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The mortality of *Oryzaephilus surinamensis* Linnaeus, 1758 (Coleoptera: Silvanidae) induced by powdered plants

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Abstract: The aim of this study was to investigate whether powdered plants of different species namely: peppermint *Mentha piperita* (L.) (Lamiaceae), wormwood *Artemisia absinthium* (L.) (Asteraceae), common sage *Salvia officinalis* (L.) (Lamiaceae), allspice *Pimenta dioica* (Linnaeus et Merrill) (Myrtaceae) and common garlic *Allium sativum* (L.) (Amaryllidaceae), added to semolina using concentrations of 1.23, 3.61, and 5.88%, influence the mortality rate in the saw-toothed grain beetle *Oryzaephilus surinamensis* Linnaeus, 1758 (Coleoptera: Silvanidae). Experiments were conducted in a laboratory at 28°C and relative humidity 60±5%. At the concentration of 1.23%, allspice seeds caused the highest mortality amongst the saw-toothed grain beetle. When concentrations of 3.61 and 5.88% were used, sage, peppermint and wormwood caused the highest statistically significant mortality of *O. surinamensis*.

Key words: *Oryzaephilus surinamensis*, powdered plants, biopesticides, pesticides

Introduction

In the protection of plants and stored products, too little attention is paid to the use of plant preparations. Instead, research efforts are focused on synthetic chemical compounds (pesticides). There is a large amount of data on toxic effects on living things caused by pesticides which affect the behaviour and neurophysiology of arthropods. The toxic effects of pesticides can take place through inhalation, consumption, or absorption (Regnault-Roger 1997; Desneux *et al*. 2007). Fumigation is one of the most effective and fastest methods used to eliminate grain pests. To date, the fumigant most often applied has been phosphine, although its use has several drawbacks. It requires a long period of aeration, is flammable at high concentrations, and since it is toxic, it can lead to the deterioration of the quality of the protected product (Santos 2006). Furthermore, the frequent application of insecticides used either as fumigants or admixtures for products, can favour the emergence of resistance in many insect species e.g. *Oryzaephilus surinamensis* Linnaeus, 1758, *Rhyzopertha dominica* (Fabricius, 1792), and *Sitophilus oryzae* (Linnaeus, 1763). Resistence, then makes the control of these pests more difficult (Pereira *et al*. 1997; Athie and Mills 2005; Lorini *et al*. 2007). The unrestricted use of synthetic insecticides involves major risks to the environment and consumers because of the insecticide residues (Shaaya *et al*. 1991, 1997; Isman 2006; Ebadollahi *et al*. 2010).

Biopesticides have attracted ever increasing attention and interest among the proponents of a friendly, safe, and integrated environmental development. At present, bioinsecticides are successfully introduced in agriculture and horticulture to control, prevent or delay the development of pests. An additional advantage of biopesticides is the fact that they appear to be safe during accidental contacts with higher animals e.g. mammals (Copping *et al*. 2000). Botanical insecticides have long been recommended as alternatives to synthetic chemical insecticides. The reasons botanical insecticides have been recommended are: herbs cause little risk to human health, the cost of production is low, and processing and use by farmers and by small enterprises is easy. Also studied was the toxicity of fumigants extracted from essential oils obtained from a number of plants. The focus was on the fumigants' potential application against stored-product insects (Shaaya *et al*. 1997). Biopesticides in the form of essential oils, e.g. pyrethrum extracted from *Tanacetum cinerariaefolium* (Trevir.) Sch. Bip., or neem from *Azadirachta indica* A. Juss. are well known on the market (Belmain *et al*. 2001; Isman 2006).

So far, natural, plant insecticides have only been utilised to a minimum extent. All biological control agents constitute only ca. 2% of crop protection substances available on the world market. In comparison, in medicine, 25% of drugs are derived from plants. This signifies that herbs have a major potential for protecting stored products. Both powdered herbs and essential oils obtained from herbs, are used for this purpose (Shaaya *et al*. 1991, 1997; Regnault-Roger 1997; Lipa and Pruszyński 2010; Souquir *et al.* 2013). Currently, after the implementation of Regulation 1107/2009 and Directive 2000/29/EC on the safe use of pesticides in European Union member states, work undertaken on the use of natural pest prevention measures has gained significance. There is an increasingly extensive search for plant species showing insecticidal properties that could be applied in combating

