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Source / Izvornik: **Journal of Stored Products Research, 2020, 86, 1 - 9**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.1016/j.jspr.2019.101565

Journal of Stored Products Research 86 (2020) 101565

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Evaluation of diatomaceous earth formulations enhanced with natural products against stored product insects

Z. Korunić [a], A. Liška [b, *], P. Lucić [b], D. Hamel [c], V. Rozman [b]

[a] Diatom Research and Consulting Inc., 14 Tidefall Dr., Toronto, ON, M1W 1J2, Canada
[b] Department for Phytomedicine, Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, 31000, Osijek, Croatia
[c] Croatian Agency for Agriculture and Food – Centre for Plant Protection, 10000, Zagreb, Croatia

ARTICLE INFO

Article history:
Received 11 November 2019
Received in revised form
17 December 2019
Accepted 25 December 2019
Available online 3 January 2020

Keywords:
Stored product insects
Natural substances
Diatomaceous earth
Essential oil
Pyrethrin

ABSTRACT

The insecticidal effect of prepared insecticide formulations labelled as Natural P, Inert Natural P and Py EC on *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* have been evaluated on wheat grains. Formulation Natural P contains diatomaceous earth (DE), amorphous silica gel (3%), pyrethrin, flax oil, lavandin essential oil (EO) and un-activated yeast. Formulation Inert Natural P contains DE, amorphous silica gel, lavandin EO and food grade bait whereas formulation Py EC contains pyrethrin, piperonyl butoxide (PBO), flax oil, polysorbate, methyl oleate and amorphous silica gel (5%). Celatom® MN-51 (diatomaceous earth – DE) was used as a standard insecticide. Inert Natural P and Natural P were applied as dust at four different doses and Py EC was applied as emulsions on grain by spraying. All three formulations showed higher insecticidal efficacy and higher progeny inhibition on all three tested insect species and had lower impact on wheat bulk density reduction compared with Celatom® MN-51. The LD_{50} and LD_{90} values of Inert Natural P were 48.7 and 163.7 ppm respectively for *S. oryzae*, 15.2 and 178.0 ppm for *R. dominica* and 115.2 and 171.3 ppm for *T. castaneum.* The LD_{50} and LD_{90} values of Natural P were 83.6 and 97.9 ppm respectively for *S. oryzae*, 19.5 and 97.9 ppm for *R. dominica* and 75.4 and 105.6 ppm for *T. castaneum.* Applied Py EC at concentration of 2.0 ppm a.i. pyrethrin exhibited 100% mortality after 2 d of *S. oryzae* and *T. castaneum* and after 6 d of *R. dominica.* In addition, all three formulations caused significant reduction of progeny (F1) population compared to control, providing promising approach of integrated pest management strategy.

1. Introduction

Diatomaceous earths (DEs) have been as viable alternatives to conventional pesticides for the control of stored product pests and have been extensively studied against wide range of species (Quarles, 1992; Korunic, 1998; Subramanyam and Roesli, 2000; Kavallieratos et al., 2007). Despite their main advantages such as low toxicity to mammals, stability (Maceljski and Korunic, 1972) and efficient insecticidal activity (Shah and Khan, 2014; Liska et al., 2015; Korunic et al., 2017), DEs cause some unwanted effects on grain related to adversely affect physical and mechanical properties of grain. Furthermore, the milling industry is reluctant to accept grain treated with DE because of its abrasive nature and possible damage to milling machinery (Losic and Korunic, 2018.).

A reduction of DE dosage could minimise the negative effect on grain, but in that case its efficacy becomes questionable. Namely, the recommended dosages that provide effective control against stored product insects are in the range from 0.5 to 1 kg t^{-1} of grain or above. To address these problems one of the main focus of intensive research is to develop new DE formulations that has enhanced activity against insects at lower dosage with no adverse effect on grain quality.

One of the solution could be combining DEs with other natural products that have insecticidal properties such as silica gels (Korunic and Fields, 1999) and botanicals (Athanassiou et al., 2009; Vayias and Stephou, 2009; Adarkwah et al., 2017). The use of amorphous silica gel effectively controls insects at significantly lower concentration in comparison with the concentrations of DE but significantly reduces bulk density and flowability of grain, as well (Korunic et al., 2017). So, the reduction of inert dusts amount of application on grain is crucial. Possible solution is mixing DE with botanicals that have different modes of action than DE. A key

* Corresponding author.
E-mail address: aliska@fazos.hr (A. Liška).

https://doi.org/10.1016/j.jspr.2019.101565

parameter of DE activity is s sorption of wax layer and abrasion of insect cuticule which consequently lead through desiccation and death of insect (Ebeling, 1971; Korunic et al., 2016). According to Ebeling (1971), death occurs when insect loose about 60% of the water content. So it takes a certain time until the insect mortality occurs, which determines DEs as slow acting insecticides. On the contrary botanicals, especially essential oils (EOs), have high toxic effect with fast penetration properties into insects which consequently interfere with biochemical and physiological functions (Lee et al., 2003; Mossa, 2016). Thus, lavender EO has repellent effect against insects and at higher concentrations is toxic to insects (Shaaya et al., 1997; Rozman et al., 2007; Germinara et al., 2017). The flower spike EO of lavender have good fumigant and contact toxicity against granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) (Germinara et al., 2017).

