# XXVI INTERNATIONAL ECO-CONFERENCE® 2022
## 21–23th SEPTEMBER

# XII SAFE FOOD

ЕКОЛОШКИ ПОКРЕТ НОВОГ САДА

**PROCEEDINGS**

NOVI SAD, SERBIA

**XXVI INTERNATIONAL ECO-CONFERENCE® 2022**

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# SPINOSAD APPLICATION IN PROCESS OF INTEGRATED PEST MANAGEMENT AGAINST *RHYZOPERTHA DOMINICA* F. IN STORED SMALL GRAINS

**Abstract**

This study aimed to evaluate insecticidal activity of Spinosad applied at doses of 0.25, 0.5, and 1.0 mg $kg^{-1}$ (ppm) on *Rhyzopertha dominica* in wheat, barley, rye, oats and triticale grains. Mortality was assessed after 7 and 14 days, while impact on progeny production was assessed after 8 weeks. In the lowest dosage after 7 days of exposure, Spinosad application affected mortality ranged from 94.5% (wheat) to 100% (barley). After 14 days, all doses of Spinosad achieved mortality of 100%. All three applied dosages of Spinosad prevented progeny emergence. Damaged grain and dust in Spinosad treated samples were not recorded which represent the most ideal small grain protection. Spinosad has been identified as a natural and promising alternative to stored-grain protectants.

**Key words:** *R. dominica, small grains, Spinosad, insecticidal activity*

## INTRODUCTION

Pest infestation has the great influence on stored grain goods. The lesser grain borer *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) is the major cause of insect damaged kernels (IDK) in wheat. *R. dominica* spends most of its life inside kernels, feeding on its endosperm, which causes damage and changes in grain physicochemical properties. Adult feeding activities produce large amounts of dockage, most of which consists of ovoid granules of undigested endosperm mixed with a finer floury part.

Application of chemical insecticides has a crucial role in the quick and efficient control of this pest, which is followed by the occurrence of undesirable residues in grains and resistance of particular populations of stored-product pests. The aforementioned problems demand the necessity of finding alternative, environmentally friendly solutions, and omit or significantly reduce negative effects of the application of synthetic insecticides. Natural originating insecticides, such as Spinosad, will contribute to solving these problems in the near perspective. Spinosad is a natural product, obtained by fermentation of soil actinomycete, *Saccharopolyspora spinosa* Mertz & Yao. Resistance of *R. dominica* to Spinosad has not yet been found (Andrić et al., 2019).

This is a primary pest which can destroy stored wheat completely. Perišić et al. (2017) were concluded that small grains – wheat, barley, rye, and triticale can be considered as "nutritionally suitable" for *R. dominica* development, but triticale is the most susceptible to attack and suitable for development of R. dominica. Based on the aforementioned, it is important to determine is the protection of all stored small grains can be uniform or it must take care of grain species, especially during application of natural originated insecticides and because of covered way of life as specificity of *R. dominica* (life cycle mainly spends in the grain feeding by grain endosperm). Special attention must be direct to the "grain effect", i.e. variation in the pest sensitivity caused by different grain species.

## MATERIALS AND METHODS

The experiment was conducted in the Center for Small Grains in Kragujevac. The tested adult population of *R. dominica* was reared on the whole wheat kernels under laboratory conditions (temperature 26±1°C and relative humidity – r.h. 60±5%.). In the experiment 2-4 weeks old adults were used. Five species of small grains were tested: wheat (Vizija variety), barley (Rekord variety), oats (Vranac variety), rye (Raša variety) and triticale (Favorit variety). All varieties originate from the Center of Small Grains Kragujevac, Serbia. Grain samples with the moisture content ranging from 11-12 % were included in the experiment.

For examination of insecticidal efficacy pesticide Laser 240 SC (active substance Spinosad-240g/L;Dow AgroSciences, Austria) was used. Efficacy of applied insecticide on small grains was determined according to methods for evaluation of the biological efficacy of insecticides in storage pests suppression (OEPP/EPPO, 2004a,b). The 0.5 kg lots of each grain samples were weighed on an analytical balance (Mettler 609-B6, Zurich, Switzerland), and placed into jars (1000 ml in volume). Afterward, grain samples were treated with 5 ml of aqueous solutions of Spinosad in amounts of 0.25; 0.5 and 1.0 mg $kg^{-1}$ (ppm) of grain. Grain labeled as a control A was treated with 5 ml of distilled water, while grain used as a control B stayed untreated. Firstly, treated grain was manually shaking for 30 seconds and afterward were mixed on a rotary mixer for 10 min.

