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THE MORTALITY OF THE LESSER GRAIN BORER *RHYZOPERTHA DOMINICA* (FABRICIUS 1792) INDUCED BY PLANT POWDERS

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Abstract

The aim of this study was to investigate the effect of powdered plants: *Salvia officinalis* L., *Artemisia absinthium* L., *Mentha piperita* L., *Allium sativum* L. and *Pimenta dioica* L. on the mortality and development of the progeny of *Rhyzopertha dominica* F. Powdered plants were used at the following weight concentrations: 1.23%, 3.61%, and 5.88%. Experiments were conducted in a laboratory at 28°C and RH 60±5%. It has been found that the effect of the tested plants depends on their concentration. Among the five species of powdered plants: *S. officinalis*, *A. absinthium*, *M. piperita*, *A. sativum*, and *P. dioica*, the highest mortality in *R. dominica* was caused by *P. dioica* at all tested concentrations and as early as after the first day of experiments. Of the tested plants, the one that may find application in the protection of stored products from *R. dominica* is *P. dioica*, which has shown insecticidal activity against this insect species.

Introduction

The loss of food produced on our planet is colossal. According to the Global Food Reports published by GUSTAVSSON (2011) and FOX (2013), it is estimated that 30–50% (1.2–2 billion tonnes) of annual world food production is lost. These figures do not include the usage of energy, water, and fertilizers in food production. Food wastage occurs throughout different stages of food production and forms a great chain of losses stretching from harvest to consumption, through transportation, storage, processing,

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packaging, finally reaching sale and food intake. Each of these stages involves losses, e.g., via spilling, dampening, and pests. Reducing this wastage, especially that of grain soon after harvest, is cheaper and more cost-effective than increasing food production. Food losses that occur during storage resulting from foraging by pests, mainly insects and mites, are difficult to evaluate, and additionally are – very diversified depending on crops, country, climatic region, as well as on the duration of the storage period of certain products. Furthermore, there is no universal method for measuring these losses. It is generally assumed that, every year, worldwide losses caused by insect and mite pests in the storage of products amount to ca. 10%. Integrated methods (Integrated Pest Management – IPM), which are considered an alternative to chemical methods, are the currently recommended and applied methods for combating pests. The former consists of preventing the occurrence of insect pests and combating them with mechanical, physical, and biological methods, coupled with limiting the use of chemical pesticides to the lowest possible level through using them only when absolutely necessary (Cox 2004, Phillips and Throne 2010, Olejarski and Ignatowicz 2011).

Since 1 January 2014, Regulation 1107/2009 and Directive 2000/29/EC on the safe use of pesticides have been in force in EU member states. Strong emphasis is placed on the widest possible use of non-chemical methods, especially biological ones. In recent years, the studies concentrated on the search for the various forms of plant-derived materials, e.g., powders, oils, or extracts that could be used to control insect pests of stored products. A great number of studies were published indicating the insecticidal effects against these insects (Jilani and Su 1983, Khan and Marwata 2003, Park et al. 2003, Isman 2006, Moreira et al. 2007, Kim et al. 2011, Padini et al. 2013). Moreover, the natural plant products are biodegradable; therefore, they are safe for the environment. Therefore, research into the activity of herbal preparations as bio-insecticides therefore seems highly relevant. Plants contain many substances that may change insect behaviour when added to food, and they also affect food absorption and cause development disturbance in insects (Nawrot 1983, Papchristos and Stamopoulos 2002).

Rhyzopertha dominica discussed in the presented study is a dangerous and widespread grain storage pest. Both adult individuals and larvae that feed on cereal grain cause extensive damage (Irshad et al. 1988, Dowdy and Mcgaughey 1992). *R. dominica* infects numerous plant products including wheat, rice, sorghum, rye, barley, maize, beans, dried vegetables, and fruits (Nawrot 2001, Phillips and Throne 2010). The aim of the tests was to investigate the mortality inducted on this pest by the use

of powdered plants, namely *Salvia officinalis* L., *Artemisia absinthium* L., *Mentha piperita* L., *Allium sativum* L., and *Pimenta dioica L.* Mer. The rate and intensity of the effects of each type of dried plant powder on the *R. dominica* population was also studied. The tests performed also had a secondary practical purpose, i.e. to indicate plant species that would show insecticidal or deterrent activity on the *R. dominica*, and could therefore prove applicable to the integrated protection of stored grain against this pest.

