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Efficacy of electrospun bionanofibers as fumigant pesticides in foodstuff storage

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Abstract

Essential oils as alternative synthetic pesticides for pest management of foodstuffs have recently received increased attention. Controlled and slow release formulations of essential oils are used to enhance their efficiency. Two volatile essential oils of *Mentha piperita* L. and *Salvia officinalis* L. were investigated for release rate and mortality percentage by fumigant toxicity against 1st instar larvae of *Plodia interpunctella*. Electrospinning was used to incorporate various concentrations of essential oils in nanofibers. The essential oils can be released from the nanofibers for long periods of time, from several days to several weeks. Poly(lactic acid) was used as a green polymer carrier and the essential oils were incorporated into the nanofibers (8–15v/v% PLA). Insecticidal bioassay revealed that oil-loaded nanofibers (NFOs) were more toxic than pure essential oils (PEOs) against tested larvae. The LC_{50} and LT_{50} of NFOs were 1.2 and 4 times, respectively, more than PEOs. Our results indicated that PEOs completely lost their insecticidal activity after 14 days, whereas at the same period, NFOs had an average of 93% mortality when applied against *P. interpunctella*. Therefore, it can be concluded that nanofibers improved the persistence of the oil. This study presents *S. officinalis* has more toxicity and *M. piperita* had more persistence for controlling the larvae of indian meal moth.

Key words: fumigant toxicity, nanofiber, persistence, *Plodia interpunctella*, storage pests

Introduction

The most destructive insect pests in stored foodstuff products are respectively Coleoptera and Lepidoptera, which are spread over a wide range of climates and are found on every continent except Antarctica (Ress 2004; Robertson 2006). Many infections of stored foodstuffs e.g. grains, nuts, dried fruits are linked to the cosmopolitan Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Johnson *et al.* 1992; Fontenot *et al.* 2012). Chemical compounds were used as simple and fast acting controls. However synthetic pesticides had negative aspects such as resistant behavior, environmental pollution and they caused some incurable diseases in humans (Desneux *et al.* 2007; Pimentel *et al.* 2009; Islam *et al.* 2010; Ali *et al.* 2012). It appears that for centuries, biodegradable essential

oils (EOs) have fought pest infections as green pesticides and have been alternative methods for integrated pest management. They also have solved problems associated with chemical pesticides (Tripathi *et al.* 2009; Werdin *et al.* 2013, 2014; Athanassiou *et al.* 2013). Some studies have been done on the use of essential oils to control insects in different stored products (Tapondjou *et al.* 2002; Sahaf *et al.* 2007; Sahaf and Moharramipour 2008; Shojaaddini *et al.* 2008). Despite the promising properties of EOs, they have many limitations such as a tendency to oxidate, low stability and release rate, and poor water solubility. Also, high concentrations are required for effective protection. These are some of the problems which have to be resolved before using them for alternative pest control (Moretti *et al.* 2002).

Moreover, high molecular weights, low vapor pressure for diffusion and penetration into commodities, high volatility, and high boiling points limit the application of essential oils in large-scale fumigation (Korunic *et al.* 1998; Rajendran and Sriranjini 2008).

An approach which might reduce many of the problems introduced above is based on the release of the essential oils (EOs) from particular carriers which (a) protect the essential oils from evaporating, (b) control their continuous release for some weeks and, finally (c) cause a homogenous distribution of the essential oils across the stored products. Essential oils are delivered via carrier-solutions, polymer derivatives, or encapsulated in solid particles (Hammer *et al.* 1999; Dorman and Deans 2000; Ojagh *et al.* 2010; Zodrow *et al.* 2012; Kavanaugh and Ribbeck 2012; Badawy and Rabea 2013). Therefore, electrospun nanofibers are prospective carriers suitable for local administration of EOs in a controlled manner. Electrospinning has been utilized to produce non-woven mats from over 100 different synthetic and natural polymer solutions (Teo and Ramakrishna 2006; Rutledge and Fridrikh 2007; Schiffman and Schauer 2007, 2008; Frey 2008; Liu *et al.* 2010; Pakravan *et al.* 2011). Polyamide 6, spun from formic acid and cellulose acetate, spun from acetone as a solvent, were chosen as carriers of pheromone to release the pheromone from nanofibers (Hellmann *et al.* 2009). Angeles *et al.* (2008) showed a successful electrospinning of oil-in-water emulsion, which featured an aqueous solution of poly(ethylene oxide) (PEO) as the continuous phase and mineral oil (a non-volatile oil) as the drop phase. Some researchers successfully incorporated and delivered essential oils from nanofiber mats (Rieger *et al.* 2014; Mori *et al.* 2015). Electrospun nanofibers are extremely interesting materials for separation sciences, including sorbents for solid-phase extraction (SPE) (Augusto *et al.* 2013). Nanofibers (NFs) sorbents possess high surface areas and adsorptive capacity. One use of NFs as SPE materials is the isolating a pesticide from aqueous solution (Augusto *et al.* 2013). Ruggieri *et al.* (2015) proved that electrospun nanofibers are the best adsorbers because of their large surface areas and tunable compositional properties. Furthermore, in recent studies, molecular imprinted polymer (MIP) nanofibers obtained by electrospinning can be used for diverse applications including drug delivery, protein detection, and other smaller molecule detection (Zaidi 2015). Imprinted polymer nanofibers can be effectively employed as filters to remove hazardous pollution substances and more robust synthetic biomimetic receptors such as pesticides from water (Ruggieri *et al.* 2015). In this research, the adsorption magnitude of MIP nanofibers was higher than the non-imprinted polymer (Ruggieri *et al.* 2015).

