Alternative Methods for in-vitro Pest Management in Serbia

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- Storage insects around the world cause great economic damage by destroying a large number of stored products.

- It is estimated that each year these pests cause post-harvest losses, from 10% in developed countries, 20% in developing countries and up to 60% in undeveloped countries.

- Red flour beetle *Tribolium castaneum* (Herbst) is one of the most economically harmful species of storage insects.
- This species feeds on numerous stored products such as stored cereals, their products, and it also causes extensive damage to oilseeds, nuts, dried fruits, spices, cocoa beans, pulses, cottonseed, feed and animal products.
- The female lays 2-18 eggs per day, a total of about 1000 eggs in a year
- Under favorable conditions, the whole development cycle lasts only 3 weeks
- Imago can live up to 3 years

- Use of insecticides in food and feed processing and storage facilities is difficult, and the resistance of this and other storage insect species to them is very high, recent research are based on finding new alternative and biological ways to protect stored products.

- Alternative methods are mainly based on better, more detailed knowledge of the biology and behavior of this pest

Goals:

- To determine development length, number and mass of the *T. castaneum* offspring at different initial population densities on different stored products
- To determine the impact of stored products volatiles in non-infested and infested (*T. castaneum*) form on the behavior of *T. castaneum*
- To determine the impact of the essential oils volatiles from different plant species on the behavior of *T. castaneum*
- Testing the durability of biodegradable packaging against attack of *T. castaneum*

Goal 1- Material and methods

- Test insect: Laboratory population of *T. castaneum* reared on wheat flour supplemented with brewer's yeast at 25 ± 1ºC and 60 ± 5%
- 8 different stored products (substrates) divided into three groups were used:

1. Carbohydrates rich substrates:

- Wheat bran
- Corn feed flour
- Coarse wheat meal

2. Protein rich substrates:

- Corn gluten meal
- Soybean concentrate
- Sunflower meal
- **3. Complete substrates:**
- Compound feed for fattening pigs
- Compound feed for laying hens
- **Control: Standard laboratory substrate (wheat flour + brewer yeast)**

- Experiment was performed in controlled conditions (30±1C and 55±10% r.h.v.), in 8 repetitions
- Insects are separated by sex in the pupal stage
- Four initial population densities of 1, 2, 5 and 10 pairs (male and female) of insects per 10 g of substrate were formed
- Pairs were left in substrates for 7 days to reproduce and then removed from it
- The development length of the offspring was monitored every day through the appearance of the first larva, the first pupa and the first imago. After eclosion of all insects, the number and body mass of offspring was determined
- All data was proccesed with one way ANOVA and Tuckey test

Goal 1- Results

Duration of *T. castaneum* development cycle on different substrates, at initial population densities of 1, 2, 5 and 10 pairs of insects

Substrates	Development duration(days±SE)			
	Population densities (pairs number/10 g of substrate)			
	1	2	5	10
Protein substrates???				
Control	22,63±0,16Ca	22,00±0,27Ca	22,50±0,19Ca	22,63±0,32Da
Wheat bran	23,25±0,16Ca	23,25±0,16Ca	23,50±0,19Ca	23,88±0,29CDa
Coarse wheat meal	26,88±0,44Ba	26,88±0,58Ba	26,13±0,48Ba	26,63±0,60Ba
Corn feed flour	31.13±0.44Aa	30,90±0,58Aa	30,63±0,50Aa	31,25±0,62Aa
Feed for pigs	24,50±0,33Ca	23,75±0,25Ca	23,75±0,16Ca	24,13±0,44Ca
Feed for hens	26,50±0,60Ba	26,13±0,35Ba	25,75±0,25Ba	26,13±0,39Ba

Each mean value is based on 8 replicates. Mean values in columns with different uppercase letters and mean values in rows with different lowercase letters are statistically significantly different (Tuckey test $p<0.05$)

Total number of *T. castaneum* offspring on different substrates at initial population densities of 1, 2, 5 and 10 pairs of insects

