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THE EFFECT OF PLANT OILS FOR REDUCING CONTAMINATION OF STORED PACKAGED-FOODSTUFFS

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Abstract: A study to determine the effect of essential oils, used together with mixed packaging, on controlling stored-grain insects, was conducted in the laboratory. Plant essential oils are one of the materials that act like a contact-fumigant, offering the prospect for use in stored product protection. Plant essential oils must have the ability to repel the insects in order to prevent penetration to foodstuffs. The objective of the present study was to test the properties of *Cinnamomum camphora* and *Syzygium aromaticum* for preventing the penetration of pest insects, including: *Sitotroga cerealella* and *Ephestia kuehniella* 5th instar larvae to packaged cereals. Ten grams of wheat and flour as foodstuffs were placed inside Cellophane packages and hung vertically in the top of a container. The repellent essential oils were used in the interior surface of containers. Each container had punctures at different parts and the insects were released around the container to determine insect penetration and the effect of the repellents. The experiments were conducted at 26±1°C, 60±5% RH in dark conditions. The highest concentration was 1.5 µl of essential oil per 0.5 ml acetone. Four days after the initiation of the experiment the results showed *S. aromaticum* had more of a repellency effect than *C. camphora* on both pests that caused contamination deduction inside the packaged foodstuffs compared with the control. However, both the essential oils were effective as protectants of cereals but *S. aromaticum* was more effective. *S. aromaticum* caused the most repellency on *E. kuehniella*. The mean of repellency of all the concentrations was 81.4%. The results demonstrated the efficacy of these essential oils for use in organic food protection. *C. camphora* and *S. aromaticum* can prevent warehouse infestation of stored-product pests.

Key words: essential oils, packaging, food preservation, stored-pests, penetration

INTRODUCTION

Plant extracts are comparatively safer to humans due to the low mammalian toxicity of the extracts. Derivatives of some plants have had temporary to restricted use in pest control or have been considered items of regional interest (Saxena 1983). Insects are the most serious pests that can contaminate food by penetration into products in warehouses. Despite modern food storage and distribution systems, most packaged food products, with the exception of canned and frozen goods, are subject to attack and penetration by insects (Mullen and Highland 1988). Most insects use their sense of olfaction to find food. There are two types of insects that attack packaged products: ‘‘penetrators’’, which are insects that can bore holes through packaging materials; and ‘‘invaders’’, which are insects that enter packages through existing holes, such as folds and seams and air vents (Highland 1984; Newton 1988). They can enter foodstuff in packages and also move from one package to another package in stores. *Sitotroga crealella* and *Ephestia kuehniella* are some of the stored-product insects that are capable of penetrating packaged food, especially cereals. Most recent research has been done in order to determine penetration abilities of various species of stored-product insects into packaged agricultural products (Cline 1978; Bowditch 1997; Allahvaisi *et al*. 2009). Research has been done on penetration of insects into different materials and yet, the effect of the repellency of essential oils on these materials was not tested. Also, whether the various parts of foodstuff packages ,for example, the top, middle or bottom, which are stored in the presence of repellents are affected differently or not, is important for how packages are placed in warehouses. The purpose of this study was to explore the possibility of using essential oils as insect repellents to prevent pests from infesting food packages.

MATERIALS AND METHODS

Insects

Two insect species, *S. cerealella* and *E. kuehniella* were reared in the laboratory at 28±1°C and 70±5% relative humidity (RH). All species had been cultured in the laboratory for over five years. Ten days old adults were used in all the experiments. *E. kuehniella* was reared on wheat

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flour mixed with 5% brewer's yeast and *S. Cerealella* was reared on whole and broken kernels of wheat.

Plant essential oils

Two commercially available essential oils were tested in this study. They were obtained from *Cinnamomum camphora* and *Syzygium aromaticum*. These oils were assayed against *S. cerealella* and *E. kuehniella* 5th instar larvae.