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harmful insects occurring in warehouses that feed on the stored products. This harmful entomofauna includes the saw-toothed grain beetle *O. surinamensis* (Coleoptera: Silvanidae), which is the subject of this study. It is currently a very common pest, and one of the most frequently found pests of grain and cereal products in Poland. Compared with other pests of stored-products, this species does not feed very intensively. However, because it lives in the bottom layers of infested food products, it is particularly difficult to detect. The species is a cosmopolitan store pest and is found in various products and habitats, particularly in mills, fodder storages, and shops (Sinha and Watters 1985; Trematerra and Sciarretta 2004; Laszczak-Dawid *et al*. 2008). The saw-toothed grain beetle is a polyphagous species that feeds primarily on ground cereal products, but also dried fruit and seeds, nuts, yeasts, sugar, tobacco, dried meat, and less often on processed foodstuffs, such as bread and confectioneries. Adult insects can also damage entomological collections (Sandner 1990; Madkur *et al*. 2013). This species is marked by high fecundity, a short developmental period, and a major migration capacity. It can easily hide in various places in the storage facilities, thus hindering the effect of insecticides, several of which it has already proven resistant to. For these reasons, it is particularly difficult to fight this species. Early detection and knowledge of the way of life and habits of pest species found in stores are necessary components of the correct procedures for checks and the protection of grain products (Wallbank and Collins 2003; Trematerra and Sciarretta 2004; Laszczak-Dawid *et al*. 2008).

The purpose of this study was to investigate whether the plant powders of peppermint *Mentha piperita* (L.) (Lamiaceae), wormwood *Artemisia absinthium* (L.) (Asteraceae), common sage *Salvia officinalis* (L.) (Lamiaceae), allspice *Pimenta dioica* (Linnaeus) Merrill (Myrtaceae), and common garlic *Allium sativum* (L.) (Amaryllidaceae), used in different concentrations, influence the mortality rates of the saw-toothed grain beetle. The conducted tests were aimed at determining plant species that would show an insecticidal effect on *O. surinamensis*, and that could be applied in the development of programmes for the integrated protection of agricultural products stored in confined facilities. These plants will be used for the extraction of chemical compounds whose effect on the studied pest should be investigated.

Materials and Methods

Experiments were carried out in a laboratory in incubators at 28°C and relative humidity (RH) 60±5%. All cultures were held in plastic containers with a 28 cm^2 bottom and a perforated lid enabling air penetration. The food used in the control cultures was semolina (40 g), and in experimental cultures semolina with powder plant admixtures. Leaves of *M. piperita*, herbs of *A. absinthium* and *S. officinalis*, seeds of *P. dioica*, and bulbs of *A. sativum* were powdered with an electric grinder. Each powdered plant was added to culture containers with the following percentage weight concentrations: 1.23, 3.61, and 5.88%. Semolina was mixed thoroughly with the plant powder. All tests were started by placing 40 adult *O. surinamensis* beetles of equal age in the culture containers. Fifteen sample variants were tested. Each sample included six repetitions. Live and dead beetles were counted after 1, 2, 7, 14 and 21 days. Dead beetles were removed from the colonies and live ones were left *in situ*. The mortality rate was calculated as a percentage share of dead individuals relative to the total number of individuals in the population per controlled time unit (Kłyś 2013).

The Kruskal-Wallis non-parametric ANOVA test showing a statistical significance at $p < 0.05$ was used to determine the significance of differences in insect mortality between the control culture and the cultures containing each plant powder admixture. The post-hoc Dunn test was applied to ascertain which cultures containing different concentrations of powdered plants show statistical differences in the insect mortality when compared to the admixture-free control culture. Differences were deemed significant in the post-hoc test when the test probability value was lower than the adopted significance level α, equalling 0.05. Calculations were made using PQStat ver.1.4.2.324 software.

Results

After the first day of the experiments, none of the powdered plants used at the concentration of 1.23%, induced saw-toothed grain beetle mortality. These results were similar to those in the admixture-free control culture. After the second day of testing, wormwood, sage, and garlic did not induce beetle mortality. After the second day of testing, peppermint caused a 5% and allspice a 1% beetle mortality. In other testing periods, i.e. 7, 14, and 21 days, this lowest plant powder concentration caused the highest beetle mortality in the case of allspice (8–36%) and peppermint (6–10%). Mortality of the saw-toothed grain beetle after adding the remaining plant species to the semolina, was low, and did not exceed 3% (Figs. 1, 2, 3, 4, 5).