Substrates	Total offspring number±SE			
	Population densities (pairs number/10 g of substrate)			
	1	2	5	10
Control	107,25±3,49Ac	185,00±6,00Ab	299,63±5,56Aa	313,38±8,66Aa
Wheat bran	111,63±7,40Ad	180,25±5,41Abc	257,63±9,41Bb	324,13±11,22Aa
Coarse wheat meal	43,00±3,94Db	56,75±4,53Dab	58,13±7,16Da	70,50±3,83Da
Corn feed flour	66,00±2,08Cd	108,50±5,40Cc	176,13±11,68Cb	248,63±4,27Ca
Feed for pigs	84,50±6,53Bd	177,88±7,13Ac	253,50±7,86Bb	325,75±8,60Aa
Feed for hens	100,00±5,27Ad	152,38±7,17Bc	239,88±7,05Bb	286,63±10,00Ba

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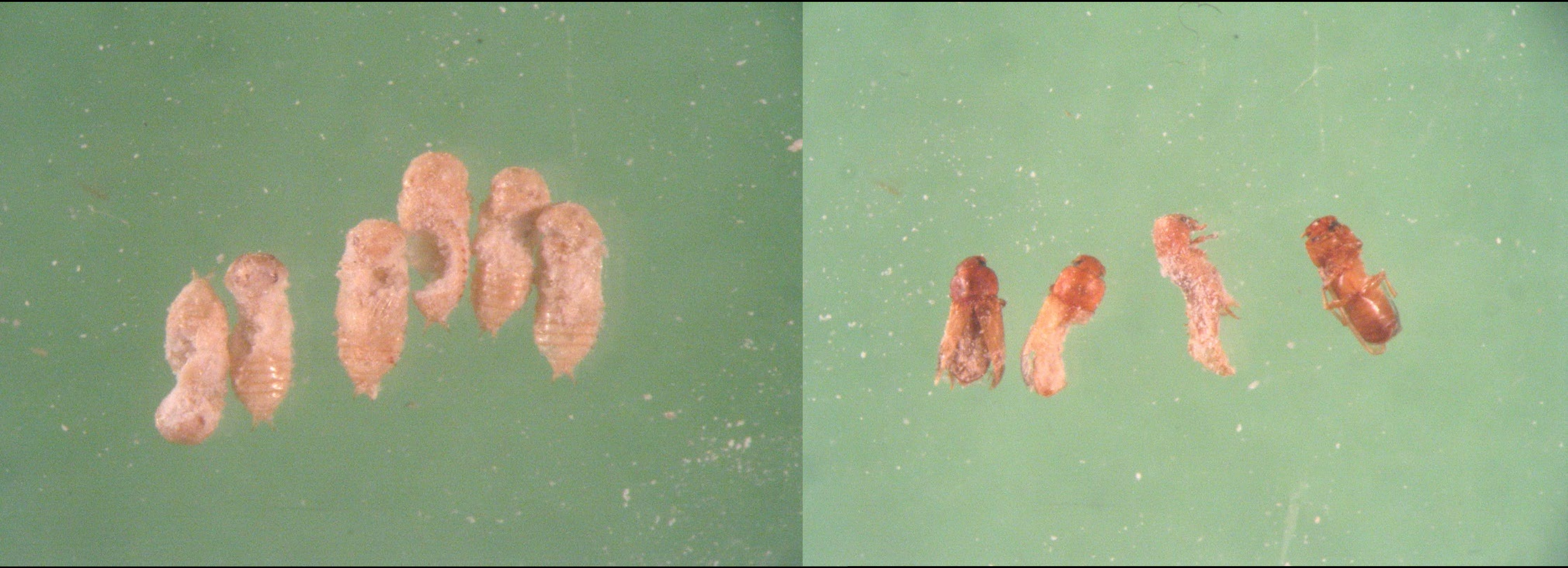


Body mass of adult *T. casatneum* offspring on different substrates at initial population densities of 1, 2, 5 and 10 pairs of insects

