500 to 1000 and more ppm (Subramanyam and Roesli, 2000).

The highest applied dose of 150 ppm of the formulation DE/Spin did not totally suppress adults and offspring of *S. oryzae*. However, that dose caused a high mortality of adult insects (97%) and a significant reduction of offspring (87%). The dose of 50 ppm totally suppressed the adults and off-

spring of *R. dominica*. The dose of the formulation DE/Spin of 50 ppm contained 45 ppm of DE and 0.5 ppm of spinosad a. s. and the dose of 150 ppm contained 135 ppm of DE and 1.5 ppm of spinosad a. s.

All three studied formulations were significantly more efficient on *R. dominica* than on *S. oryzae*. These data correspond with the results of numerous authors who have established the higher efficiency of pyrethrin, deltamethrin and spinosad on *R. dominica* in comparison with the efficiency on *S. oryzae*. However, the studied insecticide formulations generated a very high efficiency against the test insects in significantly lower doses of active substances compared with the case when those active substances were applied in separate formulations. Thus, the drawbacks of the application of DE were greatly decreased and the danger of the active substances residues in the treated goods was reduced.

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Received January, 23, 2013; accepted for printing September, 2, 2013.