Plodia interpunctella is a cosmopolitan and injurious pest of various stored agricultural foodstuffs

especially dried fruits, nuts and chocolate in warehouses. The pest attacks stored products, often as extensive mold growth which leads to considerable loss due to frequent consumer rejection.

Applying purified essential oil has some problems. For example it can poison the users, be an environmental hazard and its use is difficult to reproduce. In order to reduce these problems, slow release with minimum concentrations of EOs is the best method for stable EOs release for more than one month. To do this, the EO molecules can be distributed in sufficiently high concentrations in nanofibers (about 15–33 wt%) via electrospinning and the EOs can be released from the nanofibers for a sufficiently long time, extending over several weeks. The polymeric material poly(lactic acid) (PLA) is an aliphatic polyester of high molecular weight derived from renewable resources such as wheat, corn, sugar-cane or potato starch (Gao *et al.* 2002). The purposes of the current study were (1) the determination of the effect of PLA nanofibers loaded with two EOs vs. pure essential oil (PEO) on the mortality of *P. interpunctella* and (2) to investigate the influence of encapsulation on the persistence of the oil. Due to the effect of essential oils as fumigant pesticides, the fumigant toxicity of two volatile essential oils: *Mentha piperita* L. and *Salvia officinalis* L. was tested.

Materials and Methods

Test insects

The insect *P. interpunctella* was collected from a storage facility of nuts in Tehran. The 1st instar larvae (two-days old) of these insects were obtained from cultures maintained in the Entomology Laboratory, Research and Sciences University of Tehran for two years with no history of exposure to insecticides. They were reared under laboratory conditions, at $29\pm 1^\circ\text{C}$ and $60\pm 5\%$ relative humidity (RH) in a thermostat chamber (Vukajlovic and Pesic 2012). The standard laboratory diet (SLD) for this moth contains white cornmeal (26%), whole wheat flour (23%), glycerol (16%), honey (14%), ground dog meal (10%), brewers' yeast (5%), rolled oats (4%) and wheat germ (2%) (Silhacek and Miller 1972).

Collection and preparation of plant materials

Seeds of *S. officinalis* and *M. piperita* were collected from the field of the Medical Plant Center of Shahid Beheshti University, Tehran, Iran in May 2012. The harvested leaves were separated from other parts of the plants, cleaned and packed. Then they were dried, winnowed

and stored at -24°C until required for extraction at the beginning of the experiment in August 2012.

Extraction of essential oil

Leaves from two medicinal plants, *S. officinalis* and *M. piperita* were hydro distilled for extraction of their EO using a modified Clevenger-type apparatus. Leaves (50 g) were ground and put into a round-bottom flask over water at a temperature around 100°C . Volatile oil was collected in a reservoir after 4 h distillation. Anhydrous sodium sulfate was used to remove water after extraction. The extracted oil was stored in a refrigerator at 4°C (Sahaf *et al.* 2007). The chemical composition of the essential oil was determined by gas chromatography combined with mass spectrometry (GC-MS).

Essential oil-nanofibers preparation

We used all compounds in the following way: 50 g of an air-dried sample; 1 : 10 leaf material/water volume ratio, 4 h distillation. Anhydrous sodium sulfate was used to remove water after extraction. Oil yield (4.16% w/w) was calculated on a dry weight basis. Essential oils were stored in a refrigerator at 4°C . Poly(lactic acid) (PLA, $M_n = 90,000 \text{ g} \cdot \text{mol}^{-1}$) was dissolved in chloroform [chloroform (CHCl_3 , CAS 67-66-3)].