By combining DE and botanicals, a synergistic effects were expressed against stored product insects. Yang et al. (2010) noted that garlic (*Allium sativum* L.) EO mixed with inert dust was significantly more potent than individual treatment against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), assuming that synergistic effects could be a result of increased stress of adults due to the exposure to the oil-treated substrate which cause the cuticule more vulnerable to inert dust. Synergistic effect was also expressed in a mixture of DE and ajwain *Carum copticum* (L.) EO against *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae) and *S. granarius* (Ziaee et al., 2014). EOs accelerate insects' locomotor activity resulting in an increase in body contact with DE particles, resulting in more desiccation and death (Ziaee et al., 2019). Improved activity of DE was accomplished with the mixture of DE, silica gel, pyrethrin, piperonyl butoxide (PBO) and dill (*Anethum sowa and A. graveolens*) EO where tested formulation was more effective against stored product insects then DE alone (Korunic and Fields, 2018). Not only extracted EOs, but plant powder also can improve DE activity. Adarkwah et al. (2017a) found that combination of plant powders of *Piper guineense* (Schumacher and Thonning) and *Senna siamea* (Lam.) and DE controlled stored product insects faster than the plant powder alone.

Still, there is no practical solution of mixture of DE and botanicals that integrates both requirements; optimal insect control without comprising the grain quality. Therefore, the purpose of our research is to test the activity of three new formulations based on inert dusts diatomaceous earth and amorphous silica gel, enhanced with pyrethrin, lavandin essential oil, flax seed and food grade bait against *S. oryzae, R. dominica* and *T. castaneum* and to evaluate their impact on bulk density reduction.

2. Materials and methods

2.1. Description of the formulations

Formulation Natural P (powder) contains diatomaceous earth (DE, Celatom® MN-51) (82%), amorphous silica gel (Sipernat® 50 S) (3%), pyrethrin, flax oil, lavandin essential oil (EO) and un-activated yeast. Formulation Inert Natural P contains DE (Celatom® MN-51) (75%), food grade amorphous silica gel (Sipernat® 50 S) (20%), lavandin essential oil (EO) and un-activated yeast. The third formulation Py EC (emulsion concentrate) contains pyrethrin, piperonyl butoxide (PBO), flax oil, polysorbate 80, methyl oleate and amorphous silica gel - Sipernat® 50 S (5%). Pyrocide® 50% Pyrethrum Pale Extract (MGK Company, Minneapolis) was used for the preparation of formulation Py EC and it was diluted in solvent and emulsifier. Natural P and Inert Natural P formulations have been prepared as powder and Py EC as a concentrated emulsion. These formulations contain natural and food grade products and they belong with the categories of organic and food grade insecticides. Natural DE Celatom® MN-51 was used as a standard insecticide. It is registered as an insecticide for stored-products protection and belongs to a group of DE with medium to high efficacy against stored-products insects (73.6% amorphous silica content).

In order to attract insects with the intention of increasing the effect of DE within the formulations a food grade bait was used in a formulations composition. It consists of a powder inactive whole cell yeast, a specially grown primary torula yeast, without artificial flavours added (Lallemand Bio-Ingredients, USA).

Amorphous silicon dioxide Sipernat® 50 S, used in formulations development, is a «Generally Recognized as Safe» (GRAS) as a food additive (21 CRF 182.90 and 182.1711). It is effective against different urban pests and significantly increases the effectiveness of DE against stored grain insect pests due to its significant desiccant properties (Korunic and Fields, 1999).

Piperonyl butoxide within the Py EC composition was added as a pyrethrin synergist to prevent the insect recovery after the treatment (Ware and Whitacre, 2004).

2.2. Test insects

The insecticidal effects of the formulations have been evaluated on three important stored-grain species, two internal feeders, *S. oryzae* and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and one external feeder, *T. castaneum*.

Test insects have been reared in the laboratory at 28 ± 1 °C and 65 ± 5% r.h. on whole soft white wheat (for *S. oryzae*) and on mixture of wheat flour plus 5% brewer's yeast (by weight) (for *T. castaneum*). All adults used in the test treatments were 7–21 d-old. Twenty five unsexed adults per replicate were used in the experiments.

2.3. Commodity

Untreated Canadian Eastern Soft Red wheat clean, with low dockage and infestation free, with the initial moisture content of 13.5% acclimated for 7 d at 28 ± 1 °C and 65 ± 5% r.h. was used in all treatments. Moisture content was measured using Labtronics Moisture Meter model 919® (Labtronics, Winnipeg, Manitoba, CA).