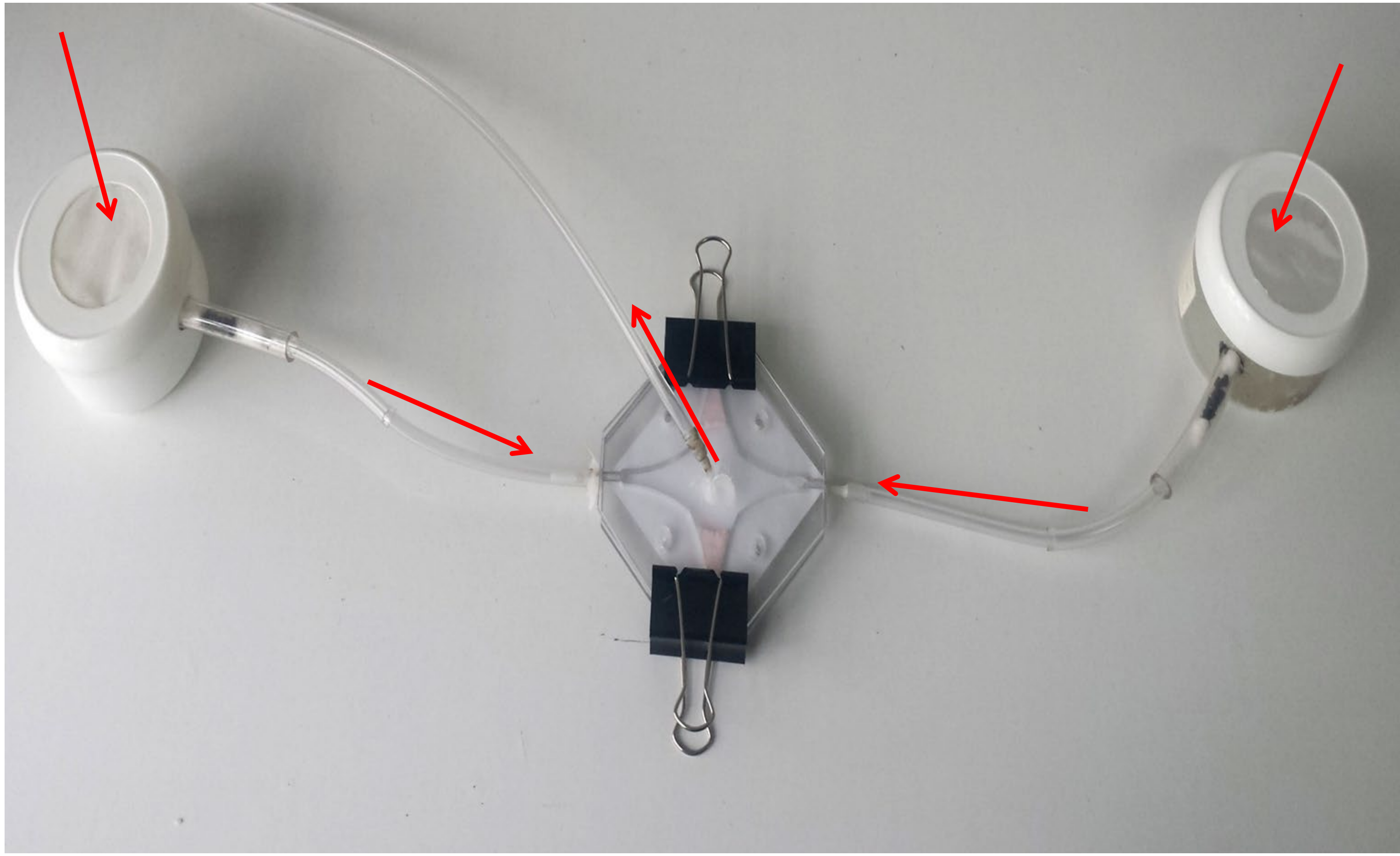
Substrates	Body mass of adult(mg)±SG			
	Population densities (pairs number/10 g of substrate)			
	1	2	5	10
Control	1,683±0,063Aa[b]	1,670±0,059Aa	1,611±0,068Aa	1,540±0,034Aa
Wheat bran	1,730±0,055Aa	1,625±0,058Aab	1,513±0,073ABb	1,385±0,053ABc
Coarse wheat meal	1,546±0,076Aa	1,540±0,065Aa	1,489±0,058ABa	1,407±0,065ABa
Corn feed flour	1,621±0,065Aa	1,611±0,060Aa	1,489±0,063ABab	1,429±0,059ABb
Feed for pigs	1,702±0,068Aa	1,674±0,063Aa	1,543±0,059ABab	1,400±0,066ABb
Feed for hens	1,584±0,076Aa	1,534±0,063Aa	1,406±0,063Bab	1,316±0,048Bb

Each mean value is based on 8 replicates. Mean values in columns with different uppercase letters and mean values in rows with different lowercase letters are statistically significantly different (Tuckey test $p<0.05$)


