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Effective Management of the *Phthorimaea operculella* (Zeller) Using PVA Nanofibers Loaded with *Cinnamomum zeylanicum* Essential Oil

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Abstract *Phthorimaea operculella* (Zeller) is one of the most common insect pests of cultivated potato in tropical and subtropical regions. In this research, a potential strategy to improve the insecticidal activity of plant essential oils for the effective management of *P. operculella* was studied. The insecticidal and residual effects of nanofiber oil (NFO) and pure essential oil (PEO) of *Cinnamomum zeylanicum* were assessed on PTM under laboratory conditions. The nanofibers were made by the electrospinning method using polyvinyl alcohol (PVA) polymer. The morphological characteristics of the nanofibers were evaluated by scanning electron microscopy and Fourier transform infrared spectroscopy. The chemical constituents of cinnamon essential oil (EO) were detected by GC/MS. Fumigant toxicity of NFO and PEO were evaluated on different growth stages (egg, male and female adults) of *P. operculella*. SEM and FTIR analyses confirmed the presence of EO on the nanofiber structure. The yield of the EO from *C. zelanicum* on the nanofibers was 1.86%. GC/MS analysis showed that cinnamaldehyde was the primary constituent (69.88%) of cinnamon EO. LC_{50} values of *C. zelanicum* EO and NFO were 4.92 and 1.76 μl/l air for eggs, 0.444 and 0.212 μl/l air for female adults, and 0.424 and 0.192 μl/l air for male adults, respectively. Fumigant bioassays revealed that NFO was more toxic than *C. zeylanicum* oil against at all stages of *P. operculella*. The residual effect of PEO and NFO was evaluated against the egg stage of the *P. operculella*. NFO lost insecticidal effectiveness 47 days after application, while the efficacy of PEO decreased 15 days after application. Our results suggest that NFO of *C. zeylanicum* can be used as an effective new tool for the management of *P. operculella*.

Resumen *Phthorimaea operculella* (Zeller) es uno de los insectos-plaga más comunes de la papa cultivada en las regiones tropicales y subtropicales. En esta investigación se estudió una estrategia potencial para mejorar la actividad insecticida de los aceites esenciales vegetales para el manejo efectivo de *P. operculella*. Se evaluaron los efectos insecticidas y residuales del aceite nanofibra (NFO) y el aceite esencial puro de *Cinnamomum zeylanicum* (PEO) en PTM bajo condiciones de laboratorio. Se hicieron las nanofibras por el método de electrogiro (electrospinning) usando el polímero de polivinil-alcohol (PVA). Se evaluaron las características morfológicas de las nanofibras mediante el microscopio electrónico de barrido y por espectroscopía Fourier transformada infra-roja. Se detectaron los constituyentes químicos del aceite esencial de la canela (EO) mediante GC/MS. Se evaluó la toxicidad fumigante del NFO y del PEO en diferentes estados de crecimiento (por ejemplo, huevo, adultos macho y hembra) de *P. operculella*. Los análisis por SEM y FTIR confirmaron la presencia de EO en la estructura de la nanofibra. El rendimiento deEO de *C. zelanicum* en las nanofibras fue de 1.86%. El análisis de GC/MS mostró que el cinnamaldehido era el constituyente

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primario (69.88%) del EO de la canela. Los valores LC_{50} del EO y NFO de *C. zelanicum* fueron 4.92 y 1.76 μl/l de aire para huevos, 0.444 y 0.212 μl/l de aire para las hembras adultas y 0.424 y 0.192 μl/l de aires para los machos adultos, respectivamente. Los bioensayos de fumigantes revelaron que el NFO era más tóxico que el aceite de *C. zelanicum* contra de todos los estadíos de *P. operculella*. El efecto residual de PEO y NFO se evaluó contra el estado de huevo de *P. operculella.* NFO perdió su efectividad insecticida 47 días después de la aplicación, mientras que la eficacia de PEO disminuyó 15 días después de la aplicación. Nuestros resultados sugieren que el NFO de *C. zelanicum* puede usarse como una nueva herramienta efectiva para el manejo de *Phthorimaea operculella.*

Keywords Potato tuber moth · Nanofiber · Essential oils · PVA · Bioassay

Introduction

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is one of the most common pests of potato, *Solanum tuberosum* L., in tropical and subtropical regions (Westedt et al. 1998; Malakar-Kuenen and Tingey 2006). The PTM originated in South America, but currently it is found in almost all potato-growing regions worldwide. PTM recently appeared as a potential economic pest of potatoes in most parts of Iran (Allahverdizadeh and Mohammadi 2016). PTM causes serious damage to stored potato through larval tunnelling and feeding, leading to partial or complete rotting due to subsequent infestation by microbial agents such as fungi and bacteria (Moawad and Ebadah 2007).