The used polymer

In this study, the permeability of transparent and flexible polymer of Cellophane with a 16.5 μm thickness was tested against stored-insect pests as the current polymer for foodstuffs packaging (Allahvaisi *et al*. 2009). This flexible packaging polymer was cut into 15x22 cm pieces with the aid of a template. Then, 8x10 cm pouches were prepared by sealing polymeric pieces with the aid of a plastic machine press for packaging 10 g foodstuffs (wheat and flour). These pouches were completely without any pores.

Essential oil concentrations

Five dilutions of each oil (0.25, 0.5, 1, 1.75, 2.75% w/w) prepared in 0.5 ml acetone and their repellent activity against *S. cerealella* and *E. kuehniella* larvae was studied. Aliquots of 1.5 ml of each dilution were sprayed on the walls of the container containing food packaging to achieve homogeneous distribution of each oil.

Penetration test

In this study, each of the prepared pouches was vertically hung, in the top of a 150 cc container. The repellent essential oils were used on the interior part of containers. Holes were made in the various parts of each container and the above mentioned insects were released inside a second container (300 cc). The first container with the food inside, was put inside the second container containing 5th instar larvae. Then, insect penetration ability and the effect of the repellents were determined. Also, a control test (water instead of oils) was used. Tests were repeated three times. The number of applied insects in any replication was 50 adults with 7–10-days-old that were starved for 24 hours. Finally, the containers were capped with a filter fine lace-mesh lid to confine the insects and prevent potential escape, and also to keep out foreign objects. The experiments were conducted at 27±1°C, 65±5% RH in dark conditions. The packages were extracted from the jars and examined daily for points of penetrations. All repellency assays were conducted in the laboratory. The pests penetrated in < 48 hours and most insect penetration usually occurred after 3 days. Since most of essential oils have knock down properties (Al-Jabr 2006), the insects that died during the experimental period were replaced by adults of the same age from the same treatments. Data recording began with the initiation of penetration. The beginning of penetration was considered to be when a puncture was created on the packaging or one insect was observed inside the packaging. Each hole created by the insects on the polymers was counted as a penetration, but the only way to determine penetration percentage was by counting the number of insects which had penetrated through packages (Allahvaisi *et al*. 2009). The daily counting of insects which had penetrated the packaging continued for 5 days. The numbers of punctures created by insects on various sites of the packages showed that arrival sites of pest insects in different parts, can be effective for determining repellent percentage of essential oils.

Data analysis

Statistical analysis of data was carried out with MSTATC and EXCEL software and Randomized Complete Design (RCD) and the means were compared with Duncan's mean test and t-test. From Arcsin√x used to monotone the data.

RESULTS AND DISCUSSION

The results show that repellent percentages of the two essential oils used in the study on the pests is different. From among the applied insects, *S. cerealella* had the most penetration points with the use of both oils. The initiation of penetration of the larvae in Cellophane polymer occurred in less than 48 hours. Many larvae penetrated packages within 48–72 hours but the most penetration took place on the fourth day. However, the pests used in the control test penetrated in less than 12 hours and the most penetration was within 48 hours. The statistical analysis revealed a significant difference among tested insects. The results showed the permeability percentage of the pests to packages with essential oils was much less in comparison with the control test. *S. aromaticum* had the most repellency effect on *E. kuehniella*, and prevented it from penetration to foodstuffs packages. No *E. kuehniella* insects, at a concentration of 2.75% w/w, penetrated through Cellophane polymer as a packaging material. This was a considerable difference compared to the control. According to the findings of this study both Cellophane polymer and essential oils had a bilateral effect on each other and significantly reduced the number of insects penetrating to the treated packages of foodstuffs (Table 1). So, *S. aromaticum's* ability to reduce penetration, in comparison to the control test, may be due to the repellent and antifeeding properties of *S. aromaticum*. Also, the results obtained in the case of *C. camphora*, were different. There was a significant difference between repellents and permeability of these pests to foodstuffs packaged with Cellophane polymer. The difference between the two essential oils was significant.