2.4. Bioassay description

The formulations Inert Natural P, Natural P and Celatom® MN-51 were applied as dusts on wheat grain in different range of doses (Inert Natural P and Celatom® MN-51 at 100, 200, 300 and 400 ppm and Natural P at 50, 75, 100 and 125 ppm). The formulation Py EC was diluted with water into four concentrations containing 0.5, 1.0, 1.5 and 2.0 ppm of pyrethrin a.i. and applied on wheat grain by spraying.

A glass jars of 500 mL volume were filled with 100 g of clean whole wheat grain (with the addition of 1% (by weight) cracked wheat for the *T. castaneum*) and determined dosages of tested formulations and Celatom® MN-51 were added. The jars were tightly closed with the lids and thoroughly shaken by hands for 30 s in order to have equally distribution of added formulations through the kernels. Jars with untreated grain served as control. After dust was settled down, 25 unsexed, 7–21 days old adults of *S. oryzae, R. dominica* and *T. castaneum* were added into each jar.

Adults' mortality and progeny reduction were evaluated. For *S. oryzae* and *R. dominica*, adults' mortality was evaluated after 2, 4, 6 d and for *T. castaneum* after 4, 6 d and 8 d of exposure. After the assessment of adult mortality, all adults were removed from the treated wheat and remained for 7 weeks and 9 weeks (for *S. oryzae* and for *R. dominica* and *T. castaneum,* respectively) in order to evaluate the emerged progeny. All treatments were replicated four times for each dose of the same formulation, separately for each insect species. The same procedure was followed for the untreated wheat which served as control. Environmental conditions during the bioassay were set at 28 ± 1 °C and 60 ± 5% r.h.

The effect of applied dosages on a bulk density was measured for each formulation and for Celatom® MN-51. Bulk density of wheat treated with formulation Py EC was measured immediately after spraying and after drying of sprayed grains during 4 h at 28 ± 1 °C and 60 ± 5% r.h. The measurement of bulk density was repeated three times for each dose of each formulation and Celatom® MN-51. The bulk density was measured using the Ohaus apparatus (Ohaus 0.5-L measure) following the procedure according to the Canadian Grain Commission (2019).

2.5. Data analysis

Mortality data of exposed and control adult insect species, progeny population and bulk density data were processed by SAS v9.3 (SAS/STAT Software 9.3 2013–2014). One-way analysis of variance of the tested variables was subjected in SAS Analyst module and a procedure ANOVA was used. Tukey's HSD ($P < 0.05$) test was used to detect differences between means of examined traits. Data obtained from each dose response bioassay were subjected to probit analysis and LD_{50} and LD_{90} values and their 95% confidence intervals were estimated using IBM SPSS Statistics (IBM Corp. Released, 2013).

3. Results

3.1. Efficacy of tested formulations against adults

Tested formulations showed insecticidal effect on tested insect species already at the beginning of the observed period and applied at the lowest dose. That was particularly expressed on *S. oryzae* where at the lowest dose all three tested formulations exhibit complete control (100% mortality) after 4 d of exposure (Table 1). Further, tested formulations achieved complete control of *R. dominica* (Table 2) and *T. castaneum* (Table 3). The exception was formulation Natural P that reached the highest mortality of 95% against *T. castaneum* at 100 ppm after 8 d of exposure. On the contrary, Celatom® MN-51 even at the highest dose and after the longest exposure time did not achieve 100% mortality of the tested insect's species.

According to the estimated lethal doses (LD_{90}) formulation Natural P was the most effective against *S. oryzae, R. dominica* and *T. castaneum* with LD_{90} 97.9 ppm, 97.9 ppm and 105.6 ppm, respectively, followed by the Inert Natural P with LD_{90} 163.7 ppm, 178.0 ppm and 171.3 ppm, respectively, while Celatom® MN-51 was at least effective with LD_{90} 352.8 ppm, 405.4 ppm and 716.8 ppm, respectively (Table 4).

3.2. Efficacy of tested formulations on progeny population

All three tested formulations successfully inhibited progeny population (Table 5) of the treated adult parents and inhibition was significant even with the lowest dose, compared to the progeny population of untreated adults. The highest reached progeny inhibition of *S. oryzae, R. dominica* and *T. castaneum* was 99.7% at 400 ppm, 97.8% at 300 ppm and 100% at 400 ppm, respectively with Inert Natural P whereas 94.8% at 125 ppm and 100% at 50 and 75 ppm, respectively with Natural P and 100% with Py EC of all three tested insect species at 0.5 and 1.0 ppm of pyrethrin.

3.3. Influence on bulk density

According to the difference in values of bulk density between untreated and treated grains (Table 6) all tested formulations (Inert Natural P, Natural P and Py EC) had a significant effect on bulk density reduction. Although comparing the bulk density of wheat threated with the highest doses, tested formulations had significantly ($df = 4$; F = 100.04; $P < 0.0001$) lower impact on bulk density reduction than Celatom® MN-51, with the exception of formulation Inert natural P (Fig. 1).