Synthetic insecticides are commonly used to control insect pests including the PTM throughout the world, but the extensive use of chemical pesticides has resulted in harmful side effects such as health risks from residues (Dikshit et al. 1985), depopulation of natural enemies (Shelton et al. 1981), and the development of resistance (Haines 1977; Llanderal-Cazares et al. 1996). There is an urgent need to find safe and effective alternatives to these chemical compounds. Plant essential oils (PEOs) or essential oils (EOs) of plants have been considered as an alternative biopesticide that could play a predominant role in integrated pest management (IPM) strategies (Sharaby et al. 2009). Thus far, the effect of EOs on PTM has not been well studied (Naghizadeh 2013; Rafiee-Dastjerdi et al. 2013). *Cinnamomum zeylanicum* Blume is a tropical tree of the Lauraceae family. The main component in this plant's essential oil is cinnamaldehyde, which has insecticidal effect (Ishii et al. 2010; Fouad 2013; Benchouikh et al. 2015).

Despite the immense potential of EOs for pest insect control, EOs have disadvantages such as their high cost of production, low vapour pressure, high volatility, low residual effect, strong odour, and phytotoxicity, which limit their applicability (Isman et al. 2010). A way to overcome these disadvantages is the incorporation of EOs into a controlled-release nanoformulation that prevents rapid evaporation and degradation; enhances stability and maintains the minimum effective dosage/application (Ghormade et al. 2011). In addition, the nanoformulation, compared to pure essential oils (PEOs) (i.e., non-formulated), is expected to be more effective against pests, less toxic towards non-target organisms, lead to reduced pesticide application, and increased residual effect of the active ingredient (Devi and Maji 2011; Anjali et al. 2012). Very few published studies are available on the insecticidal efficacy of nanoformulations loaded with EOs acting against insect pests. Negahban et al. (2012a, b) studied the fumigant properties of nano-encapsulated EOs from *Artemisia sieberi* Besser against pests of stored products. Their results showed that nano-encapsulated EOs have greater durability compared to pure EOs. Nennah et al. (2015) also evaluated the insecticidal effects of three Asteraceae essential oils and their nanoemulsions against stored product pest. They reported that the LC_{50} values decrease in the case of nanoemulsion oils in comparison to pure EOs.

In the current study, we used the electrospinning method for the production of nanofibers. Electrospinning is a simple yet versatile method for the production of polymer-based, high-performance and highly functional nanofibers that can revolutionize the structure of materials including controlled and slow release systems. Nanofibers produced by electrospinning show several important features such as a large surface-area-to-volume ratio and superior mechanical properties (Zargham et al. 2012). Nanofibers infused with EOs can also be formed by using this method. To our knowledge, this study is the first to test the efficacy of nanoformulated oil, based on polyvinyl alcohol (PVA) polymer for the control of stored product pests.

The aims of this study were to: (i) determine the chemical constituents of *C. zeylanicum* EO, (ii) evaluate the fumigant toxicity of *C. zeylanicum* PEO and NFO against different growth stages (egg, male and female adults) of PTM, (iii) investigate the residual effect of both PEO and NFO, and (iv) determine the morphological characteristics of NFO.

Materials and Methods

Insect Rearing

The initial population of PTM used in this study was collected from potato stores infested with PTM in the Ardabil province (38° 15′ 53″ North, 48° 16′ 18″ East), Iran. The colony of PTM was reared on the cultivar Agria of potato. This cultivar is the most commonly cultivated potato cultivar grown in Iran. The insect rearing was carried out under laboratory conditions

at 26 ± 2 °C, 60 ± 5% RH, and a photoperiod of 14: 10 (L: D). To obtain PTM eggs of the same age, 20–25 male-female pairs of the newly emerged moths were kept inside oviposition containers. The adults were fed using a piece of cotton imbued with a solution of 10% honey in water. The oviposition container consisted of a clear Plexiglas cylinder (15 cm in diameter and 20 cm in height) covered with fine mesh netting on the ends. Filter paper placed on the netting provided an oviposition site for the moths (Golizadeh and Zalucki 2012).

Essential Oil (EO)

Plant Materials and Essential Oil Extraction

Cinnamon bark was obtained from a local market in Ardabil, Iran. 70 g of milled bark together with 700 ml of distilled water were put into a Clevenger apparatus for 3 h of hydrodistillation. After extraction, anhydrous sodium sulphate was used to remove the water. The resulting EOs were stored in dark glass containers and kept in a refrigerator at 4 °C (Negahban et al. 2007).