In semolina, admixtures of the studied plant species were used with a 3.61% concentration. Allspice, followed by common sage, and wormwood caused over a 4% mortality rate of the saw-toothed grain beetle after a 24 h exposure (Figs. 2, 3, 4). Neither peppermint nor common garlic affected this species of beetle (Figs. 1, 5). The plant that induced the highest mortality in the saw-toothed grain beetle, from the 7th day of testing onwards in all time periods, was common sage. The mortality rate in this case fluctuated within the range of 28–43% (Fig. 3). A slightly lower mortality was noted in the semolina with an admixture of powdered allspice. The weakest effect on the saw-toothed grain beetle mortality rate, among all the tested plants at the concentration of 3.61%, was produced by garlic (Fig. 5).

A pronounced increase in the saw-toothed grain beetle mortality rate occurred when the concentrations of powdered peppermint, wormwood, and sage were elevated to 5.88%. The highest mortality rate was then obtained in the semolina with sage, amounting to 55% after 7 days, and nearly 100% after 21 days. Wormwood and peppermint also caused considerable mortality in saw-toothed grain beetles from the second testing day

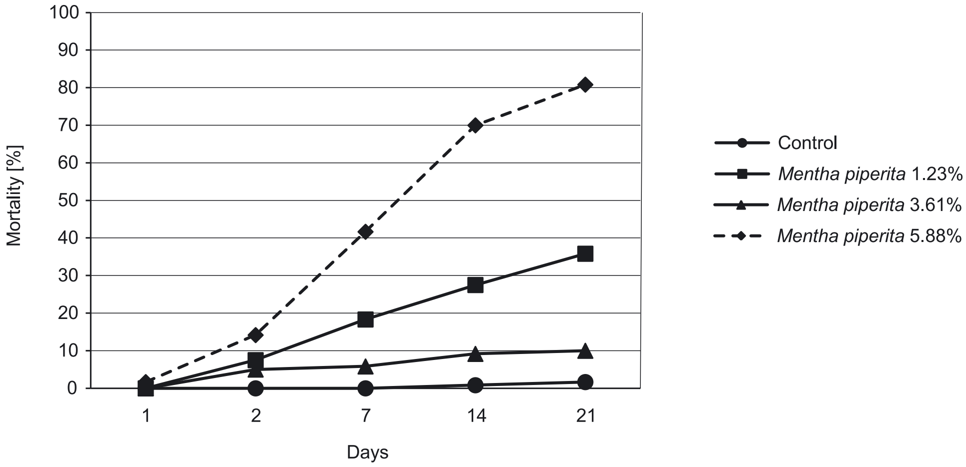

Fig. 1. Mortality of *Oryzaephilus surinamensis* caused by powdered *Mentha piperita*

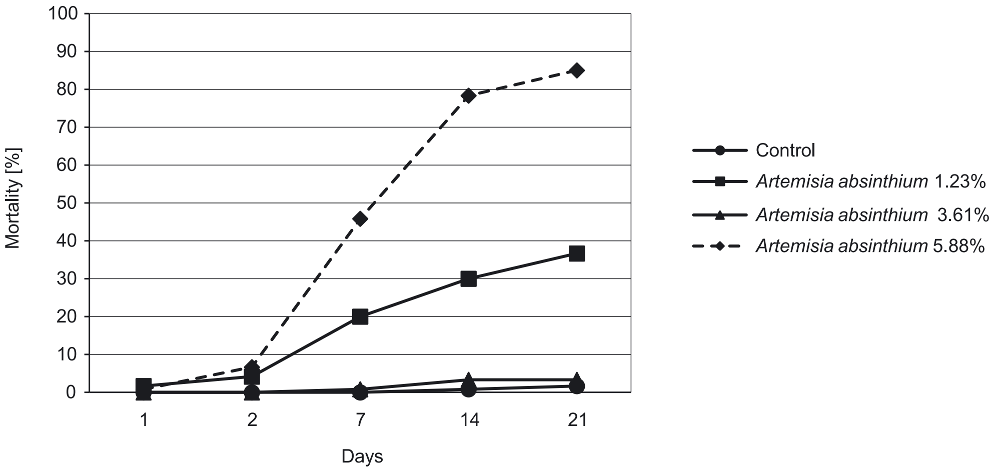

Fig. 2. Mortality of *Oryzaephilus surinamensis* caused by powdered *Artemisia absinthium*