Poly(lactic acid) (PLA, $M_w = 60,000 \text{ g} \cdot \text{mol}^{-1}$), dimethylformamide (DMF) [$\text{HCON}(\text{CH}_3)_2$, CAS 68-12-2], hexan [$\text{CH}_3(\text{CH}_2)_4\text{CH}_3$, CAS 110-54-3], chloroform (CHCl_3 , CAS 67-66-3), sodium chloride (NaCl, purity $\geq 99.0\%$, CAS 7647-14-5) and Tween 80 (Polysorbate 80, CAS-9005-65-6) were purchased from Sigma-Aldrich.

Nanofibers were prepared by electrospinning according to modifications of the methods of Hellman *et al.* (2009), Wei *et al.* (2012), Rieger *et al.* (2014), and Angeles *et al.* (2008). The latter demonstrated the ability to electrospin an oil-in-water emulsion. Poly(lactic acid) was dissolved in chloroform as a solvent. Due to the high evaporation rate of chloroform, DMF was added to the solvent to control the evaporation rate and create uniform fibers. Poly(lactic acid) dissolved in 3Chloroform/1 DMF (20 wt% PLA), yielding fiber diameters with conventional electrospinning from 50 to 350 nm, was electrospun to a viscosity of 10.7 Pa sec. For the electrical properties of the polymeric solution, NaCl with high purity was added to the solution in different percents (0–4%). On the other hand, different concentrations of essential oils of *M. piperita* and *S. officinalis* diluted by Tween 80 (Polysorbate 80) as an emulsifier were added to the prepared pre-polymer solution yielding solid nanofibers with up to 22 wt% of essential oils. The solution was mixed for 24 h at 20 rpm via an Arma-Rotator and then the solution was changed from transparent to white. Each essential

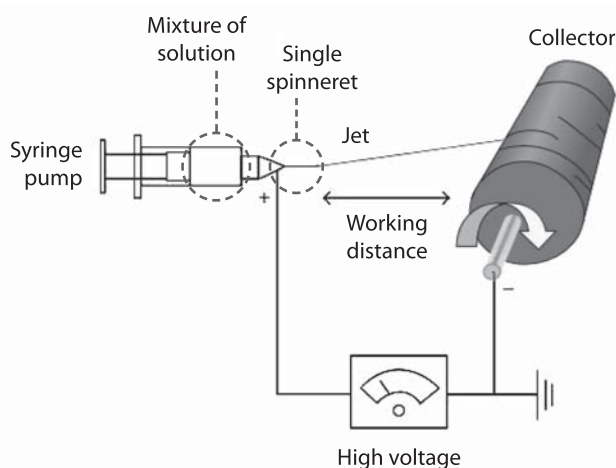


Fig. 1. Schematic diagram of the electrospinning setup (Mori *et al.* 2015)

oil/PLA solution was loaded into a 5 ml Luer-Lock tip syringe capped with Precision Glide 16, 18, 20 and 21 gauge needles, which were secured to an Ultra syringe pump.

Alligator clips were used to connect the positive anode of a high-voltage supply to the needle and the negative anode to a copperplate wrapped in aluminum foil. A constant feed rate of $60 \text{ l} \cdot \text{min}^{-1}$, an applied voltage of 15 kV (Zong *et al.* 2002), and a separation distance of 110 to 200 mm were used to electrospin essential oils/PLA solutions, respectively. The assembled electrospinning apparatus was housed in an environmental chamber with a desiccant unit to maintain a temperature of 24°C and a relative humidity of 65% (Fig. 1) (Mori *et al.* 2014).

Degradation tests

Poly(lactic acid) is a biodegradable and hydrophobic polymer. The degradation rate of the nanofiber was determined in an incubator under storage conditions. An amount of nanofibers with a determinate of EOs weight (0.05 g) was placed in an incubator. Then, we weighed them at different time intervals. The weight difference calculated by diagram slope gave the degradation rate of PLA in nanofibers.