Goal 2- Material and methods

Olfactometer experiment

- The effect of the volatiles of all substrates on *T. castaneum* beahaviour was tested both in their uninfested and infested forms. The infested substrates were exposed to insects for 15 days, after which insects were removed
- A two-armed olfactometer was used. Containers with tested infested and non-infested substrates were attached to the arms of the olfactometer using tubes. One insect at a time was tested in the olfactometer, a total of 20 insects per test. All insects were 3-5 weeks old and were starved for 24h before testing



- Preferance test
- In this test we used plastic boxes with infested and non-infested substrates on one or both sides of the box.
- One insect per box was tested, for a total of 20 insects per trial. All insects were 3-5 weeks old and starved for 24h before each test.
- The number of visits to one or the other side of the box was measured every 3 minutes for 30 minutes in total. Data has been statistically processed using the Wilcoxon paired test

- The impact of each substrate volatile was tested in three trials, the first was a choice between an empty container and a container with non-infested substrate, the second between an empty container and an infested substrate, and the third between an infested and a non-infested substrate. Then all the combinations between all the infested and uninfested substrates were tested to determine which substrate in the infested and uninfested form is the most attractive to *T. castaneum* (48 combinations in total). All data is proccesed with Wilkoxon paired test

Goal 3- Results

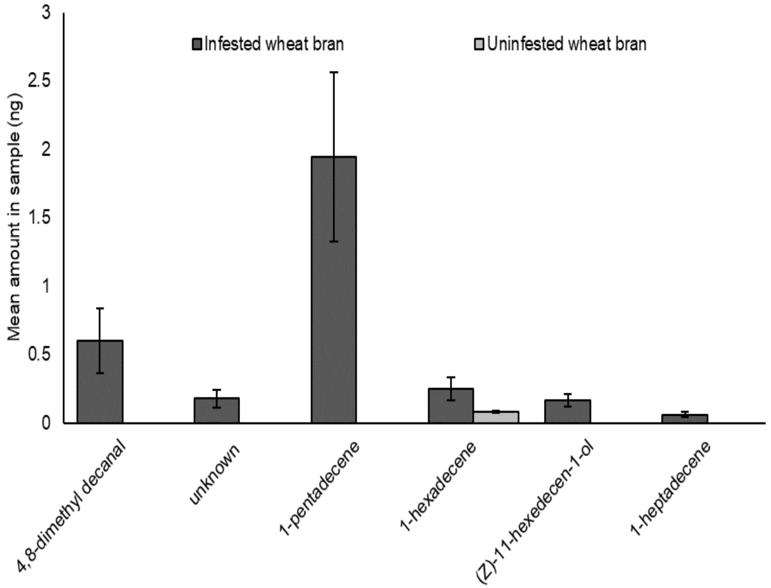
- All substrates in non-infested and infested conditions were attractive to *T. castaneum.* But when we compared, infested substrates were more attractive than non-infested ones
- Infested bran attracted insects more than any other infested substrate. Similar attractiveness of bran was shown in the preference test

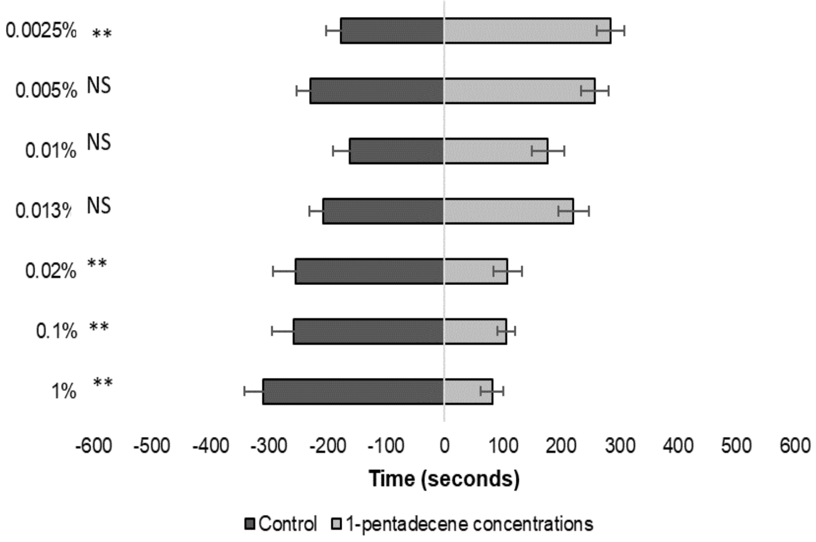
	Duration of visit in seconds.	
	Control	Uninfested bran
1	0	600
2	160	430
3	200	390
4	95	450
5	15	535
6	10	520
7	0	580
8	65	480
9	0	600
10	0	600
11	0	595
12	35	540
13	180	370
14	0	565
15	240	325
16	50	545
17	0	600
18	0	600
19	45	550
20	5	550
Total	**1100**	**10425**