On the other hand, there were significant difference between pest penetration of Cellophane polymer in both treatments and the control tests. Therefore, the reduction of the penetration by essential oils may be due to its repellent and antifeedant properties. The statistical analysis of obtained data from treated and control experiments showed that there is a bilateral effect between packaging and repellency of essential oils. Table 2 shows the mean of the insect packaging penetration in the obtained concentrations. There was more infestation of insects, and number of holes created on the top part of the packages of foodstuffs. The least infestation was on the exposed bottom part (Table 3) where there was the least air cur-

Table 1. Average penetration percentage of pest insects through Cellophane polymer with two different essential oils, after 3 days

Plant Oils	Insect Concentration	Average penetration of insects to packages at concentrations [%]				
		0.25	0.5	1	1.75	2.75
Syzygium aromaticum	*E. kuehniella*	59.8±0.22	40.1±0.7	25±0.2	10±0.32	1.76±0.32
	S. cerealella	74.3±0.18	59±0.11	31.7±0.11	16±0.22	2.6±0.34
	means	67.0±50.2 a	49.55±0.9 b	28.35±0.16 c	8±0.27 d	2.18±0.33 e
Cinnamonum camphora	*E. kuehniella*	38±0.5	26±0.11	17.3±0.32	8±0.5	0.78±0.15
	S. cerealella	53.7±0.2	34±0.34	19.9±0.2	11±0.22	4±0.45
	means	45.85±0.35 a	30±0.225 b	18.6±0.275 c	9.5±0.375 d	2.4±0.3 e

The dissimilar letters show significant differences among the concentrations ($p \leq 0.05$)

Table 2. Average penetration percentage of pest insects through Cellophane polymer with two different essential oils, after 3 days

Insect Essential oils	*E. kuehniella*	*S. cerealella*	Mean
Syzygium aromaticum	18.2±0.32	24.52±0.2	21.36±0.25 b
Cinnamonum camphora	27.33±0.35	36.72±0.2	32.02±0.27 a
The control	76±0.32	99.5±0.22	87.75±0.27 c

The dissimilar letters show significant differences among the essential oils and the control ($p \leq 0.05$)

Table 3. Mean infestation and penetration of insects from various parts of containers to packages, after 3 days

The mean of punctures on the different sites of PE packages [%]									
Treatment and the control	*Syzygium aromaticum*			*Cinnamonum camphora*			water		
Insect	top	middle	bottom	top	middle	bottom	top	middle	bottom
E. kuehniella	14.2	8.33	3.4	16.8	13.6	7.87	23	3.63	18
S. cerealella	11.74	6.6	o.82	13.1	3.31	2.5	15.7	1.68	14

rent. This means that packages in stores should be placed so that repellency reaches all parts. However, in control tests, the packaged polymer permeability was less at the middle parts. This shows the effect that essential oils have in keeping away pests (Cline 1978). The main insecticide properties of essential oils are in the plant volatile compounds (Papachristos and Stamopoulos 2002; Keita *et al.* 2001). Mullen and Mowery (2000) state that most insects enter into finished products through openings caused by sewing, folding, or damage, not by chewing through packaging. Some adult insects can pass through holes less than 1 mm in diameter, and their larvae can enter through smaller holes (Cline and Highland 1981). Therefore, the ability of chemical barriers to prevent insects from invading is more important than the prevention of penetration (Hou *et al.* 2004). *S. aromaticum* and *C. camphora* are repellents to some insects (Hussain *et al.* 2008). Our data showed that essential oils were repellent enough to reduce insect immigration into packages. Further work is needed to determine if these findings have commercial potential (Hou *et al.* 2004).