Bioassay tests

The experiments were done to determine lethal concentrations for 50% mortality of the larvae under both conditions, pure essential oils (PEOs) and formulated nanofiber oils (NFO). A series of concentrations ranging from $1.8 \text{ to } 10.64 \mu\text{l} \cdot \text{l}^{-1}$ air (PEOs) and $2.5 \text{ to } 15.84 \mu\text{l} \cdot \text{l}^{-1}$ air (NFOs) of *S. officinalis*, $2.41 \text{ to } 13.8 \mu\text{l} \cdot \text{l}^{-1}$ air (PEOs) and $3.26 \text{ to } 19.5 \mu\text{l} \cdot \text{l}^{-1}$ air (NFOs) of *M. piperita* were tested on 1st instar larvae for determination of LC_{50} of the fumigant. Then,

25 larvae (1–3 days old) were introduced into each vial and placed in 280 ml glass vials with screw lids. Filter papers (Whatman No.1) and EOs nanofiber were placed separately under the surface of the screw caps and impregnated with an appropriate concentration of the oil to prevent insects from contacting the oil or EOs nanofiber. Empty glass vials were the oil control and non-loaded nanofiber for EOs nanofiber. The caps were screwed on tightly and the vials were placed in an incubator set at $27\pm 1^\circ\text{C}$ and $55\pm 5\%$ RH in continuous darkness. Mortality for each exposure time was evaluated independently (Robertson et al. 2007) and concentrations were replicated for each exposure time separately. Each concentration and control was replicated several times. A mortality count was made 24 h after exposure. Exposure periods were 24 and 48 h and then the number of dead and live insects in each glass vial was counted 40 days after initial exposure to EOs and NFOs. The observations were recorded once every 48 h. When no movement was observed, the insects were considered dead. Each concentration was replicated five times. Probit analysis (Finney 1971) was used to estimate LC_{50} values. The release rate experiment was continued until the oil or EOs nanofiber lost their insecticidal effect.

Release rate bioassay

The highest concentration of PEOs was from the experiment of fumigant toxicity ($= 72.64 \mu\text{l} \cdot \text{l}^{-1}$ air) on 1st instar larvae of the insect tested in some separate glass vials. The conditions of the release rate experiment were the same as the ones described for the fumigant toxicity bioassay. Although, at the beginning of the fumigant toxicity tests up to 24 h, the selected concentration into both formulation of PEO and NFO was put in the glass vials without insect. Then, after the time the insects were moved to glass vials and after 24 h, the dead insects were counted, the lids of glass vials were closed until their counting. Each vial was a replication and was removed after counting of insects. This process was repeated for 15 days for PEO but mortality was rarely observed. Then, the first mortality count was made 24 h after exposure. The mortality for 40 days was continued. The mortality for NFO for 40 days and longer was nearly constant. The determination of

release rate continued as the PEO or NFOs significantly decreased their insecticidal effects.

Statistics

No mortality was observed in the control group, so there was no need to correct the data for normal mortality of the control. For the mortality percent, the square root of Arc Sine transformation was used to stabilize the variance and normalize the data (Osborne 2010), however non-transformed data are presented in the tables. The data were analyzed using one-way analysis of variances (ANOVA) and Tukey's test was used to separate means, at 0.05 probability (SPSS 2007). Probit analysis (Finney 1971) was used to estimate LC_{80} and PT_{50} values (persistence of the oil) and its 95% confidence limits using SPSS software (SPSS 2007).

Results

The best diameter of NFOs was studied. Various parameters including the percentage of salt, DMF, the concentration of solution and the space of needle (in 20 gauge) to aluminum page can be determiner to create optimal diameter (Table 1). The best diameter of fibers is 58 nm when 14% of EOs is loaded. Scanning electron microscope (SEM) images consist of nanofibers with relatively uniform thickness as non-woven webs that essential oil droplets incorporate into these nanofibers (Fig. 2).

The degradation rate of poly(lactic acid)/essential oils nanofibers is more than poly(lactic acid) nanofibers. The results show that LC_{50} for NFOs (*S. officinalis* = $12 \mu\text{l} \cdot \text{air}^{-1}$ and *M. piperita* = $15.8 \mu\text{l} \cdot \text{air}^{-1}$) is higher than PEOs (*S. officinalis* = $10.87 \mu\text{l} \cdot \text{air}^{-1}$ and *M. piperita* = $13.58 \mu\text{l} \cdot \text{air}^{-1}$). These values are calculated after 24 h exposure time. So, this results seem to be logical because the releasing is so low in first days and it gradually increase. Outputs are shown in Fig. 4. In the figure is showed the significant differences among the concentrations. Therefore, according to the LC_{50} s, the fumigant toxicity of *M. piperita* is significantly more than *S. officinalis* (Tab. 2).