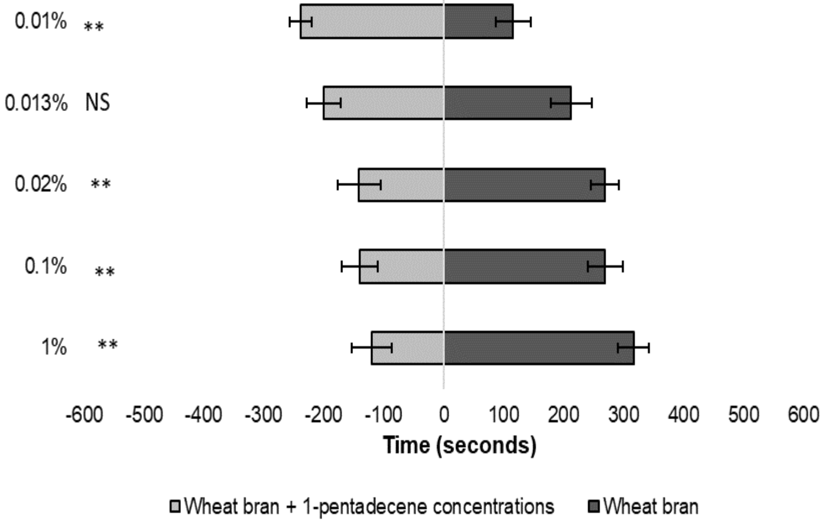
	Duration of visit in seconds.	
	Control	Infested bran
1	225	290
2	0	570
3	55	515
4	15	540
5	285	315
6	0	600
7	20	85
8	110	225
9	0	595
10	0	585
11	0	600
12	0	600
13	0	515
14	0	570
15	0	460
16	40	410
17	70	515
18	70	490
19	0	540
20	60	485
Total	**950**	**9505**

	Duration of visit in seconds	
	Infested bran	Uninfested bran
1	265	70
2	210	240
3	600	0
4	580	0
5	490	40
6	600	0
7	560	0
8	420	140
9	600	0
10	450	40
11	570	0
12	305	55
13	240	175
14	415	0
15	480	60
16	95	20
17	410	60
18	335	170
19	100	65
20	360	90
Total	**8085**	**1225**

- In order to determine which chemical compound in the infested substrate attracts this species, infested and non-infested samples were sent to Sweden for volatiles collection. By the "headspace" method with the help of appropriate filters, and the chemical analysis of the collected volatiles was performed by gas chromatography - mass spectrometry (GC-MS)
- The major chemical compound detected in the headspace of infested wheat bran, but not detected in uninfested bran, was 1-pentadecene (defensive secretion)