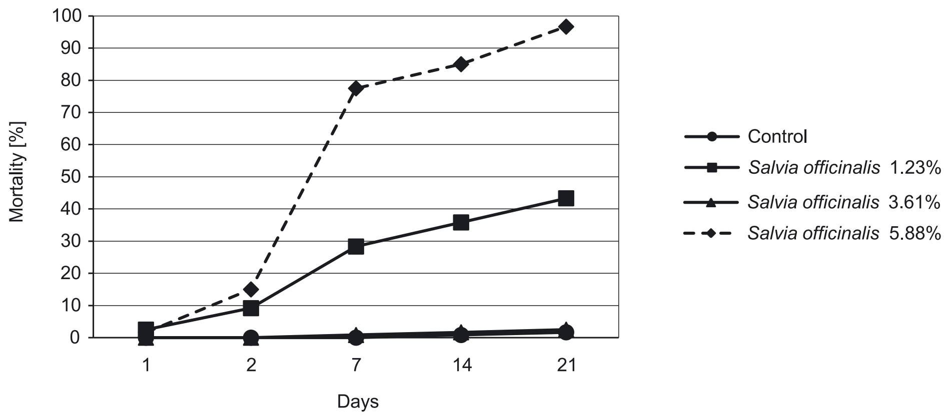

Fig. 3. Mortality of *Oryzaephilus surinamensis* caused by powdered *Salvia officinalis*

onwards, especially when used with the concentration of 5.88% (Figs. 1, 2, 3). The lowest mortality was, once again, caused by garlic.

Beetles in the control culture showed a 100% survival rate after 1, 2 and 7 testing days, with their mortality rate still registering below 1% after 14 and 21 days.

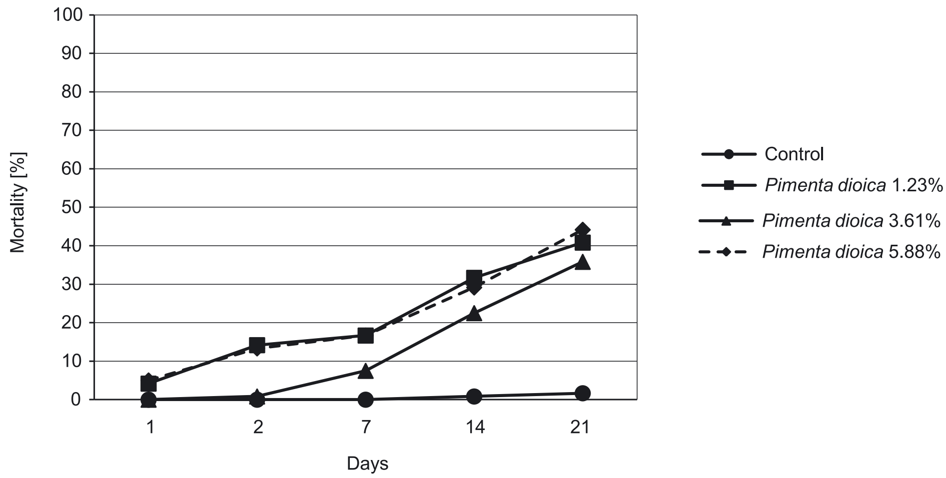

Fig. 4. Mortality of *Oryzaephilus surinamensis* caused by powdered *Pimenta dioica*