Plastic vessels (200 ml in volume) were filled with 50 g of treated, as well as untreated grain, and placed in a thermostat chamber with controlled conditions, 26

±1°C temperature and 60 ±5% r. h. After 24 h, 25 adults of *R. dominica* were released into each vessel (except in control B) and the vessel was topped with a cotton cloth and fixed with a rubber band. Four plastic vessels was set up for every variant, i.e. four replications. Insect mortality was determined after 7 and 14 days of contact with treated or untreated grain types. After the last mortality count, both dead and living adults were removed and all vessels were returned to the incubator for 8 additional weeks under the same (described) conditions.

After 8 weeks, progeny emergence/suppression was determined by counting insects in treated and control grain samples. During $F_1$ generation counting, whole grain, damaged grain, and dockage from each vessel were separated and weighed on an analytical scale (Mettler 609-B6, Zurich, Switzerland). The whole procedure was repeated twice. Recorded data of insecticide efficacy were expressed as percentage mortality with calculated standard error (% ± SE). Before analysis, the percentage of mortality was transformed using *arcsine*, while data for the amount of insect-damaged grains and dockage were transformed by a square root. Differences in means for all data were statistically analyzed with one-way ANOVA and Tukey-Kramer (HSD) post hoc test (at $P = 0.05$).

## RESULTS

In the lowest dosage, after 7 days of exposure, Spinosad application affected mortality from 94.5% (wheat) to 100% (barley). The high mortality of *R. dominica* (≥98%) with dosages application of 0.5 and 1 mg $kg^{-1}$ in all tested grain species was achieved. Spinosad application in the dosage of 1 mg $kg^{-1}$ caused 100% of the *R. dominica* mortality, except in triticale where mortality was slightly lower (99.5%). In the present research, Spinosad application caused 100% of *R. dominica* mortality after 14 days of exposure, for every applied rate, in all examined grain species (Table 1).

*Table 1. Mortality of R. dominica adults (% ± SE) after 7 and 14 days of exposure in different grain types treated with Spinosad*

| Insecticide | Dosage ($mgkg^{-1}$) | Mortality (%±SE) after exposure in small grains | | | | |
|---|---|---|---|---|---|---|
| | | Wheat | Barley | Rye | Oat | Triticale |
| | | After 7th day of exposure | | | | |
| Spinosad | 0.25 | 94.5±0.2$^{b*}$ | 100.0±0.0$^{a}$ | 97.5±0.2$^{b}$ | 96.5±0.3$^{b}$ | 95.5±0.2$^{b}$ |
| | 0.5 | 99.0±0.2$^{a}$ | 100.0±0.0$^{a}$ | 99.5±0.0$^{ab}$ | 99.5±0.0$^{ab}$ | 98.0±0.0$^{ab}$ |
| | 1.0 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 99.5±0.0$^{a}$ |
| Control A | 0 | 2.0±0.1$^{c}$ | 1.0±0.1$^{b}$ | 2.0±0.1$^{c}$ | 1.0±0.1$^{c}$ | 1.0±0.1$^{c}$ |
| *F* | | 719.30 | 91823 | 457.78 | 341.83 | 266.37 |
| *P* | | < 0.05 | < 0.05 | < 0.05 | < 0.05 | <0.05 |

| | | After 14th day of exposure | | | | |
|---|---|---|---|---|---|---|
| Spinosad | 0.25 | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] |
| | 0.5 | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] |
| | 1.0 | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] | 100.0±0.0[a] |
| Control A | 0 | 2.0±0.1[b] | 1.0±0.1[b] | 2.0±0.1[b] | 1.0±0.1[b] | 1.0±0.1[b] |
| *F* | | 686458 | 918203 | 686458 | 918203 | 91203 |
| *P* | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

* Within every period of exposure, values across columns marked with same letters do not show a statistical difference; Tukey-Kramer (HSD) test for P>0.05; df=7.56. Control A – untreated, infested grain with *R. dominica*

Ten weeks after the treatment, all three applied dosages of the examined insecticide prevented progeny emergence. The suitability of grain species for *R. dominica* development was varying. The greatest progeny number in control samples was determined in triticale (432.5), followed by rye (177.1) and wheat (128.5), while oats had the lowest suitability (36.4) (Table 2).