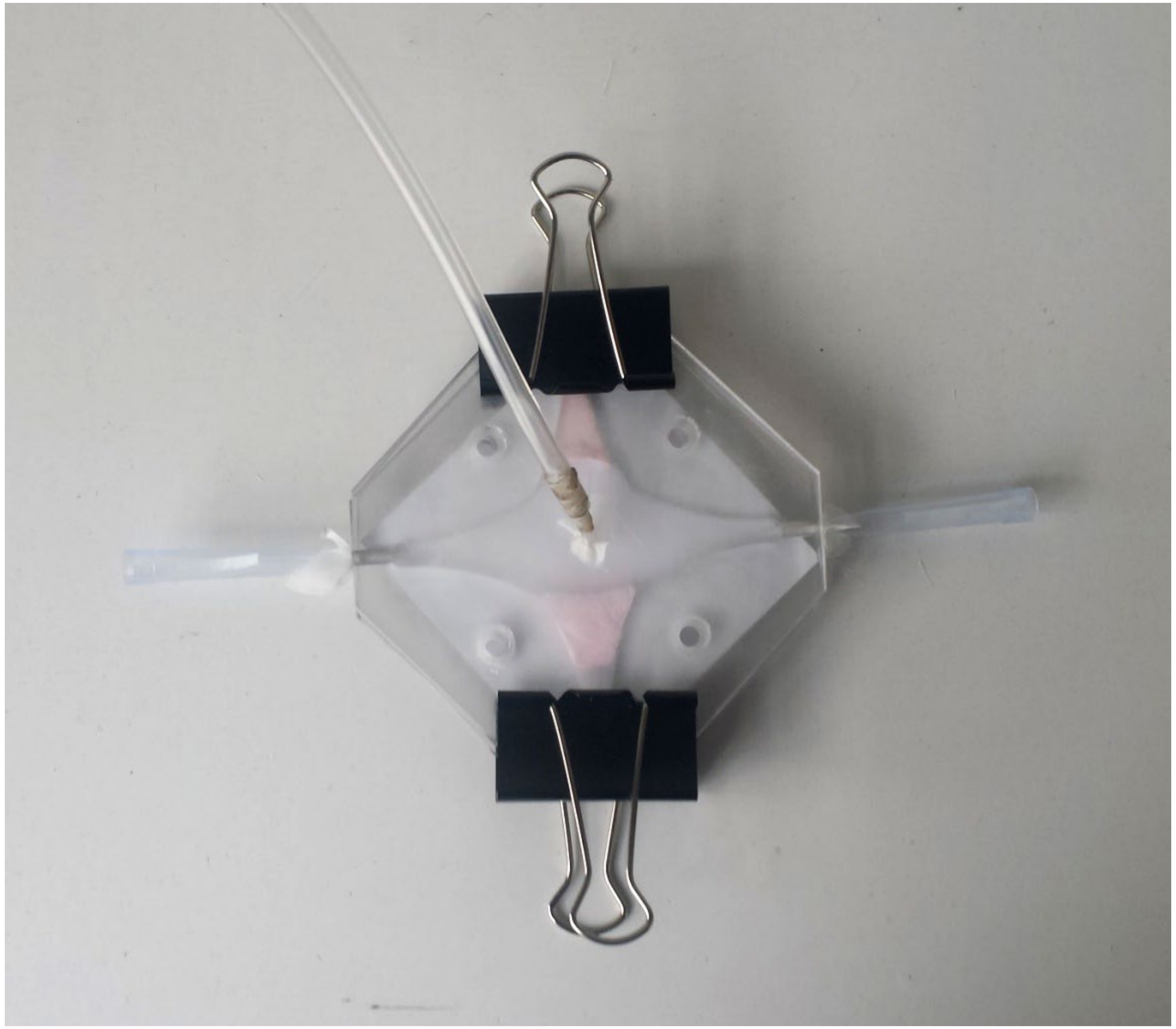
Goal 3- Material and methods

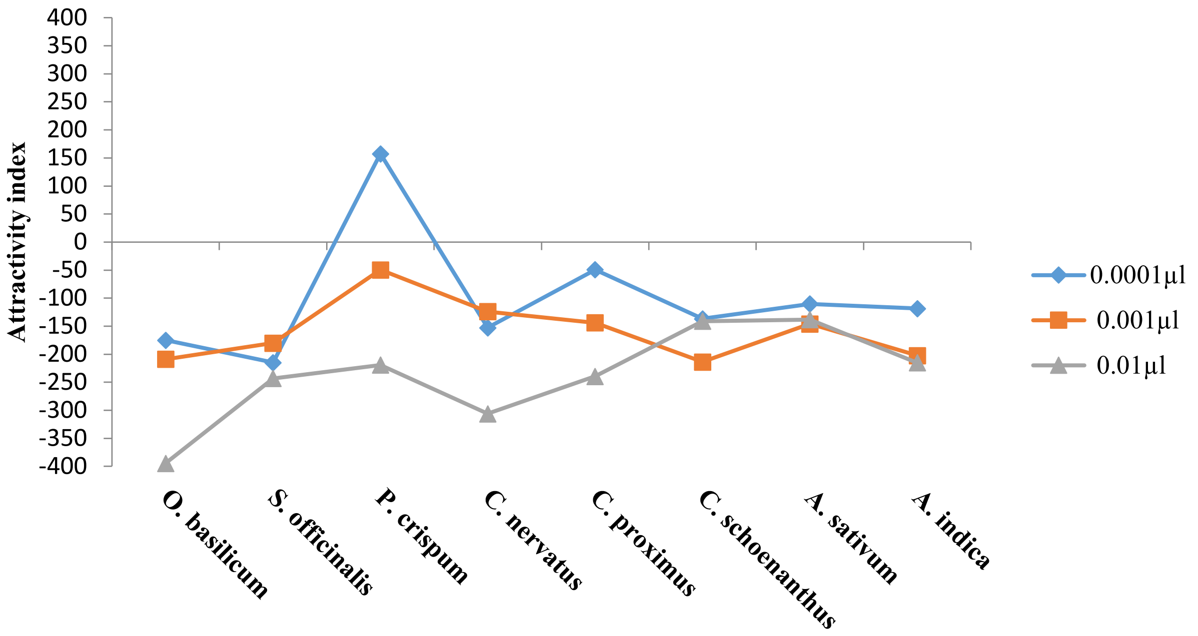
- Commercial essential oils of plants were used in the experiments: basil (Ocimum basilicum), sage (Salvia officinalis), parsley seeds (Petroselinum crispum), (Probotanic, Serbia), garlic extract (Allium sativum), (Institute for Medicinal Plants "Josif Pančić", Serbia) i essential oil of three types of lemongrass from Sudan (Cymbopogon nervatus, Cymbopogon proximus and Cymbopogon schoenanthus) The bioinsecticide NeemAzal containing 1% extract of the *Azadirachta indica* plant (manufacturer Trifolio-M GmbH, Germany) was used as a standard in the research.
- In the experiment, solutions of tested plant extracts and essential oils obtained by diluting them in n-hexane in 3 concentrations (0.01, 0.1 and 1%) were used. N-hexane was used as a control