Materials and Methods

Mass cultures of the *R. dominica* were first established in order to obtain individuals of equal age, which were subsequently used in the experiments. According to previous research, wheat is considered the most suitable food and habitat for the development of the *R. dominica* (KŁYŚ 2006). Therefore, with the use of analytical scales, 100 g doses of wheat were placed in a number of plastic containers with a 50 cm^2 bottom and perforated lid enabling air penetration. Eighty individuals of this species were then transferred to each container with wheat. Culture containers were placed in incubators in 28°C and RH 60±5 %. After four weeks, all adult individuals were carefully removed using tweezers, while eggs, larvae, and pupae were left in the wheat. Individuals of equal age were obtained after another four weeks and used in the experiments. The experiments were carried out in plastic, 28 cm^2 bottom containers closed with perforated lids. All tests were started by transferring same-aged adult individuals (20 males and 20 females) to culture containers.

Control cultures were held, using clean wheat as a substrate, as well as a set of experimental cultures with wheat containing dried plant additives as substrate. Dried leaves of *M. piperita*, herb *S. officinalis*, bulbs of *A. sativum*, herb *A. absinthium*, and seeds of *P. dioica* were powdered with an electric grinder. Each type of dried plant material and up to 40 g of wheat was added to containers in the following amounts: 0.5 g, 1.5 g, and 2.5 g, which gave the respective percentage weight concentrations: 1.23%, 3.61%, and 5.88%. 40 g of wheat was weighed out and added to closed plastic containers, which were perforated to enable constant air inflow. The wheat was thoroughly mixed with plant powders. *R. dominica* individuals were then transferred to containers, with 40 insect individuals per each container, thus creating 15 sample variants. Six repetitions of each sample were performed. Control samples were placed in a separate incubator in the same temperature and humidity conditions as experimental

samples, to prevent the effect of volatile substances on the insect mortality. The population status was monitored after 1, 2, 7, 14, and 21 days. Live and dead insects were counted. Dead insects were removed and live ones were left in the culture.

In order to investigate the effect of applied plant powders on the progeny of *R. dominica* on 21 day all beetles were removed and the cultures were left in the incubator. After 50 days the population was re-monitored.

To assess the effect of the added plants on the studied populations, a Kruskal-Wallis ANOVA test showing the statistical significance of differences was employed, with the use of PQStat ver.1.4.2.324. software assuming a significance level $\alpha = 0.05$. The Dunn test was the *post-hoc* test performed after the rejection of zero-hypothesis by the Kruskala-Wallis test.

The mortality rate, representing the percentage share of dead individuals relative to the total number of individuals in the population per controlled time unit, was calculated from the following formula (KŁYŚ 2013):

$$\frac{\overline{x}_d}{\overline{x}_d + \overline{x}_l} \cdot 100\%$$

$\overline{x}_d$ – mean number of dead insects
$\overline{x}_l$ – mean number of live insects.

Results

After the first day of testing, the highest mortality of *R. dominica* was recorded in wheat with the admixture of powdered *P. dioica*, and the lowest was found in wheat with the admixture of *M. piperita* at all concentrations (1.23%; 3.61%; 5.88%). The 1.23% concentrations of *M. piperita*, *S. officinalis* and *A. sativum* did not cause any mortality in *R. dominica* after the first day of testing. The insect mortality caused by these plants used at other concentrations did not exceed 2% in wheat with *M. piperita*, it equalled 4% in wheat with *A. sativum*, and 7.5% in wheat with *S. officinalis* (Figures 1*a–e*).