*Table 2. Average number of the R. dominica progeny in grains (±SE) ten weeks after insecticide Spinosad treatment*

| Insecticide | Dosage (mg kg$^{-1}$) | Progeny number average (±SE) in small grains | | | | |
|---|---|---|---|---|---|---|
| | | Wheat | Barley | Rye | Oat | Triticale |
| Spinosad | 0.25 | 0 | 0 | 0 | 0 | 0 |
| | 0.5 | 0 | 0 | 0 | 0 | 0 |
| | 1.0 | 0 | 0 | 0 | 0 | 0 |
| Control A | | 128.5±1.0 | 82.1±0.8 | 177.1±0.8 | 36.4±0.3 | 432.5±0.8 |
| *F* | | 31.81 | 25.12 | 41.81 | 20.21 | 50.89 |
| *P* | | > 0.05 | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

– Control A – untreated, infested grain with *R. dominica*

The smallest mass of damaged grain was found in infested, untreated oats (0.44 g), while the greatest mass of damaged grains was weighed in triticale (10.26 g). The presence of dockage in all treated samples with Spinosad was not recorded. The highest mass of the dockage was measured in a control sample of triticale infested with *R. dominica* (5.2 g), followed by rye (3.8 g), significantly lower in wheat (2.04 g) and barley (1.05 g), and the smallest in oats (0.27 g) (Table 3.).

*Table 3. Mass of damaged grains and the dockage (g±SE) after sieving of the R. dominica progeny from Spinosad treated grains*

| Insecticide | Dosage, $mgkg^{-1}$ | Mass of damaged grains and the dockage (g±SE) in grain types | | | | |
|---|---|---|---|---|---|---|
| | | Wheat | Barley | Rye | Oat | Triticale |
| | | Damaged grains | | | | |
| Spinosad | 0.25 | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] |
| | 0.5 | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] |
| | 1.0 | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] | 0±0.0[a] |
| Control A | 0 | 6.83±0.3[b] | 3.04±0.2[b] | 6.85±0.2[b] | 0.44±0.1[b] | 10.26±0.2[b] |
| *F* | | 33.77 | 14.08 | 18.04 | 3.53 | 23.29 |
| *P* | | > 0.05 | > 0.05 | > 0.05 | > 0.05 | > 0.05 |
| | | Dockage | | | | |
| Spinosad | 0.25 | 0.00[a] | 0.01±0.0[a] | 0.05[a] | 0.00a | 0.01[a] |
| | 0.5 | 0.00[a] | 0.01[a] | 0.00[a] | 0.00a | 0.00[a] |
| | 1.0 | 0.00[a] | 0.00[a] | 0.01[a] | 0.00a | 0.00[a] |
| Control A | 0 | 2.04±0.1[b] | 1.05±0.1[b] | 3.80±0.2[b] | 0.27±0.0[b] | 5.20±0.2[b] |
| *F* | | 29.75 | 24.35 | 47.78 | 10.47 | 11.04 |
| *P* | | > 0.05 | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

* Within every period of exposure, values across columns marked with same letters do not show a statistical difference; Tukey-Kramer (HSD) test for P>0.05; df=7.56;
– Control A – untreated, infested grain with *R. dominica*

## DISCUSSION

In the present research, Spinosad caused 100% mortality of *R. dominica* after 14 days of exposure, for every applied rate, in all examined grain species. Numerous studies determined complete control of *R. dominica* in wheat with Spinosad, after 14 days of exposure (Fang et al., 2002; Vayias et al., 2009; Vayias et al., 2010; Athanassiou et al., 2011; Subramanyam et al., 2012; Nayak and Daglish, 2017).

Athanassiou et al. (2008) recorded that the mortality of *R. dominica* increased with dosage, exposure interval of Spinosad, and temperature. In present study the highest dose was 1 ppm. This dose was recommend for used to discriminate between susceptible and resistant populations for future resistance monitoring programs (Nayak and Daglish, 2017).