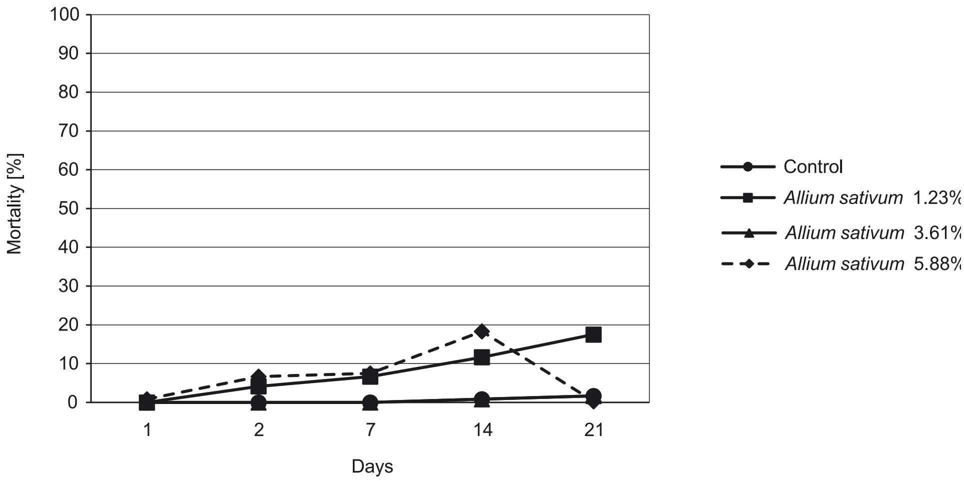

Fig. 5. Mortality of *Oryzaephilus surinamensis* caused by powdered *Allium sativum*

The Kruskal-Wallis test indicated no significant differences in the insect mortality after the first day of the experiments between the control culture and the cultures containing admixtures of particular plant species at different concentrations ($H = 60.37$, $p > 0.05$). However, in the subsequent four time intervals, i.e. after 2, 7, 14 and 21 days of the experiments, the Kruskal-Wallis test showed significant differences ($p < 0.0001$).

On the second day of the study, the post-hoc Dunn test revealed significant differences in the insect mortality rate between the control and the cultures with 5.88% peppermint ($p = 0.0190$), 5.88% sage ($p = 0.0053$), and allspice concentrations of 3.61% ($p = 0.0022$) and 5.88% ($p = 0.0037$). On the 7th day of the study, significant differences were noticed between the control group and cultures containing 5.88% peppermint ($p = 0.0019$), 5.88% wormwood ($p = 0.0004$), 3.61% sage ($p = 0.0083$), and 5.88% sage ($p < 0.0001$). On the 14th day of the experiments, the mortality of the insects was different between the control and the cultures containing 5.88% peppermint ($p = 0.0003$), 5.88% wormwood ($p = 0.0003$), 3.61% sage ($p = 0.0276$), and 5.88% sage ($p = 0.0001$). On the last day of the study, three significant differences in mortality were noted between the control group and the insect cultures containing 5.88% peppermint ($p = 0.0004$), 5.88% wormwood ($p = 0.0003$), and 5.88% sage ($p < 0.0001$) (Table 1). Significant differences in insect mortality between cultures with admixtures of various herb species, at different concentrations, are presented in table 1.

Discussion

Constant efforts are made to improve the methods of integrated protection of stored foodstuffs (integrated pest management – IPM), designed to ensure a high quality of stored products, while making sure the use of chemical agents is kept to a minimum. The integrated method primarily involves the use of natural factors for limiting the development of pests. Attention is also paid to the considerable potential of chemical substances found in plants. Research that seeks to discover plants and their ingredients with insecticidal, antifeedant, and/or repelling activity, have gained momentum over the last twenty years (Nawrot and Harmata 2012).

Table 1. Significant differences in the mortality of *Oryzaephilus surinamensis* caused by powdered plants of different species. Kruskal--Wallis test (H), followed by the Dunn post-hoc test (p*)