The relative toxicity calculated by LC_{50} shows that the ratio of LC_{50} of NFOs to PEOs is about 1.05 and 1.44

Table 1. Parameters for optimal nanofiber thickness of diameter

Concentration [%]	Space [cm]	DMF [%]	Salt [%]	Average diameter [μm]
22.0	12.1	16.2	3.9	0.058

DMF – Dimethylformamide

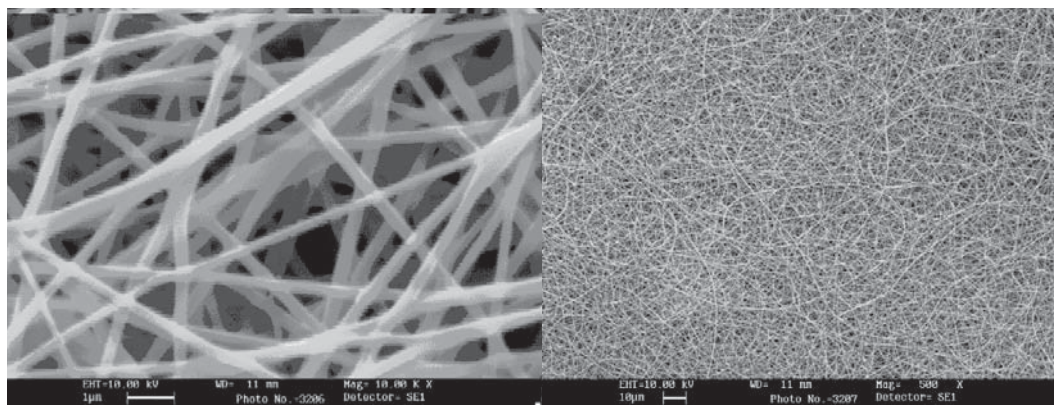


Fig. 2. Scanning electron microscope (SEM) photographs of the diameter distribution of the electrospun nanofibers of the different formulations

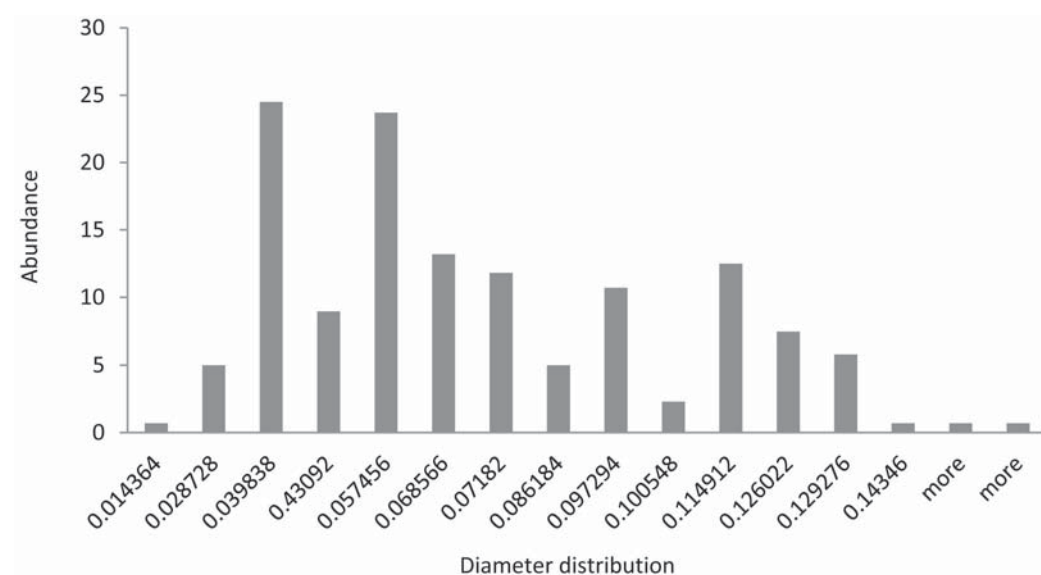


Fig. 3. The optimal diameter distribution

Table 2. Fumigant toxicity of NFOs and PEOs against 1st instar larvae of *P. interpunctella* after exposure at 27°C and 65% RH

Plant	Type	N	LC ₅₀ (95% fiducial limits)	Slope±SE	df	p-value	Chi square (χ ²)	Time [h]
<i>Salvia officinalis</i>	NFO	625	12.0 (11.4–12.7)	1.9±0.2	4.51	0.86	8	24
	PEO	625	10.87 (9.75–13.8)	7.8±1.0	1.24	0.78	3	24
<i>Mentha piperita</i>	NFO	625	15.8 (14.8–16.5)	2.0±0.1	1.75	0.75	7	24
	PEO	625	13.58 (10.8–16.2)	7.5±1.0	1.75	0.75	3	24