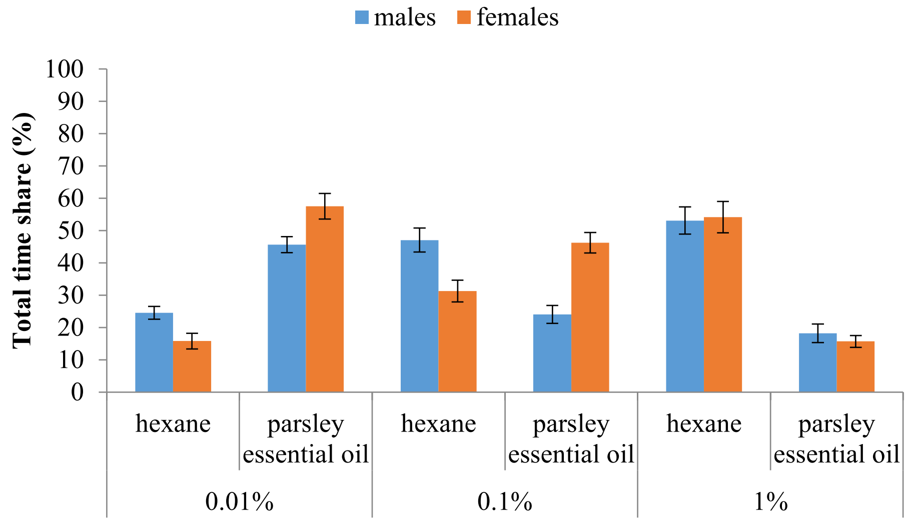



- A two-armed olfactometer was used
- Plastic tubes with strips of filter paper were attached to the arms of the olfactometer
- Micropipette applied n-hexane or tested substances in different concentrations in amounts of 10 µl
- One insect at a time was tested in the olfactometer, a total of 20 insects (repetitions) per trial
- 10 minutes followed





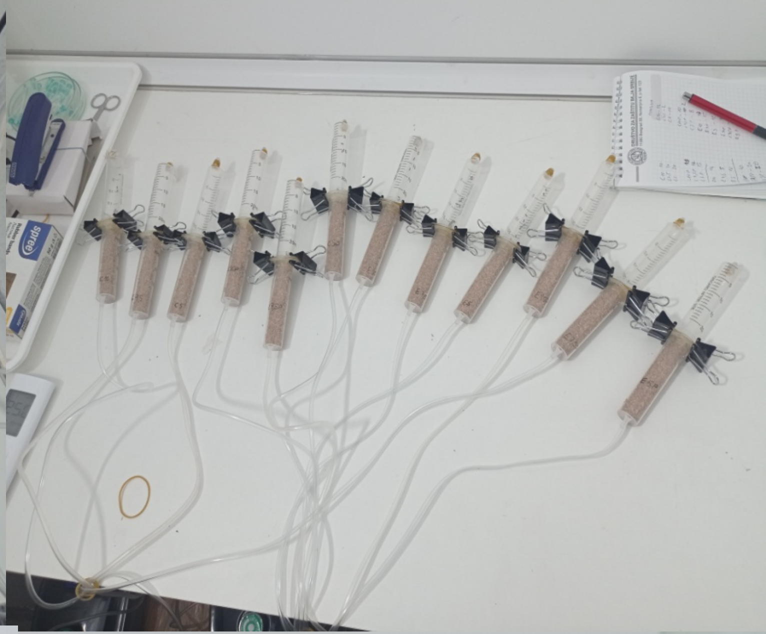
Attractivity index
400
350
300
250
200
150
100
50
0
-50
-100
-150
-200
-250
-300
-350
-400
O. basilicum
S. officinalis
P. crispum
C. nervatus
C. proximus
C. schoenanthus
A. sativum
A. indica
0.0001µl
0.001µl
0.01µl