Days of research									
1		2		7		14		21	
plants [%]		plants [%]		plants [%]		plants [%]		plants [%]	
H = 60.37 p > 0.05	p*	H = 79.89 p < 0.0001	p*	H = 88.36 p < 0.0001	p*	H = 86.82 p < 0.0001	p*	H = 86.20 p < 0.0001	p*
the control–plant	ns	CTR–M 5.88	*	CTR–M 5.88	**	CTR–M 5.88	***	CTR–M 5.88	***
		CTR–S 5.88	**	CTR– Ar 5.88	***	CTR–Ar 5.88	***	CTR–Ar 5.88	***
		CTR–P 3.61	**	CTR–S 3.61	**	CTR–S 3.61	*	CTR–S 5.88	***
		CTR–P 5.88	**	CTR–S 5.88	***	CTR–S 5.88	***	M 1.23–M 5.88	*
plant–plant	ns	M 5.88–Ar 1.23	*	M 1.23–S 5.88	*	M 1.23–S 5.88	*	M 1.23–Ar 5.88	*
		M 5.88–S 1.23	*	M 5.88–Ar 1.23	**	M 5.88–Ar 1.23	**	M 1.23–S 5.88	**
		M 5.88–Al 1.23	*	M 5.88–S 3.61	**	M 5.88–S 1.23	***	M 5.88–Ar 1.23	*
		Ar 1.23–S 5.88	**	M 5.88–Al 1.23	**	M 5.88–Al 1.23	***	M 5.88–S 1.23	*
		Ar 1.23–P 3.61	**	Ar 1.23–Ar 5.88	**	Ar 1.23–Ar 5.88	**	M 5.88–S 5.88	**
		Ar 1.23–P 5.88	**	Ar 1.23– S 3.61	*	Ar 1.23–S 5.88	***	Ar 1.23–Ar 5.88	**
		S 1.23–S 5.88	**	Ar 1.23–S 5.88	**	Ar 5.88–S 1.23	***	Ar 1.23–S 5.88	***
		S 1.23–P 3.61	**	Ar 5.88–S 1.23	**	Ar 5.88–Al 1.23	***	Ar 5.88–S 1.23	***
		S 1.23–P 5.88	**	Ar 5.88–Al 1.23	***	S 1.23–S 5.88	**	Ar 5.88–Al 1.23	***
		S 5.88–P 1.23	*	S 1.23–S 3.61	*	S 3.61–Al 1.23	*	S 1.23–S 5.88	***
		S 5.88–Al 1.23	**	S 1.23–S 5.88	***	S 5.88–Al 1.23	***	S 5.88–Al 1.23	***
		P 1.23–P 3.61	*	S 3.61–Al 1.23	**	S 5.88–Al 3.61	*	S 5.88–Al 3.61	*
		P 1.23–P 5.88	*	S 5.88–Al 1.23	***				
		P 3.61–Al 1.23	**	S 5.88–Al 3.61	*				
		P 5.88–Al 1.23	**						

CTR – the control; M – *Mentha piperita;* S – *Salvia officinalis;* Ar – *Artemisia absinthium*; Al – *Allium sativum;* P – *Pimenta dioica*
significant differences: * 0.05 > p > 0.01; ** 0.01 > p > 0.001; *** p < 0.001
ns – not significant

The effect of powdered herbs, for example of *S. officinalis*, *A. absinthium*, lavender *Lavandula officinalis* Mill., and *M. piperita*, on the saw-toothed grain beetle, was studied by Kłyś (2006). She investigated the influence of these herbs at a 1.2% weight concentration on the said species of beetle, over a long term period of 250 days. This time period included several generations. She then noted the strong inhibitory effect of peppermint and wormwood on the development of a population of this pest. Lavender and common sage caused the highest mortality amongst saw-toothed grain beetle which was confirmed by a high mortality rate, reaching 80%. Peppermint caused the lowest level of mortality in the beetle after 40 days, and the highest after 160 days. In the presented paper, common sage applied at the same concentration but for the shorter period of 21 days, produced a very low 3% level of mortality in this beetle. It can therefore be inferred, that insecticidal properties of sage increase over time. Similarly, peppermint generated a very low 10% mortality rate.

Popoola (2013) also found in his studies, that insecticidal properties of plants strengthened over time. He tested the effectiveness of plant powders acting on the saw-toothed grain beetle feeding on date fruits *Phoenix dactylifera* (L.). He investigated the influence of garlic bulbs *A. sativum*, onion bulbs *A. cepa*, and dried red bell pepper fruit *Capsicum annum* (L.) for the control of *O. surinamensis*. The bioinsecticides were applied as powders and whole forms in three concentrations of 1.25, 2.50, and 5.00 g, for three and six weeks. The fecundity, emergence, and mortality rate of the saw-toothed grain beetle were studied. The application of whole red pepper as a boinsecticide was more potent than the powdered form. All the plants used, displayed insecticidal properties that increased in a time-dependent manner. In the mentioned study, the effect of garlic on the saw-toothed grain beetle was stronger than that of onion or pepper.