Statistical analysis of the obtained data performed using the Kruskal-Wallis test showed statistically significant differences ($H = 74.60$, $p < 0.0001$) on the first day of experiments. When analysing the results with the post-hoc Dunn test, statistically significant differences were found between the mortality of R. dominica kept in the control culture (without herbal additives) and the mortality of these insects kept with *P. dioica* at all concentrations used: 1.23% ($p = 0.0373$); 3.61% ($p = 0.0030$)

and 5.88% ($p = 0.0019$). When comparing the mortality of insects between cultures containing different concentrations of powdered plants, thirteen significant differences were found on the first day. In all compared pairs, statistically significant differences in the insect mortality were noted in the presence of *P. dioica* (Table 1).

Table 1

Significant differences in the mortality of *Rhyzopertha dominica* (Fabricius 1792) caused by powdered plants of different species

Kruskal-Wallis Test, $\alpha = 0.05$														
Day of research														
1			**2**			**7**			**14**			**21**		
*	**	***	*	**	***	*	**	***	*	**	***	*	**	***
C- P1				C- P1		C- S3			C- S2				C- S2	
	C- P2				C- P2			C- P1		C- S3			C- S3	
	C- P3				C- P3			C- P2			C- P1			C- P1
M1- P1				M1- P1			C- P3				C- P2			C- P2
	M1- P2				M1- P2	M1- S3				C- P3				C- P3
	M1- P3				M1- P3			M1- P1		M1- S3			M1- S3	
M3- P2				Ar1- P1				M1- P2			M1- P1			M1- P1
M3- P3				Ar1- P2			M1- P3				M1- P2		M1- P2	
Ar1- P2				Ar1- P3		Ar1- S3				M1- P3			M1- P3	
Ar1- P3				S1- P1			Ar1- P1			Ar1- S3			Ar1- P1	
S1- P1					S1- P2			Ar1- P2		Ar1- P1			Ar1- P2	
	S1- P2				S1- P3		Ar1- P3			Ar1- P2		Ar1- P3		
	S1- P3					S1- S3			Ar1- P3			S1- S3		
P1- Al1							S1- P1			S1- S3				S1- P1
	P2- Al1							S1- P2			S1- P1			S1- P2
	P3- Al1						S1- P3				S1- P2		S1- P3	
							P1- Al1			S1- P3			P1- Al1	
							P2- Al1			P1- Al1			P2- Al1	
										P2- Al1		P3- Al1		

Control/Plant: C – control, M – *Mentha piperita* (Linnaeus 1753), S – *Salvia officinalis* (Linnaeus 1753), Ar – *Artemisia absinthium* (Linnaeus 1753), Al – *Allium sativum* (Linnaeus 1753), P – *Pimenta dioica* (*Linnaeus* 1753, Merrill)

Weight concentrations of powdered plant: 1–1.23%, 2–3.61%, and 3–5.88%.

Significant differences:

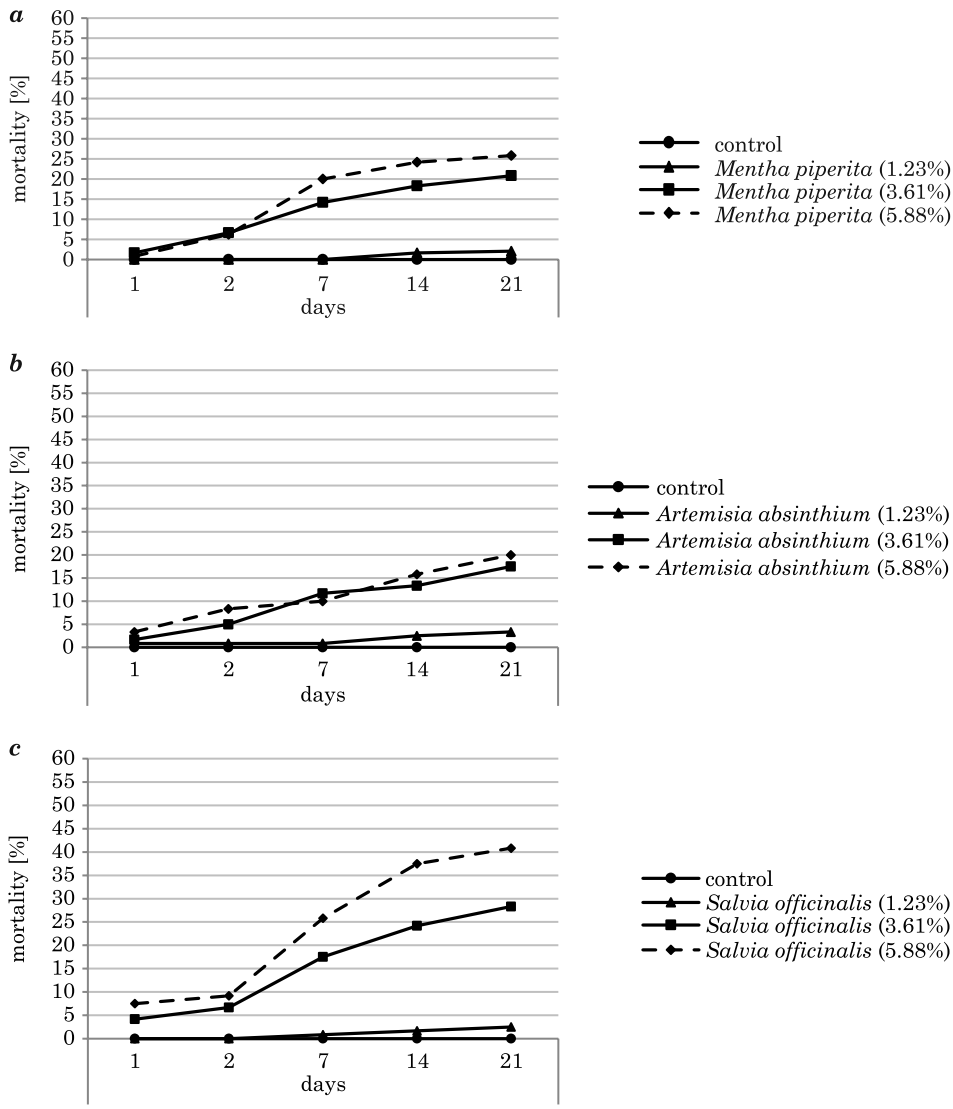
* $0.05 > p > 0.01$;

** $0.01 > p > 0.001$;

*** $p < 0.001$

In the further experimental time periods, i.e. after 2, 7, 14, and 21 days, the highest mortality was caused by *P. dioica*, followed by *S. officinalis*, *M. piperita*, *A. absinthium*, and *A. sativum*. Insects kept in the con-

trol culture containing wheat without plant additives presented a 100% survival rate in all experimental time periods (Figures 1 *a–e*). The mortality of the *R. dominica* in wheat with 5.88% admixtures of *M. piperita*, *A. absinthium*, *S. officinalis*, and *A. sativum* showed a time-dependent increase and was the highest compared to other concentrations of plant powders (Figures 1 *a–d*).