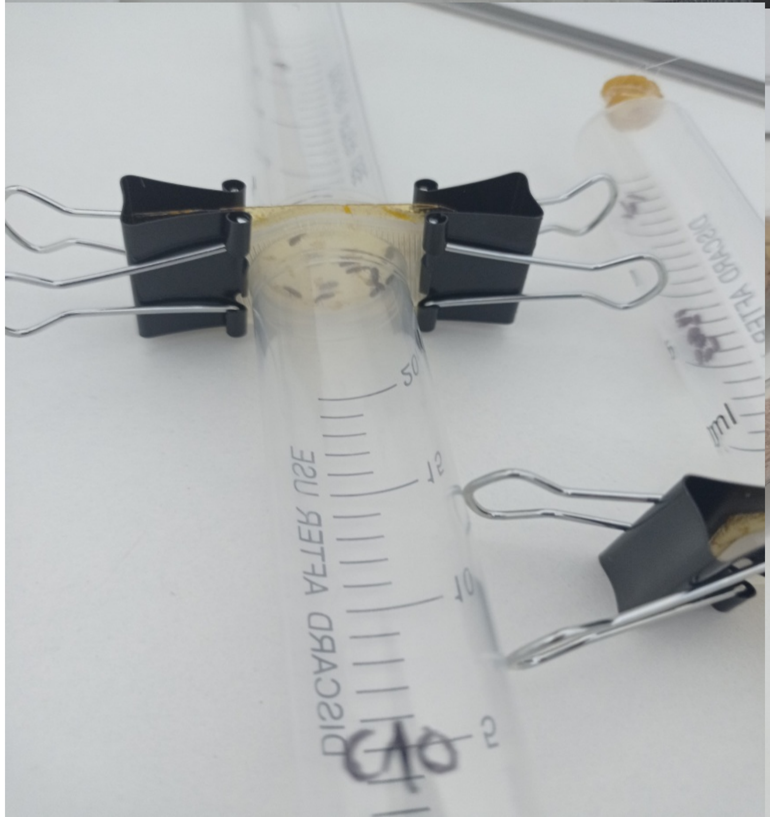


males
females
Total time share (%)
100
90
80
70
60
50
40
30
20
10
0
hexane
parsley essential oil
hexane
parsley essential oil
hexane
parsley essential oil
0.01%
0.1%
1%

Testing the durability of biodegradable packaging against attack of *T. castaneum*

- The packaging is made from corn protein zein
- Variants with or without sage extract impregnated in the package





Conclusion

- The results of these researches can help us in determining priorities for the protection of stored products, as well as in eliminating unnecessary costs of protecting those products in which the development cycle of this pest is not possible (high protein content substrates).
- The results have led us to the conclusion that high-protein substrates, could be used in storage facilities as a barrier around store products that is highly susceptible to infestation by *T. castaneum*. This would significantly improve existing pest management, making it safer and more eco-friendly.
- The research results also show that in all cases where they were examined, infested substrates had a higher attractiveness than non-infested substrates. This high attractive potential could be used in the future to monitor the presence and abundance of this economically significant pest.
- The results of the research showed that the all examined essential oils exhibit a significant repellent potential at all tested concentrations and that in the future it could be used as repellents of natural origin in relatively low concentrations. On the other hand, the essential oil of the parsley in a concentration of 0.01% could be used as an attractant, i.e. to monitor the abundance of *T. castaneum* in storage facilities.

Thank you!