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of EO

GC-MS analysis was carried out on a GC 6890 N (Agilent, USA) equipped with a split injector and MS 5973 N mass selective detector system. Chromatographic separation was carried out in an HP5ms capillary column (30 m × 0.25 mm, 0.25 μm in film thickness). The MS acted in the EI mode (70 eV). Helium (99.99%) was used as the carrier gas with a flow rate of 1 mL min^{-1}. Column pressure was 8.75 PSI. The injector temperature was set at 150 °C, the column temperature programme started at 10 °C for 3 min, increased by 10 °C min^{-1} to 120 °C, by 10 °C min^{-1} to 150 °C, and by 7 °C min^{-1} to 240 °C, and was maintained for 5 min. Identification of spectra was carried out by studying their fragmentation and comparing with standard spectra present in the library of the instrument (Adams 2001).

Nanofiber

Preparation of Nanofiber

Pure polyvinyl alcohol (PVA) polymer supplied by the Kuraray Co., Ltd. (Japan), was dissolved in distilled water at 10% (*w*/*v*) concentration, and the solution stirred using a magnetic stirrer (MTOPS, MS300HS model, Turkey) for 3 h until the polymer was completely dissolved.

An electrospinning apparatus (manufactured by FannavaranNano-meghyas Company) was used for the electrospinning process (Sill and Recum 2008). Five ml of the solution was put into a syringe, which was connected to a metal needle. An adjustable DC power supply was held at the tip of the needle as an anode, and the aluminium collector was rotated as a cathode. The feed rate of the syringe pump was set at 0.5 mL/h, and the working distance from the spinneret to the aluminium collector was 15 cm under an applied voltage of 17 kV. After the electrospinning, the collected nanofiber samples were kept in an oven at room temperature for two hours to dry. In the next step, the desired concentration of EO was loaded on the produced nanofibers.

Characterization of the Nanofibers

Morphological Characterization The morphology of the nanofibers that were loaded and non-loaded with EO was evaluated by scanning electron microscopy (SEM) (LEO 1430VP, Germany) at an accelerating voltage of 15 kV. Before scanning electron microscopy observation, all samples with a mat size of 10 × 10 mm^2 were attached to metallic stubs coated with gold by ion sputtering for 120 s under a current of 15 mA and vacuum pressure (Polaron, SC 7690, UK). Several positions were selected randomly on the SEM images (30,000× magnification) of the nanofiber mats and examined with imaging software (ImageJ, National Institutes of Health, Bethesda, MD) to determine whether there were any morphological changes in the structure of NFO.

Fourier Transform Infrared (FTIR) Spectroscopy Fourier transform infrared (FTIR) spectroscopy (PerkinElmer, Spectrum RXI, USA) was applied to characterize the presence of particular chemical bonds in the nanofibers before and after the loading with EO. The wave number region for the analysis was 4000–400 cm^{-1} (in the mid-infrared range).

Bioassays

Fumigant Toxicity

The fumigant effects of EO were evaluated as described by Negahban et al. (2007). Bioassay tests were conducted on eggs and adult (male and female) stages (<24 h old) of PTM. Glass vials containing 250 ml were used for the experiments. Twenty-five individuals of each stage of PTM were subjected to each treatment dose. Filter paper (Whatman No. 1) and non-loaded nanofibers (NLN) were placed under the surface of screw caps and injected with an appropriate concentration of the EO. Preliminary dose-setting trials were carried out to obtain the highest and lowest concentrations causing 80% and 20% mortality during treatment (Robertson et al. 2007). Concentrations of PEO and NFO were 3.2–6.8 and 1.24–2.16 μl/l air for egg stage, 0.15–0.88 and 0.07–0.36 μl/l air for male adult stage and 0.16–1 and 0.08–0.4 μl/l air for female adult stage, respectively. The control treatment included insects that were exposed to nanofibers without EO. Four

replicates of each treatment and control were set up in experiments. Cap-glass vials were covered with Para film so as to prevent any escape of the EO. Experiments were carried out in an incubator that was set at 26 ± 2 °C, 60 ± 5% RH and photoperiod of 14: 10 (L: D). Mortality was recorded 24 h after treatment in the adult stage and after hatching in the egg stage.