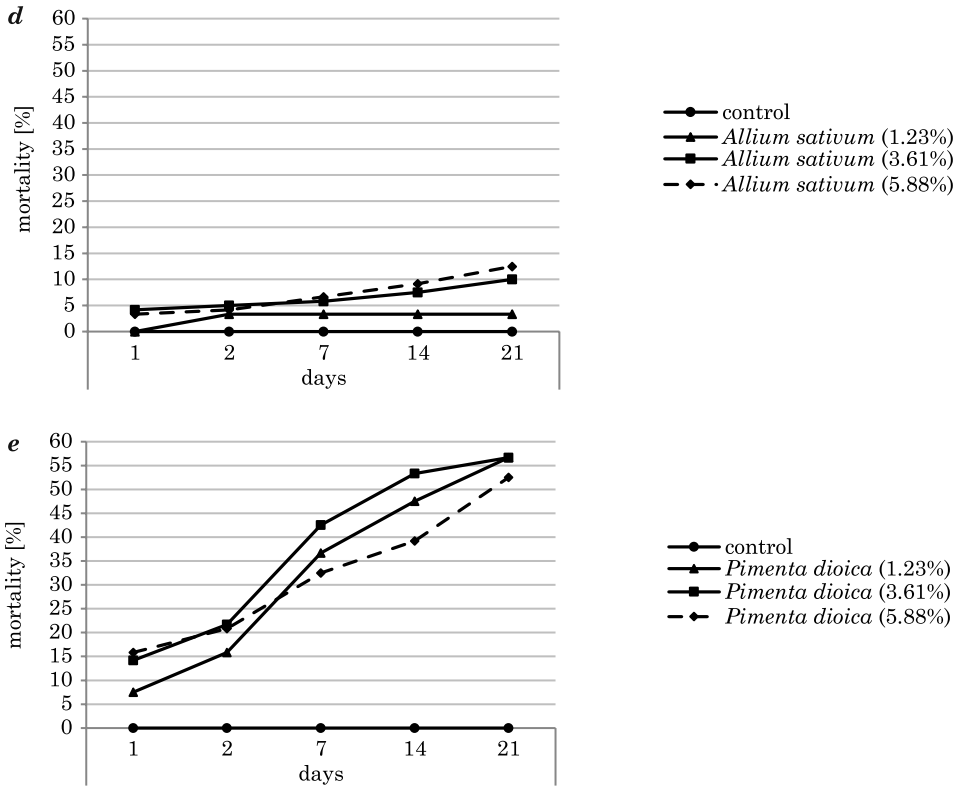


Figure 1. Mortality of *Rhyzopertha dominica* (Fabricius 1792) caused by: *a* – *Mentha piperita* (Linnaeus 1753); *b* – *Artemisia absinthum* (Linnaeus 1753); *c* – *Salvia officinalis* (Linnaeus 1753); *d* – *Allium sativum* (Linnaeus 1753); *e* – *Pimenta dioica* (Linnaeus 1753, Merrill)

It was also found that the mortality of *R. dominica* rose over time in wheat containing the admixtures of each plant at all concentrations used. Another trend discovered was the fact that the insect mortality usually increased in accordance with the concentration of plant powders. One exception was the culture in wheat with 3.61% *P. dioica*, where the mortality rate of insects was higher than that with 5.88% *P. dioica*, starting from the second day of testing, and reached from 21.67% to 56.67% (Figure 1 *e*).

While comparing mortality rates of insects in the control group (free of plant additives) with 1.23%, 3.61%, and 5.88% *P. dioica*, cultures, statistically significant differences were indicated. Significant differences were noted after 7, 14, and 21 days of testing between the control and the 5.88% *S. officinalis culture* ($p = 0.0120$, $p = 0.0019$, $p = 0.0016$). On the last two days of testing, significant differences were also shown between the mortality of the *R. dominica* in the control culture and the mortality in wheat

containing 3.61% of *S. officinalis* ($p = 0.0380$, $p = 0.0244$). The comparison of mortality rates of insects between cultures containing powdered plants at various concentrations provided 43 significant differences, where each compared pair included *P. dioica*. Among these differences, 15 were highly significant ($p < 0.001$) – Table 1.

S. officinalis, *M. piperita* and *P. dioica* caused a 100% reduction offspring of *R. dominica*. *A. absinthium* reduced the number of progeny of *R. dominica* compared to the control culture by 35, 37 and 40 and *A. sativum* respectively by 30, 35 and 39 (depending on the concentration used) – Table 2.

Table 2

The number of progeny individuals (mean) obtained after 50 days of removal of parents

Plants powder	Dose [%]			Control
	1.23	3.61	5.88	
S. officinalis	0	0	0	45
A. absinthium	10	8	5	
M. piperita	0	0	0	
A. sativum	15	10	6	
P. dioica	0	0	0	

Discussion

Natural plant substances have become increasingly important as prophylactic measures in the protection against the pests of stored products. Numerous reports on plants that constrain the development of grain storage pests can be found in the scientific literature. Besides oils, water and alcohol extracts, or their combinations (Tripathi et al. 2002, Mishra et al. 2006, Rozman et al. 2007, Derbalah and Ahmed 2011, Manzoor et al. 2011), plant powders have been studied in terms of their effects on storage pests, which can be insecticidal, deterrent and/or reductive to offspring (Sharaby 1989, Srinivasan et al. 2003, Koona and Njoya 2004, Kłyś 2004, Govindan and Nelson 2009, Wawrzyniak and Wrzesińska 2009, Chayengia et al. 2010, Kłyś 2011, Lu and Shi 2012).