Our study combined research on the efficiency of Spinosad on all small grains (wheat, barley, rye, oats, and triticale). By examining the application of Spinosad on different food commodity, Fang et al. (2002) were among the first who found a difference in the efficiency of Spinosad in the control of *R. dominica* on four classes of wheat (hard red winter, hard red spring, soft red winter and durum wheat). During the examination of grain species (wheat, barley, rice, and maize), depending on the efficacy of Spinosad, Athanassiou et al. (2008) and Vajas et al. (2009) revealed that *R. dominica* mortality was the lowest in maize, after 7 days of exposure. After 7 days of exposure, Chintzoglou et al. (2008) recorder the same lower mortality on maize (80%) treated with 0.025 ppm of a.i. and no differences of Spinosad efficacy in wheat and barley. Listed authors determined no significant differences in mortality of *R. dominica* after 14 days of exposure in comparison with the commodities. Athanassiou and Kavallieratos (2014) investigated the Spinosad efficacy on *R. dominica* in the applied rate of 1 ppm on wheat, barley, rye, and maize and reported that overall mortality ranged from 92.8 to 100 % after 7 days of the exposure. After 14 days of exposure, all adults were dead in all treatments, which is in agreement with our results.

The influence of commodity (wheat, barley, rice, rye, and maize) on the efficiency of spinosad was determined in controlling of *Sitophilus oryzae* L., *Prostephanus truncatus* (Horn), *Tribolium confusum* (Jacquelin du Val), *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* (Stephens), *Plodia interpunctella* (Hubner). Although tested small grain species differed in properties, Spinosad effectiveness in exterminated small grains was not different. According to Athanassiou et al. (2008) the "grain effect" is manifesting at the most in pest species less susceptible to Spinosad, while in *R. dominica*, as very susceptible, differences between different grain species could be masked.

Present study revealed that the examined insecticide prevented progeny emergence. Similar results were reported by Vayas et al. (2009), Athanassiou et al. (2011) and Nayak and Daglish (2017), who draw the same conclusions for Spinosad application in dosages of 0.1, 0.5 and 1 mg $kg^{-1}$, after 10 weeks of exposure. Athanassiou and Kavallieratos (2014) revealed that offspring emergence was ≤0.4 adults/vial in all tested commodities (wheat, barley, rye, and maize), with total suppressed only in wheat.

Present results show that *R. dominica* progeny production and feeding damage varied considerably depending on the small grain species, so the influence of commodity on the development of this pest was significant. In the present study, the highest progeny was found in untreated triticale, as well as the mass of damaged grains and dockage. The amounts of damaged kernels and dockage were in correlation with the emergence of progeny in untreated, infested samples. Damaged grain and dust in Spinosad treated samples were not recorded which represent the most ideal small grain protection and especially in the world where permitted limit for wheat is 2.5 damaged grain/100 g of total grain.

## CONCLUSIONS

In this laboratory bioassays, Spinosad application was efficient in the suppression of *R. dominica* adults in small grains. All three dosages of examined insecticide were prevented progeny emergence and that is very important parameter for making decision about long protecting potential in stored grains. Untreated grain samples, infested with *R. dominica*, in relation to the other tested samples, showed the biggest change in mass of damage grain and dockage which clearly show a positive aspect of the use of Spinosad.

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# ПРИМЕНА *SPINOSAD-а* У ИНТЕГРАЛНОЈ ЗАШТИТИ СТРНИХ ЖИТА ОД *RHYZOPERTHA DOMINICA* F.

**Сажетак**

Истраживање је спроведено у циљу оцене инсектицидне активности Спиносада, примењеног у дозама од 0,25, 0,5, и 1,0 мг кг$^{-1}$ (ппм) на *Рхузопертха доминица* у зрну пшенице, јечма, ражи, овса и тритикалеа. Смртност је процењена након 7 и 14 дана, док је утицај на стварање потомства оцењен након 8 недеља. При најнижој дози након 7 дана излагања, примена Спиносада је изазвала смртност од 94.5% (пшеница) до 100% (јечам). Након 14 дана, све дозе Спиносада оствариле су смртност од 100%. Све три примењене дозе Спиносада спречиле су појаву потомства. У узорцима третираним Спиносадом није забележена појава оштећених зрна и прашине што представља савршену заштиту зрна стрних жита. За Спиносад је утврђено да представља природну и одговарајућу алтернативу синтетичким инсектицидима стрних жита.

**Кључне речи:** *Rhyzopertha dominica, стрна жита, Спиносад, инсектицидна активност*

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