4. Discussion

Among dust formulations, Natural P was more effective than Inert Natural P against all three tested insect species. Since those two formulations differ in the composition of active ingredients, we assumed that the enhanced activity of formulation Natural P was accomplished due to flax oil and pyrethrin in its composition. It is well known for the knockdown effect of pyrethrins and hyperactivity and convulsions in most insects, as a result of neurotoxic action similar to that of many synthetic organochlorine pesticides (El-Wakil, 2018). In addition, vegetable oils have both ovicidal and larvicidal effect (Credland, 1992), which could explain the complete progeny inhibition of *T. castaneum* in our investigation even though the mortality of parents wasn't 100%. In order to evaluate the exact mode of action and a possible synergistic effect, a further study should be done with the flax oil and pyrethrin alone.

Besides the addition of botanical components, prepared formulations consist also of food grade bait in order to ensure the better grain consumption by insects. While insects are not highly attracted to the base material, especially when it is treated with insecticidal material (Barbara and Capinera, 2008). Better grain consumption consequently leads to a higher intake of insecticidal material into insects' digestive tract thus enabling internal desiccation by DEs particles, for which is known that abrasive properties could also damage the insects' gut (Jackson and Webley, 1994; Losic and Korunic, 2018). Presumably, food grade bait within the composition of Inert natural P and Natural P formulations attracted insects which kept them in the contact with dust formulations for longer period or induced higher grain consumption which led to higher mortality of adult insects.

In our previous study (Liska et al., 2018), two grain protectant formulations (F1H and F2H), based on inert dust originated from Croatia, laurel leaves, lavender EO, corn oil, silica gel and pyrethrin, almost completely suppressed *R. dominica* population in wheat and corn after 6 months period of storage. In addition, formulation F2H, with pyrethrin in its composition, was slightly more effective than the formulation F1H, without pyrethrin. The potential additive effect was also reported by Adarkwah et al. (2017) combining some botanical food by-products and DE against stored grain beetles where the mixture had a faster activity than tested botanical powders alone. Khorrami et al. (2018) investigated synergistic and antagonistic interactions in combination of ethanolic extracts of five medicinal plants and DE against adults of *T. castaneum* demonstrated that essential oils and ethanolic extracts synergized the performance of DE. Addition of, not only the whole essential oils, but isolated monoterpenoids itself also improved efficacy of DE against stored product pests. Thus, eugenol and cinnamaldehyde enhanced efficacy of DEs Protect-It™ and SilicoSec® against *S. oryzae* and *Callosobruchus maculatus* (Fab.) (Islam et al., 2010). Pierattini et al. (2019) observed synergistic effect on the toxicity of DE and EOs, where particular that effect was very distinct for *Foeniculum vulgare* (Mill.) EO that showed the lowest efficacy alone, but applied together with DE, the toxicity increased about three times. According to Athanassiou and Arthur (2018), in mixture,

Table 1
Mean (% ± SD) mortality of *Sitophilus oryzae* adults after 2, 4 and 6 d of exposure to treated wheat grains with tested formulations and Celatom® MN-51.

Treatment	Dose (ppm)	Mean (% ± SD [a]) mortality		
		2 d of exposure	4 d of exposure	6 d of exposure
Inert natural P	0	4.0 ± 5.6 d	4.0 ± 5.6 b	6.0 ± 6.9 b
	100	43.0 ± 6.8 c	100.0 ± 0.0 a	100.0 ± 0.0 a
	200	82.0 ± 8.9 b	100.0 ± 0.0 a	100.0 ± 0.0 a
	300	92.0 ± 7.0 ab	100.0 ± 0.0 a	100.0 ± 0.0 a
	400	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	F	153.83	1152.00	736.33
	P	<.0001	<.0001	<.0001
Natural P	0	4.0 ± 5.6 e	4.0 ± 5.6 b	6.0 ± 6.9 b
	50	47.0 ± 8.2 d	100.0 ± 0.0 a	100.0 ± 0.0 a
	75	74.0 ± 5.2 c	100.0 ± 0.0 a	100.0 ± 0.0 a
	100	87.0 ± 5.0 b	100.0 ± 0.0 a	100.0 ± 0.0 a
	125	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	F	190.83	1152.00	736.33
	P	<.0001	<.0001	<.0001
Py EC	0	4.0 ± 5.6 c	4.0 ± 5.6 b	6.0 ± 6.9 b
	0.5 Py	80.0 ± 5.6 b	100.0 ± 0.0 a	100.0 ± 0.0 a
	1.0 Py	94.0 ± 5.2 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	1.5 Py	98.0 ± 2.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	2.0 Py	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	F	342.75	1152.00	736.33
	P	<.0001	<.0001	<.0001
Celatom® MN-51	0	4.0 ± 5.6 c	4.0 ± 5.6 e	6.0 ± 6.9 e
	100	13.0 ± 6.8 bc	21.0 ± 6.8 d	23.0 ± 5.0 d
	200	24.0 ± 3.3 b	45.0 ± 6.8 c	58.0 ± 8.3 c
	300	44.0 ± 10.3 a	65.0 ± 8.9 b	77.0 ± 6.8 b
	400	55.0 ± 6.8 a	82.0 ± 8.3 a	96.0 ± 3.3 a
	F	37.13	73.41	138.85
	P	<.0001	<.0001	<.0001

[a] Means in the same column within each treatment followed by the same letters are not significantly different (for all treatments $df = 4$; Tukey's HSD, $P = 0.05$).