The efficiency of plant powders affecting the *R. dominica* was studied, inter alia, by Kalinović and co-authors (Kalinović et al. 2002). They demonstrated that an effective and natural insecticide for this species is *Thymus vulgaris* L., which caused the complete extinction of the population on the 11th day of exposure when applied in powdered form at 7.5% concentration. Tests conducted by Kłyś (2013) did not confirm such a high activity of *T. vulgaris* used at lower (1%) concentration. It caused an 80%

mortality rate in the *R. dominica*, and only after 30 days of exposure. In the presented study, it was confirmed that the effectiveness of the applied herbs depended on the concentration used. The efficacy of the insecticidal effect of *S. officinalis*, *A. absinthium* and *A. sativum* increased in each time interval along with the increase of concentration.

ASHOURI and SHAYESTEH (2010) tested the effect of powdered *Capsicum annuum* L. fruit on the *Rhyzopertha dominica* and *Sitophilus granarius*. A 5% dosage of pepper fruit caused a mortality rate of approximately 60% in both species on the 14th day of testing. *Piper nigrum* L. seed powder applied at 0.5% concentration reduced the population of *S. granarius* to null as early as after five days, while the *R. dominica* was sensitive to *P. nigrum* to a lesser extent. The latter insect species showed a 100% mortality rate after 14 days *P. nigrum* of testing. In addition, *P. nigrum* significantly hindered the development of new generations.

In turn, KŁYŚ (2013) proved that powders from other plant species: *Chrysanthemum cinerariaefolium Vis.* and *Origanum majorana* L. had stronger insecticidal and deterrent effects on the *R. dominica* compared with *C. annuum* and *P. nigrum* used in the study by ASHOURI and SHAYESTEH (2010). *Ch. cinerariaefolium* (1%) caused a 100% mortality rate in the *R. dominica* already after one day of testing, and marjoram caused a 90% mortality rate during the first three months of experiments, which later reached 100%. Also at 0.5% concentration, *Ch. cinerariaefolium* inhibited reproduction in the *R. dominica* and caused near-total mortality. When used at lower concentrations (0.25%, 0.125%, and 0.06%), *Ch. cinerariaefolium* also hampered the reproduction and development of the *R. dominica* population. KŁYŚ (2004) also showed that *Salvia officinalis* L. and *Artemisia absinthium* L. exerted an antifeedant effect on *R. dominica*.

In the presented paper, a powder from *P. dioica* has been found to show strong insecticidal properties, causing a statistically significant mortality in *R. dominica* at the three concentrations used (1.23%, 3.61%, 5.88%) after only one day of experiments. However, no statistically significant effect of powders from *M. piperita*, *A. absinthium*, and *A. sativum* on the mortality of the *R. dominica* was noted, which applies to all tested time periods and all concentrations used. KALINOVIĆ and co-workers (2002) studied the influence of *Lavandula angustifolia* Mill. powder, used at a higher (7.5%) concentration than that of *Pimenta dioica* in this study, on the *R. dominica*. They found that the addition of *L. officinalis* caused a 100% mortality rate in the *R. dominica* after 11 days of exposure.

Conclusions

Out of five species of powdered plants: *S. officinalis*, *A. absinthium*, *M. piperita*, *A. sativum*, and *P. dioica*, used at three weight concentrations (1.23%, 3.61%, and 5.88%), *Pimenta dioica* caused the highest mortality of *R. dominica*. The mortality of *R. dominica* kept in wheat containing different plant species showed a time-dependent increase. The mortality of insects usually grew in accordance with the increase of herb concentrations. *S. officinalis*, *M. piperita* and *P. dioica* caused a 100% reduction offspring of *R. dominica*.

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