Residual Effect

LC_{99} values that were determined from the fumigant toxicity tests were used for the residual effect experiments. All of the other aspects of the residual effect experiments of PEO and NFO were the same as described above for the fumigant toxicity assays. Twenty-five eggs of PTM were introduced to each vial every 2 days following the initial treatment. Starting at 5 days post exposure, mortality was recorded daily for 15 days for the PEO treatments, and for 47 days in the case of NFO. Four replications were made for each exposure. The residual effect experiment was continued until the treatments lost their insecticidal effects.

Data Analysis

Data were tested using PROC GENMOD (Robertson et al. 2007; SAS Institute 2002), and data analysis were conducted using the PROC PROBIT to compute LC_{50} (median lethal concentration), LC_{99}, and PT_{50} (median persistence time of the oil) values on standard and log scales with associated 95% fiducial limits using the SAS programme (SAS Institute 2002). Graphs were plotted using Excel software (2010 version).

Results

Chemical Constituents of EO from *C. zelanicum*

The yield of the EO from *C. zelanicum* was 1.86% (*w*/*v* based on dry weight). A total of 45 components from the EO of *C. zelanicum* were identified, accounting for 99.89% of the total EO (Table 1). The major constituents in the oil were cinnamaldehyde (69.88%), followed by naphthalene-1,2,3,4-tetrahydro-1,6-dimethyl-4-(1. (5.45%),naphthalene-1,2,4a,5,6,8a–hexahydro-4,7-dimethyl (2.41%), paramethoxycinnamic aldehyde (2.33%), tau. Muurolol (2.25%), α-copaene (2.11%), 1,3-benzodioxole (1.67%) and L-limonene (1.62%).

FTIR

The functional groups present in PVA, EO, and PVA-EO were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph obtained from an FTIR spectrophotometer. The FTIR spectrum of PVA nanofiber is shown in Fig. 1a. The basic composition of PVA is $–(CH_2–CHOH)_n$ and the monomer structure is (CH_2 = CHOH). Fig. 1a clearly reveals the major peaks associated with PVA. The peak observed at approximately 1100 cm^{-1} is attributed to the presence of terminal polyvinyl groups, whereas the peak 1376 cm^{-1} shows $–CH_2$, and the peak at 1735 cm^{-1} indicates the –C = O carbonyl stretching bond. Furthermore, the peak obtained at 2941 cm^{-1} indicates a -CH stretching bond and the peak at 3327 cm^{-1} is a hydrogen bonded –OH group. Fig. 1b clearly reveals the major peaks associated with EO. The infrared spectrum of PEO showed absorption bands located at 3336 and 3506 cm^{-1} (− OH), 2816 and 2744 cm^{-1} (−CH), 1891 and 1811 cm^{-1} (−C = O stretching), between 1678 and 1495 cm^{-1} (−C = C), between 1178 and 1007 cm^{-1} (−CO) and between 689 and 845 cm^{-1} (−C = C). The infrared spectrum of the PVA-EO (Fig. 1c) showed absorption bands at 2940, 2817, 2744, 1732, 1677–1495, 1376, 1179–1007 and 847–689 cm^{-1}.

SEM

SEM was used to investigate the effect of EO loading on the morphology of the electrospun nanofibers. Agglomeration in the fibers loaded with EO was observed by SEM (Fig. 2). Moreover, the SEM images showed the formation of the layers in between the fibers. The obtained diameter of the nanofibers was 79.596 nm.

Fumigant Bioassay

LC_{50} values of *C. zelanicum* EO and NFO at the egg stage of the PTM were 4.92 and 1.76 μl/l air (equivalent 1.23 and 0.44 μl), respectively. For the female adult stage, these values were 0.444 and 0.212 μl/l air (equivalent 0.111 and 0.053 μl), respectively. Moreover, the LC_{50} values of EO and NFO at the male adult stage were 0.424 and 0.192 μl/l air (equivalent 0.106 and 0.048 μl), respectively (Table 2). A statistically significant difference in the toxicity of EO and NFO treatments was found at the egg and adult growth stages of PTM, as inferred by the lack of overlap in the LC_{50} confidence intervals. Also, the LC_{99} values of EO and NFO at the egg stage were 13.08 and 3.84 μl/l air, respectively. These values were 4.84 and 1.56 μl/l air, for the female adult stage, and 4.2 and 1.23 μl/l air for the male adult stage, respectively (Table 2). The insecticidal activity of EO and NFO in the fumigant bioassays depended on their concentrations. Furthermore, there was a positive correlation with the increased susceptibility of the PTM growth stages and EO concentration, resulting in an increased mortality rate with an increase in oil concentration (Fig. 3).