Table 2
Mean (% ± SD) mortality of *Rhyzopertha dominica* adults after 2, 4 and 6 d of exposure to treated wheat grains with tested formulations and Celatom® MN-51.

Treatment	Dose (ppm)	Mean (% ± SD [a]) mortality		
		2 d of exposure	4 d of exposure	6 d of exposure
Inert natural P	0	3.0 ± 3.8 c	8.0 ± 3.3 d	19.0 ± 6.8 d
	100	11.0 ± 6.8 c	63.0 ± 7.6 c	62.0 ± 5.2 c
	200	39.0 ± 11.9 b	71.0 ± 5.0 bc	75.0 ± 3.8 b
	300	41.0 ± 8.2 b	78.0 ± 8.3 b	90.0 ± 6.9 a
	400	91.0 ± 5.0 a	98.0 ± 2.3 a	100.0 ± 0.0 a
	F	80.18	135.04	146.57
	P	<.0001	<.0001	<.0001
Natural P	0	3.0 ± 3.8 d	8.0 ± 3.3 c	19.0 ± 6.8 b
	50	74.0 ± 8.3 ab	88.0 ± 7.3 b	100.0 ± 0.0 a
	75	61.0 ± 5.0 b	98.0 ± 4.0 a	100.0 ± 0.0 a
	100	80.0 ± 10.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	125	81.0 ± 6.8 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	F	87.81	397.80	562.37
	P	<.0001	<.0001	<.0001
Py EC	0	3.0 ± 3.8 c	8.0 ± 3.3 c	19.0 ± 6.8 b
	0.5 Py	55.0 ± 7.6 a	70.0 ± 10.6 b	93.0 ± 6.0 a
	1.0 Py	41.0 ± 3.8 b	85.0 ± 6.8 a	100.0 ± 0.0 a
	1.5 Py	47.0 ± 6.8 ab	92.0 ± 3.3 a	100.0 ± 0.0 a
	2.0 Py	58.0 ± 8.3 a	91.0 ± 6.8 a	100.0 ± 0.0 a
	F	48.47	110.09	306.12
	P	<.0001	<.0001	<.0001
Celatom® MN-51	0	3.0 ± 3.8 d	8.0 ± 3.3 d	19.0 ± 6.8 c
	100	31.0 ± 3.8 c	37.0 ± 3.8 c	65.0 ± 10.0 b
	200	49.0 ± 3.8 b	52.0 ± 5.2 b	60.0 ± 5.6 b
	300	67.0 ± 5.0 a	80.0 ± 3.3 a	85.0 ± 5.0 a
	400	73.0 ± 10.0 a	88.0 ± 3.3 a	91.0 ± 3.8 a
	F	95.76	291.60	73.45
	P	<.0001	<.0001	<.0001

[a] Means in the same column within each treatment followed by the same letters are not significantly different (for all treatments $df = 4$; Tukey's HSD, $P = 0.05$).

Table 3
Mean (% ± SD) mortality of *Tribolium castaneum* adults after 4, 6 and 8 d of exposure to treated wheat grains with tested formulations and Celatom® MN-51.

Treatment	Dose (ppm)	Mean (% ± SD [a]) mortality		
		4 d of exposure	6 d of exposure	8 d of exposure
Inert natural P	0	1.0 ± 2.0 c	1.0 ± 2.0 d	2.0 ± 2.3 c
	100	2.0 ± 2.3 c	2.0 ± 2.3 d	6.0 ± 2.3 c
	200	1.0 ± 2.0 c	28.0 ± 5.6 c	38.0 ± 10.6 b
	300	77.0 ± 8.2 b	90.0 ± 7.0 b	97.0 ± 2.0 a
	400	99.0 ± 3.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	F	782.89	510.49	358.55
	P	<.0001	<.0001	<.0001
Natural P	0	1.0 ± 2.0 c	1.0 ± 2.0 c	2.0 ± 2.3 d
	50	1.0 ± 2.0 c	7.0 ± 3.8 c	15.0 ± 3.8 c
	75	20.0 ± 3.3 b	28.0 ± 3.3 b	42.0 ± 8.3 b
	100	68.0 ± 7.3 a	83.0 ± 6.8 a	95.0 ± 3.8 a
	125	68.0 ± 3.3 a	82.0 ± 8.3 a	95.0 ± 3.8 a
	F	281.69	219.04	322.03
	P	<.0001	<.0001	<.0001
Py EC	0	1.0 ± 2.0 c	1.0 ± 2.0 d	2.0 ± 2.3 d
	0.5 Py	5.0 ± 2.0 c	7.0 ± 3.8 d	16.0 ± 3.3 c
	1.0 Py	22.0 ± 8.3 b	44.0 ± 8.6 c	80.0 ± 8.6 b
	1.5 Py	33.0 ± 11.0 b	60.0 ± 3.3 b	96.0 ± 3.3 a
	2.0 Py	64.0 ± 8.6 a	86.0 ± 6.9 a	100.0 ± 0.0 a
	F	47.01	165.16	253.11
	P	<.0001	<.0001	<.0001
Celatom® MN-51	0	1.0 ± 2.0 c	1.0 ± 2.0 c	2.0 ± 2.3 c
	100	14.0 ± 2.3 b	15.0 ± 3.8 b	19.0 ± 3.8 b
	200	16.0 ± 7.3 b	16.0 ± 3.3 b	30.0 ± 5.2 b
	300	20.0 ± 6.5 ab	20.0 ± 3.3 b	35.0 ± 13.2 b
	400	28.0 ± 6.5 a	34.0 ± 7.6 a	60.0 ± 8.6 a
	F	13.14	38.32	30.86
	P	<.0001	<.0001	<.0001