NFO – nanofiber essential oil; PEO – pure essential oil; N – number of insects

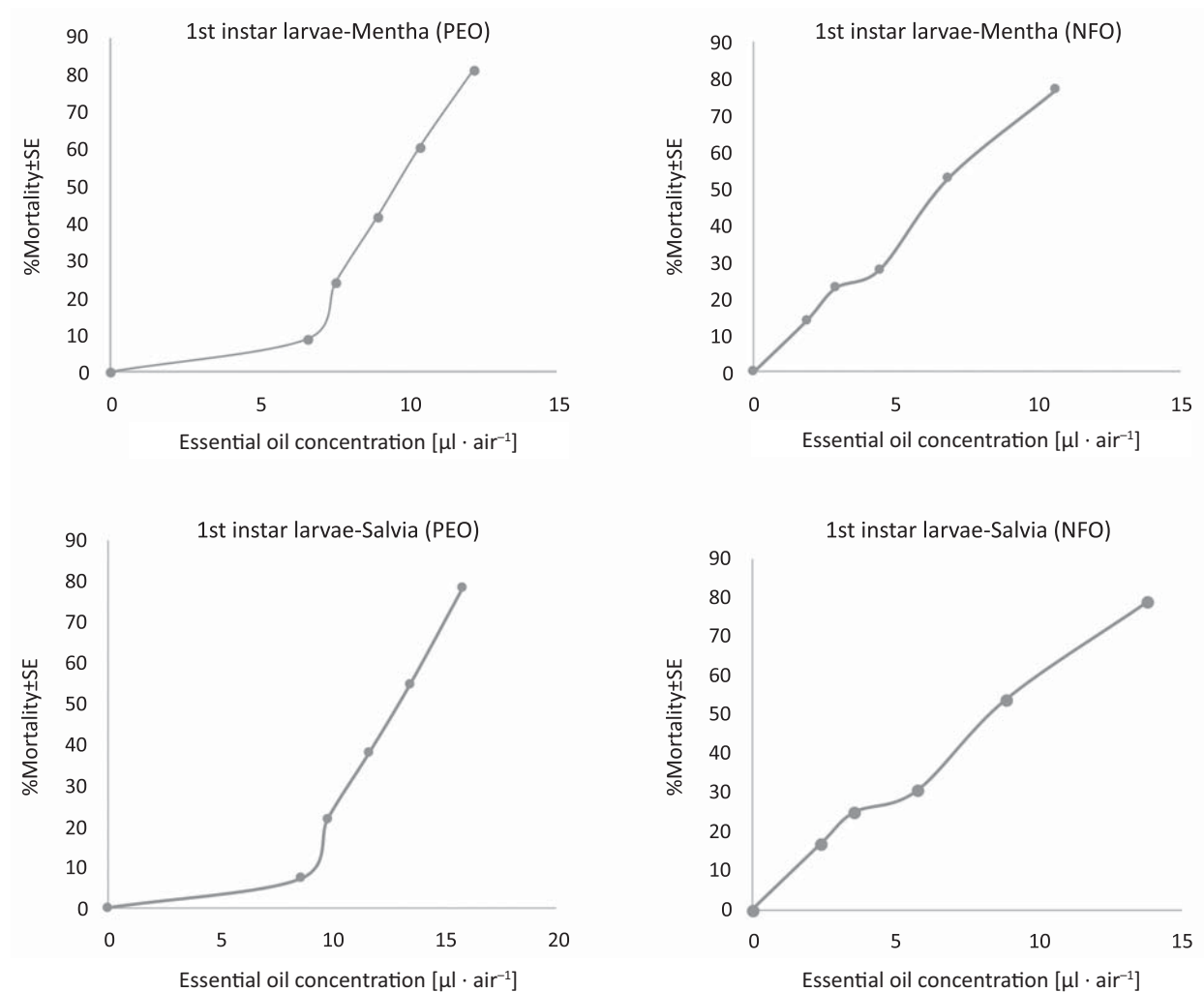
for *S. officinalis* and *M. piperita*, respectively. The 95% fiducial limits are 0.86 to 1.30 for *S. officinalis*. These values of *M. piperita* are 1.19 to 1.78. The results show that there is a significant difference between the LC₅₀s of PEO and NFO. Their regression lines of EOs (*S. officinalis* ($x^2 = 3.087$, $df = 1$, $p\text{-value} = 0.142$) and *M. piperita* ($x^2 = 1.9$, $df = 1$, $p\text{-value} = 0.167$) are parallel.