Table 1 Chemical analysis of essential oils of *C. zelanicum* by GC-MS

Compound	RT*	Percentage	Formula
α- Pinene	4.7	0.12	$C_{10}H_{16}$
Benzaldehyde	5.16	0.13	C_7H_6O
L-Limonene	6.39	1.62	$C_{10}H_{16}$
1-Octanol	7.06	0.11	$C_8H_{18}O$
Linalool	7.59	0.2	$C_{10}H_{18}O$
β-Thujone	7.74	0.08	$C_{10}H_{16}O$
Benzenepropanol	8.85	0.77	$C_9H_{10}O$
α- Terpinene	9.34	0.19	$C_{10}H_{16}$
Benzene, 1-methoxy-4-(2-propenyl)	9.49	0.74	$C_{10}H_{12}O$
Cinnamaldehyde	12.56	69.88	C_9H_8O
(+)-Cyclosativene	13.24	0.26	$C_{15}H_{24}$
α- Copaene	13.44	2.11	$C_{15}H_{24}$
Aromadendrene	13.70	0.13	$C_{15}H_{24}$
α- Amorphene	13.80	0.13	$C_{15}H_{24}$
(−)-Isosativene	14.09	0.2	$C_{15}H_{24}$
Trans- caryophyllene	14.28	0.31	$C_{15}H_{24}$
Bergamotene	14.47	0.14	$C_{15}H_{24}$
Trans-cinnamyl Acetate	14.82	0.46	$C_{11}H_{12}O_2$
α- Caryophyllene	14.99	0.13	$C_{15}H_{24}$
Para Methoxy Cinnamic Aldehyde	15.08	0.16	$C_{10}H_{10}O_2$
Naphthalene, 1,2,3,4,4a,5,6,8a- octahydro-7-methyl…	15.45	0.63	$C_{10}H_8$
Curcumene	15.54	0.59	$C_{15}H_{22}$
Naphthalene, 1,2,4a,5,6,8a- hexahydro-4,7-dimethyl…	16.00	2.41	$C_{10}H_8$
Naphthalene, 1,2,3,4,4a,5,6,8a- octahydro-7-methyl…	16.28	0.24	$C_{10}H_8$
Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1…	16.54	5.45	$C_{13}H_{18}$
Para Methoxy Cinnamic Aldehyde	16.84	2.33	$C_{10}H_{10}O_2$
Naphthalene, 1, 2-dihydro-1,1, 6-tetra…	16.97	0.42	$C_{13}H_{16}$
Elemicin	17.14	0.45	$C_{12}H_{16}O_3$
Nerolidol	17.29	0.21	$C_{15}H_{26}O$
Guaiene	17.48	0.11	$C_{15}H_{24}$
Caryophyllenyl alcohol	17.60	0.3	$C_{15}H_{26}O$
(+)-Spathulenol	17.75	0.9	$C_{15}H_{26}O$
Benzene, 1,2,3,4-tetramethoxy-5…	17.96	0.47	$C_{13}H_{18}O_4$
Curcumene	18.26	1.08	$C_{15}H_{22}$
Apiol	18.53	0.32	$C_{12}H_{14}O_4$
Cubenene	18.67	0.94	$C_{15}H_{24}$
Tau. Muurolol	18.96	2.25	$C_{15}H_{26}O$
α- Cadinol	19.17	0.51	$C_{15}H_{26}O$
α- Humulene	19.37	0.29	$C_{15}H_{24}$
1,3- Benzodioxole	19.62	1.67	$C_7H_6O_2$
Vulgarol B	19.94	0.11	$C_{15}H_{24}O$
Nephthalene-6-ethyl-1,2,3…	20.05	0.13	$C_{12}H_{16}$
Benzylbenzoate	21.02	0.07	$C_{14}H_{12}O_2$
Z-7-Hexadecanal	21.39	0.05	$C_{16}H_{32}O$
Calamenol	21.67	0.09	$C_{15}H_{22}O$

*Retention time (min)