[a] Means in the same column within each treatment followed by the same letters are not significantly different (for all treatments $df = 4$; Tukey's HSD, $P = 0.05$).

Table 4
Lethal doses LD_{50} and LD_{90} of tested formulations and Celatom® MN-51 toward adults of three tested insect species.

Treatment	95% Confidence Limits of doses for formulations and Celatom® MN-51 Estimate LD_{50}[a]/LD_{90}[a]	Lower Bound for LD_{50}/LD_{90}	Upper Bound for LD_{50}/LD_{90}	Chi-square tests[b]
	Sitophilus oryzae			
Inert natural P	48.7/163.7	9.8/141.6	72.1/218.7	Chi-sq = 25.12 *df* = 14 *P* = 0.033
Natural P	83.6/97.9	45.1/91.2	59.7/107.3	Chi-sq = 9.42 *df* = 14 *P* = 0.803
Py EC	-/ 0.8	-/ 0.6	-/ 1.0	Chi-sq = 8.13 *df* = 14 *P* = 0.882
Celatom® MN-51	188.5/352.8	167.6/325.9	207.0/389.1	Chi-sq = 9.09 df = 14 *P* = 0.825
Rhyzopertha dominica				
Inert natural P	15.2/178.0	−29.70/154.0	22.96/214.9	Chi-sq = 9.17 *df* = 14 *P* = 0.820
Natural P	19.5/97.9	−34.6/91.2	34.4/107.3	Chi-sq = 11.06 *df* = 14 *P* = 0.681
Py EC	-/ 1.5	-/ 1.3	-/ 2.1	Chi-sq = 16.58 *df* = 14 *P* = 0.279
Celatom® MN-51	39.9/405.4	−78.1/346.1	100.5/520.9	Chi-sq = 15.59 *df* = 14 *P* = 0.339
Tribolium castaneum				
Inert natural P	115.2/171.3	106.6/157.8	124.6/189.6	Chi-sq = 5.69 *df* = 14 *P* = 0.974
Natural P	75.4/105.6	69.4/98.3	80.9/116.2	Chi-sq = 26.10 *df* = 14 *P* = 0.025
Py EC	-/ 1.2	-/ 1.1	-/ 1.3	Chi-sq = 14.11 *df* = 14 *P* = 0.441
Celatom® MN-51	358.1/716.8	317.3/599.1	422.6/947.1	Chi-sq = 12.13 *df* = 14 *P* = 0.596

[a] LD values are expressed as parts per million (ppm).
[b] Pearson Goodness of-Fit Test (Probit).

Table 5
Progeny (F1) population of three tested insect species after parent exposure to wheat treated with tested formulations and Celatom® MN-51.