In the nanofibers made of PLA the degradation rate was more than PLA. In the investigation of persistence of EOs, the curve of PEOs descended with storage time (Fig. 5). The curve of NFOs showed that the first mortality occurred 24 h after the beginning of the experimer and the mortality percentage of insects increased with the pass of time. The release rates of PEOs and NFOs

Table 3. LT_{50} of release rate of fumigant toxicity of NFOs and PEOs (at a concentration of $72.64 \mu\text{l} \cdot \text{air}^{-1}$) against 1st instar larvae of *P. interpunctella*

Plant	Type	N	p-value	χ^2 (df)	Slope \pm SE	LT_{50} [day]
<i>Salvia officinalis</i>	PEO	625	0.99	0.68(8)	-5.6 ± 0.7	4.8(4.2–5.3)
	NFO	625	0.99	0.99(6)	-4.3 ± 0.7	26.1(24.3–28.4)
<i>Mentha piperita</i>	PEO	625	0.98	0.94(8)	-4.3 ± 0.3	6.2(5.6–6.8)
	NFO	625	0.98	1.29(6)	-3.6 ± 0.3	22.7(20.4–24.6)

PEO – pure essential oil; NFO – nanofiber essential oil; N – number of insects

**Fig. 4.** A comparison of fumigant toxicity of nanofiber essential oil (NFO) and pure essential oil (PEO) against 1st instar larvae of *Plodia interpunctella* for both essential oils of *Mentha piperita* and *Salvia officinalis*

over time are presented in Table 3 and Figure 5. The impact of PEOs volume was clearly enhanced during the first hours after exposure. The curve of PEOs descended with storage time.

The greatest mortality percentage occurred during days 10 to 18 and at this period of time the slope of curve was very low. During the next days, there was a significant decrease in the mortality of the larvae and release of essential oils. The release of essential oils and thus

mortality continued and gradually became constant. Then, the releasing of EOs continued over 40 days.

On the twelfth day, the mortality percentage of larvae exposed to PEOs of *M. piperita* and *S. officinalis* was 11% and 5%, respectively. However, at the same time the mortality percentage for NFOs of the mentioned plants was maximum and the recorded volumes were 98% and 88%, respectively. There was a significant difference between LT_{50} s of the pure and formulated

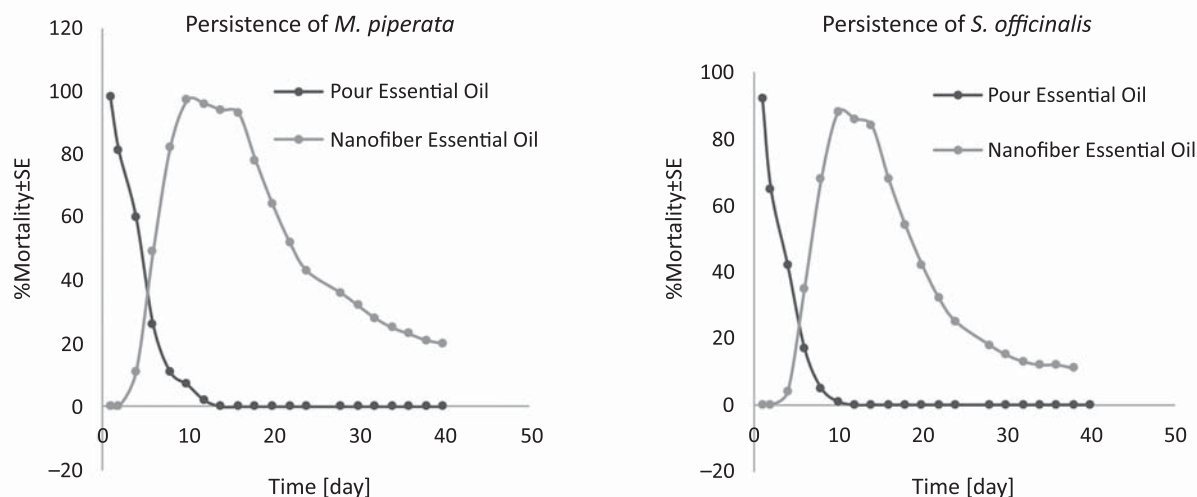


Fig. 5. The compatibility of the release rate of essential oils (EOs) in both pure essential oil (PEO) and nanofiber essential oil (NFO) against *P. interpunctella*

essential oils (Tab. 3). Finally, fumigant toxicity and release rate of *M. piperita* were more than that of *S. officinalis*. After 40 days of exposure, the per cents of adult emergence in both pure and nanofiber essential oils were 62.22 and 24.9 for *M. piperita* and 38.3 and 82.84 for *S. officinalis*.