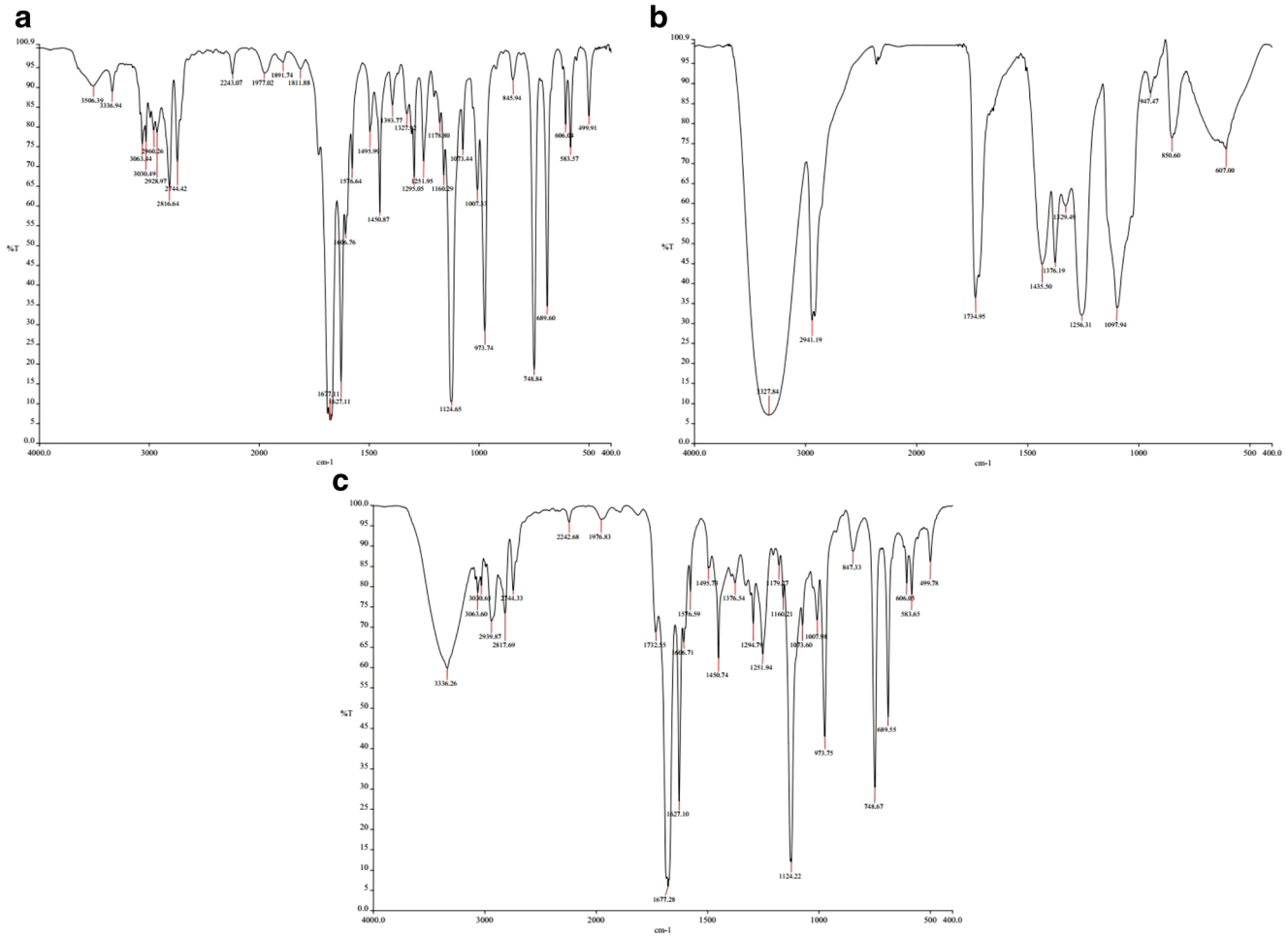

Fig. 1 FTIR spectra for **a** *C. zelanicum* EO, **b** PVA nanofiber **c** NFO

Residual Effect

The residual effect of EO and NFO over time is shown in Fig. 4. The insecticidal effectiveness of EO and NFO decreased with increasing storage time. The insecticidal potential of *C. zelanicum* EO was high after 3 days of storage and caused 92% mortality, while mortality only reached 29% when EO was stored for 5 days. During the same periods, NFO treatment induced 99% and 98% mortality, respectively. PT_{50} values of *C. zelanicum* EO and NFO at the egg stage of the PTM were 4.60 and 18.27 days, respectively.