Hundreds of materials have been investigated for use in insect-resistant packaging, such as synthetic pyrethroids, natural botanical antifeedants, and silica gel (Laudani and Davis 1955; Watters 1966; Highland *et al.* 1984; Bloszyk *et al.* 1990). Highland *et al.* (1984) showed that insects did not infest cereal–grain packed bags treated with permethrin. Included in the construction of the multiple-wall bags was a barrier layer that prevented the migration of permethrin into the cereals. Hou *et al.* (2004) suggested that both the penetration and the envelope tests showed that Diethyl-m-toluamide (DEET) has great potential for preventing the infestation of packaged goods. This repellent is mainly used for protecting humans from biting flies (Hou *et al*. 2004). Hussain *et al*. (2008) believe that *S. aromaticum* and *C. camphora* are repellents to some insects. Our data suggest that *S. aromaticum* had the most repellency effect on tested pest insects and it might also be effective on jute bags, which are commonly used in many developing countries for grain storage.

The packaging would have to be modified to prevent the essential oil contamination of the finished product, and the contamination of neighboring products by the volatile action of essential oils. Barriers developed for preventing the migration of pyrethrins into packaging, or similar barriers may prevent the contamination of food by DEET (Hou *et al*. 2004) and essential oils.

CONCLUSIONS

The results of this study show that using packaging and repellents together can significantly reduce the pest insect penetration to packaging of foodstuffs in the warehouse. The use of both packaging and repellents would also be a safe method for Integrated Pest Management (IPM) programmers which could further reduce the application of the synthetic chemicals and prevent the infestation of the stored-product pests.

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POLISH SUMMARY

WPŁYW OLEJKÓW ROŚLINNYCH NA OGRANICZENIE WYSTĘPOWANIA SZKODNIKÓW MAGAZYNOWYCH W PRZECHOWYWANYCH PRODUKTACH SPOŻYWCZYCH

W warunkach laboratoryjnych prowadzono badania nad zwalczaniem szkodników magazynowych zasiedlających produkty spożywcze przechowywane w opakowaniach. Roślinne olejki eteryczne działają podobnie jak środki kontaktowe i fumiganty, tym samym stwarzając możliwość wykorzystania ich w ochronie przechowywanych produktów spożywczych. Olejki roślinne powinny charakteryzować się właściwościami odstraszającymi owady aby zapobiegać penetracji i zasiedlaniu produktów spożywczych. Celem prezentowanych badań było określenie przydatności olejków eterycznych pochodzących z roślin *Cinnamonum camphora* oraz *Syzygium aromaticum* do zapobiegania zasiedlaniu produktów zbo-

żowych przez 5 pokolenie larwalne szkodników *Sitotroga cerealella* i *Ephestia kuehniella*. Mąkę pszenną o wadze 10 g umieszczono w celofanowych opakowaniach, które następnie zawieszono w górnej części cylindrycznego pojemnika, zaopatrzonego w dodatkowe otwory. Olejki roślinne rozprowadzano na wewnętrznej powierzchni ścian każdego pojemnika. Celem określenia penetracji i zasiedlania testowanego produktu spożywczego oraz odstraszającego działania olejków roślinnych szkodniki uwalniano wokół pojemnika. Doświadczenia prowadzono w temperaturze 26 ± 1° C i wilgotności względnej 65 ± 5% bez dostępu światła. Najwyższa koncentracja olejku roślinnego wynosiła 1,5 μl/0,5 ml acetonu. Po upływie 4 dni olejek pochodzący z roślin *S. aromaticum* dawał lepsze efekty odstraszania owadów niż olejek z roślin *C. camphora* w stosunku do obu testowanych szkodników. Chociaż obydwa testowane olejki roślinne były skuteczne, to olejek z roślin *S. aromaticum* dawał lepsze efekty odstraszania w przypadku szkodnika *E. kuehniella*. Średnia wartość odstraszania dla wszystkich zastosowanych koncentracji wynosiła 81,4%. Wyniki przedstawionych badań wskazują na przydatność roślinnych olejków eterycznych z roślin *C. camphora* i *S. aromaticum* w ekologicznej ochronie produktów spożywczych przed szkodnikami magazynowymi.