Discussion

Nanofibers as nanoscaffolds are widely used in tissue engineering. In this study, the impact of released EOs from polymeric carriers was investigated. Electrospun nanofibers were favorable carriers for this study. They provide high stiffness and strength by strong chain and crystal orientations induced by the spinning process, thus enhancing the fiber stability during use as a layer on the products or through foodstuff sacks (Huang *et al.* 2003; Hellmann *et al.* 2009). A diameter of 58 nm was the best diameter when 14% of EOs was loaded. According to the results, the insecticidal impact of *S. officinalis* continued to be more than *M. piperita* but the persistence of *M. piperita* was more than *S. officinalis*. Finally, the results of LC_{50} s proved the NFOs can be in average 4 time more effective on control of the larvae in comparison with PEOs. The result was similar to the findings of Mori *et al.* (2015) who believed that this decrease in thickness of diameter may be because the electric conductivity of the solution decreased with the addition of essential oil, and resulted in an increase of the nanofibers' diameters.

Therefore, the small diameter of such nanofibers was advantageous since the strength of fibers increases with a decrease in the diameter (Griffith 1921). Furthermore, small fiber diameters which fall within the nanometer scale, having unique fibers with more contact

space, reduce the resistivity with respect to airflow/storm considerably due to the Knudsen effect (Maze *et al.* 2007). Thus, nanofibers are the best carriers of EOs in toxicology research. This research showed that: 1) webs known as nanofibers with defined diameters were a suitable choice of carriers to keep the EOs for more than 40 days, 2) the calculated LC_{50} for control of *P. interpunctella* in both NFO and PEO for fumigant toxicity showed that the LC_{50} for NFO formulation was significantly more than for PEO. As a result essential oils loaded through nanofibers as a carrier enhance its toxicology properties, reduce the feeding of larvae and extend the time of larvae development by controlling their respiratory systems to moderate the hard conditions. This period for PEO is longer than NFO since the insects exposed to NFO in the pupa stage had the greatest mortality; 3) have a continuous and controlled release of insecticide instead of explosive release.

This research demonstrates that initial mortalities of insects as a result of the releasing of EOs occurred after 48 h and we observed the greatest release on the tenth day. There was a constant release of NFOs after 40 days; however, PEOs had the greatest release in the initial hours of the first day and decreased quickly hours later. After day 14 the volume of release declined to cause no mortality. According to the results, the insecticidal impact of *S. officinalis* continued to be more than *M. piperita* but the persistence of *M. piperita* was more than *S. officinalis*. Finally, the results of LT_{50} s proved the NFOs can be in average 4 times more effective on control of the larvae in comparison with PEOs. In these investigations the liquid essential oils were incorporated into the nanofibers to more than 14 wt% of polymeric solution. Also, in this research the essential oils were released from the nanofibers under storage conditions over a period of several weeks, until the amount of release stayed constant.

However, the poly(lactic acid) polymer, an electrospun-nanofiber, is a biodegradable polymer which has a high capacity to load green pesticides such as essential oils. When used it is not hazardous and the release rate continues for a period of time if the material is chosen appropriately. As a result, when the polymer decomposed and was destroyed, the release of EOs from the PLA nanofiber took place. Therefore, this offers a new formulation of pesticides electrospun through nanofibers and the volume of its impact is related to many factors such as chemical combinations, the fibers' diameters and the method of application of the formulation. Finally, the new formulation of essential oils loaded in nanofibers can be a textile layer inside foodstuff sacs that control *P. interpunctella* by both fumigation and contact toxicity for a prolonged period of time. Reducing the dosing frequency responsible of the stability and strength of insecticides through nanofibers makes it possible to obtain more toxicity. Thus, the innovative strength of PLA packaging with insecticidal properties had a direct impact on consumer health by creating safer and more wholesome packaged foods up to 40 days for essential oils and improved the insecticidal toxicity. During the slow release, the larvae decreased their nutrition to control their respiratory system, creating unfavorable conditions for them because the growth and development period of the larvae was longer.

We found that there was still a significant amount of EOs being released through PLA nanofibers even after 40 days, making it very profitable to use essential oils to protect foodstuffs against stored pests. In this study, nanofibers as carriers of essential oils were investigated as to their ability to: 1) protect the active ingredient of these pesticides from evaporation or other adverse effects; 2) enhance their toxicity, and 3) determine slow release rate of insecticide. In the new technology of developing nanofibers extensive research has recently been devoted to the fabrication of molecular imprinted polymer nanofibers. These nanofibers offer longer release of imprinted compounds than some polymer scaffolds which can lead to burst release and less effective life time, and high loading capacity with easy release. Thus, due to their high selectivity and stability, in the future we can apply them in pesticide formulations which will have greater selectivity and stability and efficiency than non-imprinted polymer nanofibers. The results of this study and similar research provide active packaging which has certain extraordinary and vital functions that provide an inert barrier between products and their external conditions.

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