Discussion

Electrospinning is a well-known method to produce fibers from different materials on a nano-scale (Teo and Ramakrishna 2006). Nanofibers show several important characteristics such as a large surface-area-to-volume ratio, high porosity, and superior mechanical properties. In this study, we used electrospinning to produce PVA nanofibers containing plant essential oils for PTM control. The results of FTIR spectra showed the establishment of the functional groups of EOs on the structure of the PVA. In the case of the PVA-EO spectrum, functional groups associated with the structure of PVA included peaks at: 1376 cm^{-1} ($-CH_2$), 1732 cm^{-1} (−C = O) and 2940 cm^{-1} (−C-H). Also, functional groups associated with the EO structure included peaks at: 2817 and 2744 cm^{-1} (−C-H), 1495–1677 cm^{-1} (−C = C), 1007–1179 cm^{-1} (−CO) and 847–689 cm^{-1} (−C = C). These results are consistent with results reported by Sasipriya et al. (2013). Their results confirmed the existence of functional groups of (−O-H) [3200–3570 cm^{-1}], (−C-H) [2850–3000 cm^{-1}] and (−C = O) [1735 cm^{-1}] in the structure of PVA. Several studies and our study are associated with functional groups of cinnamon EO (Yan-qun et al. 2013; Bizuneh 2014). The images of scanning electron microscopy (SEM) also demonstrate the establishment of EO in the structure of the PVA-nanofiber. Changes in the structure of the nanofibers loaded with EO demonstrate the entrapment of EO molecules in the nanofiber structure.

Based on LC_{50} values, a significant reduction was observed in the amount of EO found in the formulated PVA nanofiber. These results were consistent with the results of Negahban

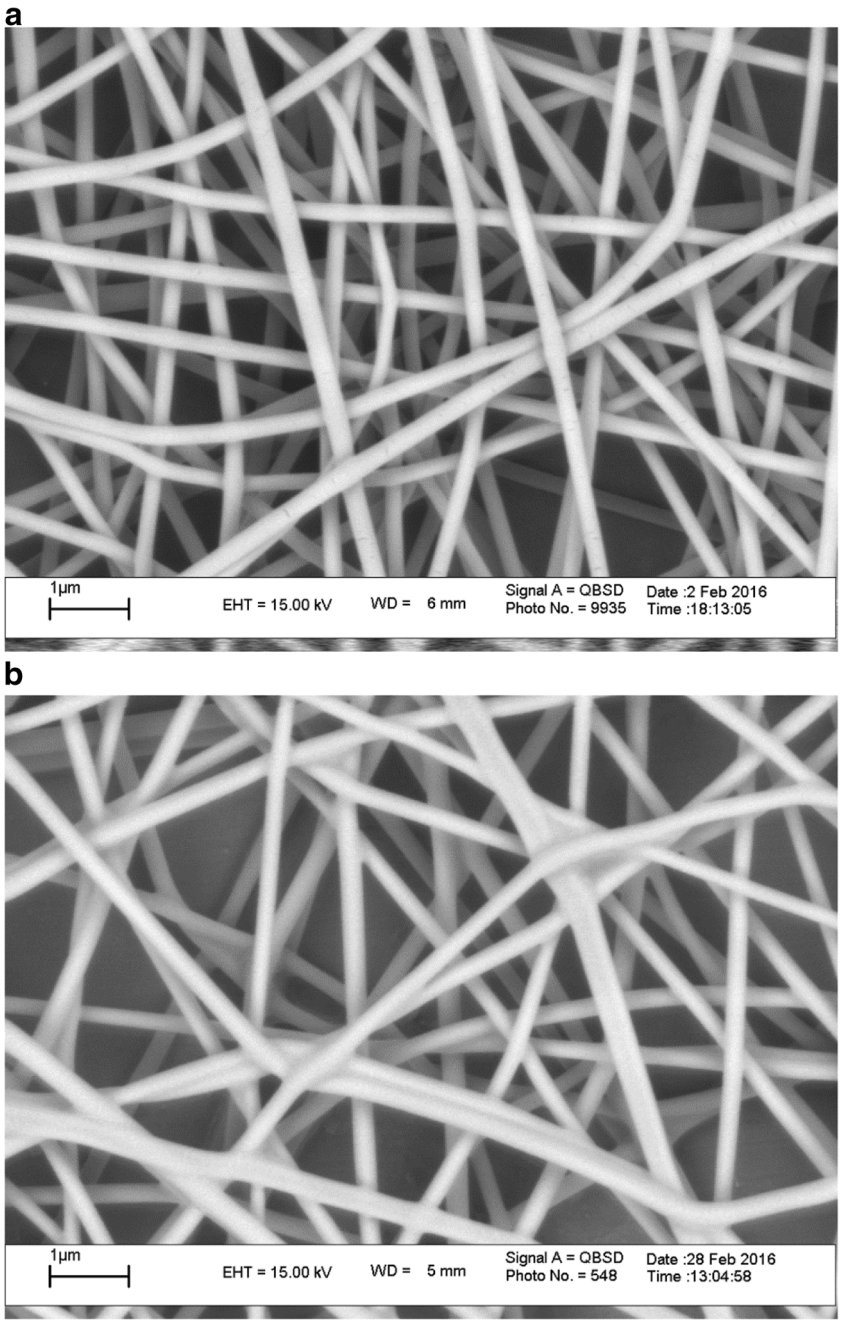

Fig. 2 Scanning Electron Microscopy (SEM) image of nano-fiber PVA without oil **a** and nano-fiber PVA with oil **b**

et al. (2012a, b). They studied the fumigant toxicity of PEO and NFO of cumin on *Tribolium*, and reported that the LC_{50} values of 32.12 ppm and NPO was 16.25 ppm for PEO and NPO, respectively. These low LC_{50} values as well as the low NFO, suggests that the use of EOs in the formulation is cost-effective.