Treatment	Dose (ppm)	*S. oryzae*		*R. dominica*		*T. castaneum*	
		Number of adults Mean±SD[a]	Inhibition (%)	Number of adults Mean±SD[a]	Inhibition (%)	Number of adults Mean±SD[a]	Inhibition (%)
Inert natural P	0	402.7 ± 40.3a	-	46.5 ± 13.4 a	-	59.5 ± 6.3 a	-
	100	117.5 ± 11.7 b	70.8	11.2 ± 4.6 b	75.8	10.7 ± 3.1 b	82.0
	200	83.2 ± 8.9 b	79.3	9.2 ± 5.2 b	80.1	4.5 ± 1.3 bc	92.4
	300	2.5 ± 2.1 c	99.4	1.0 ± 0.8 b	97.8	3.0 ± 3.6 bc	95.0
	400	1.2 ± 0.9 c	99.7	0.2 ± 0.5 b	95.5	0.0 ± 0.0 c	100
	F	295.34		31.51		192.69	
	P	˂.0001		˂.0001		˂.0001	
Natural P	0	402.7 ± 40.3 a	-	46.5 ± 13.4a	-	59.5 ± 6.3 a	-
	50	93.2 ± 35.2 b	76.8	0.0 ± 0.0 b	100	0.7 ± 0.9 b	98.7
	75	45.7 ± 27.7 bc	88.6	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100
	100	32.0 ± 6.2 c	92.1	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100
	125	21.0 ± 13.7 c	94.8	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100
	F	134.46		48.32		341.19	
	P	˂.0001		˂.0001		˂.0001	
Py EC	0	402.7 ± 40.3a	-	46.5 ± 13.4a	-	59.5 ± 6.3 a	-
	0.5 Py	0.7 ± 0.9 b	99.8	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100
	1.0 Py	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100
	1.5 Py	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100
	2.0 Py	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100	0.0 ± 0.0 b	100
	F	398.38		48.32		351.10	
	P	˂.0001		˂.0001		˂.0001	
Celatom® MN-51	0	402.7 ± 40.3 a	-	46.5 ± 13.4 a	-	59.5 ± 6.3 a	-
	100	130.2 ± 20.1 b	67.7	21.5 ± 7.4 b	53.8	58.5 ± 21.5 a	1.0
	200	91.0 ± 16.8 bc	77.4	5.0 ± 1.6 c	89.2	9.7 ± 3.3 b	83.6
	300	78.2 ± 5.7 c	80.6	4.2 ± 0.9 c	90.9	11.2 ± 4.3 b	81.1
	400	62.2 ± 3.8 c	84.5	1.7 ± 2.4 c	96.2	2.0 ± 0.8 b	96.6
	F	170.56		29.25		30.16	
	P	˂.0001		˂.0001		˂.0001	

[a] Means in the same column within each treatment followed by the same letters are not significantly different (for all treatments $df = 16$; Tukey's HSD, $P = 0.05$).

Table 6
Bulk density (kg hL^{-1}) of wheat treated with tested formulations and Celatom® MN-51 and bulk density differences in regard to bulk density of untreated wheat.

Treatment	Dose (ppm)	Wheat bulk density[a] (kg hL^{-1} ± *SD*)	Bulk density difference (kg hL^{-1})
Inert natural P	0	81.3 ± 0.3 a	-
	100	78.3 ± 0.05 b	−3.0
	200	77.2 ± 0.1 c	−4.1
	300	75.6 ± 0.2 d	−5.7
	400	74.6 ± 0.6 e	−6.7
	$df = 4$; F = 199.89; *P* ˂.0001		
Natural P	0	81.3 ± 0.3 a	-
	50	78.7 ± 0.1 b	−2.6
	75	77.9 ± 0.01 c	−3.4
	100	76.7 ± 0.04 d	−4.6
	125	75.9 ± 0.03 e	−5.4
	df=4; F = 783.73; *P* ˂.0001		
Py EC	0	81.3 ± 0.3 a	-
	after treatment[b]	79.9 ± 0.1 b	−1.4
	after drying[b]	80.3 ± 0.2 b	−1.0
	df=2; F = 44.74; *P* = 0.0002		
Celatom® MN-51	0	81.3 ± 0.3 a	-
	100	77.6 ± 0.2 b	−3.7
	200	76.7 ± 0.05 bc	−4.6
	300	75.8 ± 0.1 c	−5.5
	400	74.0 ± 0.5 d	−7.3
	$df = 4$; F = 103.36; *P* ˂.0001		

[a] Means in the same column within each treatment followed by the same letters are not significantly different (Tukey's HSD, $P = 0.05$).
[b] Bulk density measured immediately after spraying; **Bulk density measured after drying of sprayed grains.

inert dusts absorb EOs thus enabling their rapid breakdown which leads to prolongation of insecticidal activity of oils, and at the same time, accelerate the mortality caused by DE. In line with that, our results showed that at the same applied dose (400 ppm) formulations Inert natural P and Natural P had about two times stronger insect activity after only 2 d than DE alone. That was particularly expressed against *S. oryzae* and *T. castaneum.* Stronger and faster mortality induced by two formulations consequently led