Powdered Monnier's snowparsley *Cnidium monnieri* (L.) Cusson 1782 also acted as an insecticide on the saw--toothed grain beetle. Application of a 6.4% concentration caused an 88% mortality rate after 3 days, in a population of the studied pest, and inhibited the development of the next generation by 100%. Powdered bark from the tree of heaven *Ailanthus altissima* (Mill.) showed a similar potency at this concentration level. Lesser galangal *Alpinia officinarum* (Hance), also known as Thai ginger, caused a 12% mortality rate in the saw-toothed grain beetle and reduced the development of a subsequent generation (Lü and Shi 2012).

Kłyś (2013) investigated the influence of the following powdered herbs: caraway *Carum carvi* (L.), herb Robert *Geranium robertianum* (L.), basil *Ocimum basilicum* (L.), dense mullein *Verbascum densiflorum* (Bert.), common thyme *Thymus vulgaris* (L.), marjoram *Origanum majorana* (L.), and Dalmatian pyrethrum *Chrysanthemum cinerariaefolium* (Vis.), on the population dynamics, mortality, and sex structure in the saw-toothed grain beetle, rice weevil, and lesser grain borer. In these tests, she determined the concentrations of particular herbs which allowed for examining their effects on several generations of the discussed insect over a long period of 8 months. Out of the seven species of herbs used at a 1% concentration, Dalmatian pyrethrum resulted in a 100% mortality rate in the saw-toothed grain beetle, followed by 50% for the herb Robert. Furthermore, herb Robert, common thyme, and marjoram, all used at a 1% concentration, significantly affected the mortality of the saw-toothed grain beetle females. Similar results were also obtained by the author when using 0.5% herb Robert. Dalmatian pyrethrum (1%; 0.5%) and herb Robert (1%) inhibited the reproduction of the saw-toothed grain beetle.

Adding powdered plants to the food not only increases the mortality among insects but affects the sex structure of their populations (Kłyś 2012).

In the studies by Al-Qahtani *et al.* (2012), all the plant powders used, namely ginger *Zingiber afficinale* (Roscoe), fennel *Foeniculum vulgare* (Miller), and green cardamom *Elettaria cardamomum* (L.), also displayed insecticidal activity against adult *O. surinamensis* beetles when added to flour. The recorded mortality of beetles in the colonies containing 4, 2, 0.5, 0.25, and 0.125% ginger was 66.6, 63.2, 61.4, 59.5, and 49.15%, respectively. The insect mortality in cardamom-treated colonies amounted to 68.4, 63.2, 50.9, 42.1, and 40.3%, respectively. With an admixture of fennel to flour, the lowest insect mortality was noted, namely: 63.2, 54.4, 47.4, 45.6, and 26.3%, for the respective concentrations. Ginger showed the strongest insecticidal potency, with the lowest lethal concentration level, LC_{50} = = 0.14 g · 100 g^{-1} of flour. It was followed by cardamom, which killed half of the insect population at the level of 0.4 g · 100 g^{-1} of flour. Fennel displayed the lowest lethal dose activity, LC_{50} = 0.7 g · l00 g^{-1} of flour.

Apart from plant powders, the effects of plant substances (e.g. in the form of oils, water, and alcohol extracts, and their combinations) on the saw-toothed grain beetle are being tested (Shaaya *et al.* 1991; Moreira *et al.* 2007; Shah *et al.* 2008; Ebadollahi *et al.* 2010; Benelli *et al.* 2012; Madkour *et al.* 2013; Padin *et al.* 2013; Najafabadi *et al.* 2014). The above-described studies indicate that herbs used in various forms may be potential insecticides/allies in the combat against storage pests. They prove effective when used in small amounts, work promptly, and unlike the wide range of traditionally used pesticides, they pose no threat to foodstuffs and to the environment.

Conclusions

1. Out of the five plant species: *M. piperita*, *A. absinthium*, *S. officinalis*, *P. dioica*, and *A. sativum*, used at the weight concentration of 1.23%, it was the allspice seeds which induced mortality in the saw-toothed grain beetle *O. surinamensis*. However, the highest statistically significant mortality rate in *O. surinamensis* of all the plants used at the concentrations of 3.61 and 5.88%, was caused by common sage, peppermint, and wormwood.
2. For common garlic, no significant effect on saw-toothed grain beetle mortality was found for any of the concentrations used, relative to the control culture.

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