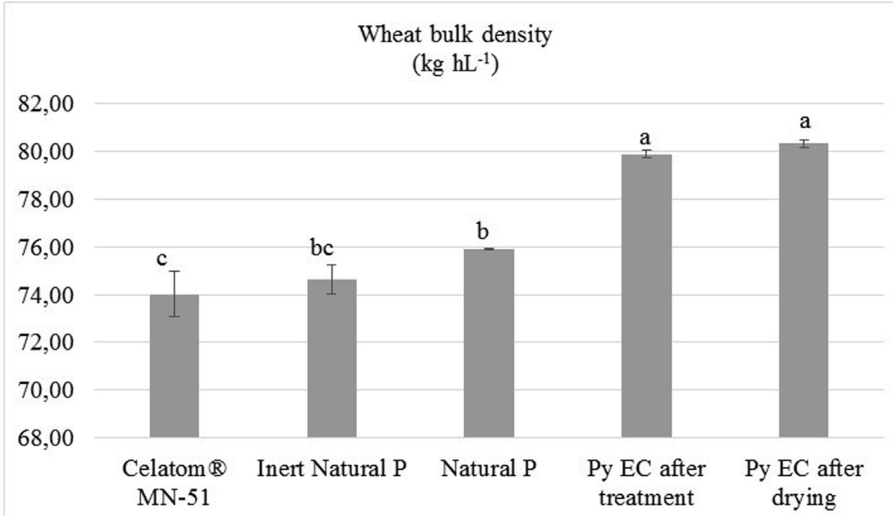

Fig. 1. Bulk density mean values (±SD) (kg hL^{-1}) of wheat treated with the highest doses of tested formulations (Inert Natural P 400 ppm; Natural P 125 ppm and Py EC 2.0 ppm) and Celatom® MN-51 (400 ppm). Means in the column followed by the same letters are not significantly different (Tukey's HSD, *P* = 0.05).

to higher inhibition of progeny population in regard to DE alone.

Athanassiou et al. (2004) and Athanassiou and Kavallieratos (2005) by using PyriSec formulation that combined DE with pyrethrin against adults of *S. oryzae*, *R. dominica* and the confused flour beetle, *T. confusum* found that, for a satisfactory level of mortality, the dose rate should be > 500 ppm. However, current study revealed that according to the generated LD_{90} doses of the tested dust formulations (Inert Natural P and Natural P), effective doses were below 200 ppm against all three tested species.

Previous studies with several DE formulations indicated that adults of *Tribolium* spp. are among the most DE-tolerant species and *S. oryzae* and *R. dominica* are considered more susceptible than *Tribolium* spp. (Arthur, 2000; Fields and Korunic, 2000; Athanassiou et al., 2003, 2004, Athanassiou and Kavallieratos (2005)). Our results also indicate that the *T. castaneum* is more tolerant to Celatom® MN-51 and to all three tested formulations than *S. oryzae* and *R. dominica.* Differences in sensitivity between insect species to DE could be explained by several factors, differences in epicuticule (thickness, hairy or flat structure, rugosity, thickness of wax layer), the size and shape of insects, mobility through the grain mass, ability of water loss recover (Ebeling, 1971; Rigaux et al., 2001; Losic and Korunic, 2018). Since *T. castaneum* has very flat skin surface and is less agile species (Rigaux et al., 2001) that could be the one of reasons for lower mortality in our treatments.

Bulk density reduction is one of the significant negative effects of DE usage on grain. Additionally, more effective inert dusts at lower doses equally reduce bulk density as less effective inert dusts at much higher doses (Liska et al., 2017). It is a great challenge to exceed that demerit of DE in grain treatments. Comparing the bulk density values of wheat treated with Celatom® MN-51, all three tested formulations had lower impact on bulk density reduction although the bulk density was still significantly reduced comparing with the bulk density value of untreated wheat. It could be considered that this effect by the use of DEs is unavoidable. Even with the small doses from 10 to 50 ppm of DE, the bulk density reduced (Korunic, 2016). Although the bulk density is considered as one of the categories of grain quality, the real reduction in grain quality by DE is questionable. Namely, Bodroža-Solarov et al. (2012) found significant improvements of rheological parameters of wheat treated with inert dusts, such as moisture absorption in the non-infested wheat and flour (dough), and particularly through a rise in dough energy. But even though, as long as the grain market includes bulk density in quality categorization and final price of cereals, its reduction will be a limiting factor for grain trade. Thus mixing DE with other compounds that enhance its activity and at the same time minimise the bulk density reduction, would enable a wider adoption of DE to control stored-product insect pests and make it more acceptable by grain industry and farmers.

However, prior to the introduction of developed formulations regarding placing on the market, a regulatory approval in accordance with Regulation (EC) No 1107/2009 should be obtained for each constituent substance in the formulation, as well as for the mixture. Testing is required to determine if these formulations fulfil demands, among others, stability of the formulation, effect on non-target organisms, human health and operator exposure as well as environmental safety and efficacy on the target organisms.

Although there is interest in many institutions for researches and performing assessments and future usage often they have no human resources, capacity and proper funding and it can be assumed that's why only few basic substances have been approved.

The interest should be to organise cooperation between interested institutions and researchers to divide studies that will give them opportunity to focus on the one kind of studies of the same substance or mixture of substances like physical-chemical properties, influence on non-target organisms or the environment, efficacy etc. and finally combine results and together apply for the approval and placing on the market.

For the future registration procedure it should be expected that the complexity of the requested studies should be simplified what could increase the number of basic substances as well as low-risk active substances.

Declaration of competing interest

All authors declare that there are none interest to declare.

Also by this declaration all authors confirm that this manuscript has not been published elsewhere and it is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to Journal of Stored Products Research.

CRediT authorship contribution statement

Z. Korunić: Conceptualization, Methodology, Investigation, Writing - original draft, Supervision. **A. Liška:** Data curation, Writing - review & editing, Writing - original draft. **P. Lucić:** Writing - review & editing. **D. Hamel:** Writing - original draft, Visualization. **V. Rozman:** Writing - review & editing, Writing - original draft.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit-sector.

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