Several studies (Rafiei-Karahroudi et al. 2010; Katila et al. 2014) have indicated the effectiveness of cinnamon EO in pest insect control.,This insecticidal efficacy is likely related to the mode of entry of essential oils. The primary constituents of EOs such as monoterpenes show maximum insecticidal efficacy through the insect respiratory system (Prates et al. 1998). Kim et al. (2003) also state that the insecticidal activity of essential oils is caused by their fumigant mode of action and prove to be toxic after entering the insect's body via the respiratory system. The fumigant toxicity assays of PEO and NFO indicated that the adult stage was more sensitive than the egg stage (i.e., the adult stage has a lower LC_{50} compared with the egg stage). These results were consistent with the results Karaboürklü et al. (2011). Our results also indicated a difference in sensitivity between male and female adults. This difference in sensitivity may be due to differences in terms of size (weight) and the amount of fat in the body (Weaver et al. 1994; Papachristos and Stamopoulos 2002; Papachristos et al.

Table 2 Toxicity of *C. zelanicum* EO and NFO to growth different stages of *Phthorimaea operculella*

Growth stages	Treatments	n	Slope ± SE	χ^2(df)	Lethal concentrations (μl) or [μl/l air]	
					LC_{50} (95% FL)	LC_{99} (95% FL)
Egg	EO	480	5.5 ± 0.6	4.88^{ns} (18)	1.23 (1.17–1.31) [4.92]	3.27 (2.72–4.31) [13.08]
	NFO	480	6.95 ± 0.83	5.63^{ns} (18)	0.44 (0.42–0.46) [1.76]	0.96 (0.82–1.23) [3.84]
Female adult	EO	480	2.24 ± 0.24	5.82^{ns} (18)	0.111 (0.097–0.13) [0.444]	1.21 (0.78–2.38) [4.84]
	NFO	480	2.68 ± 0.29	8.87^{ns} (18)	0.053 (0.047–0.059) [0.212]	0.39 (0.27–0.68) [1.56]
Male adult	EO	480	2.34 ± 0.26	2.84^{ns} (18)	0.106 (0.093–0.12) [0.424]	1.05 (0.68–2.05) [4.2]
	NFO	480	2.88 ± 0.31	3.58^{ns} (18)	0.048 (0.043–0.053) [0.192]	0.308 (0.218–0.52) [1.23]

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 2002)

2004). The results of Heydarzadeh and Moravvej (2012) and Zandi-Sohani et al. (2012) are consistent with the current results. The results of fumigant toxicity showed further losses in all growth stages following an increase in the concentration (Fig. 3) and these results were consistent with the results of Yang et al. (2009).

Several studies have shown that the insecticidal effects of EOs are associated with the chemical constituents that are found in EOs (Regnault-Roger et al. 1993; Lamiri et al. 2001; Aslan et al. 2004; Ayvaz et al. 2010). It is possible to understand the structure of EO constituents with the help of gas chromatography/mass spectrometry (GC/MS). GC/MS analysis showed that cinnamaldehyde is the primary constituent (69.88%) of cinnamon EO. Lee et al. (2008) have demonstrated the insecticidal activity of a cinnamaldehyde compound on *Sitophilus oryzae* (Coleoptera: Curculionidae). Their results showed that in residual bioassays, cassia and cinnamon oils exhibited good insecticidal activity. These results and ours indicate that the cinnamon oils merit further study as potential fumigants for the control of *S. oryzae*. Our GC/MS analysis showed that tau. Muurolol, α-copaene, L-limonene, bergamotene, curcumene, (+)-spathulenol, α-caryophyllene, elemicin, and α-humulene are also significant constituents of cinnamon EO. These results are consistent with the results of other researchers showing related constituents in EOs: Jayaprakasha et al. (2003) (linalool, (E)-cinnamaldehyde, α-copaene, spathulenol, benzyl benzoate), Boniface et al. (2012) (α-pinene, linalool, cinnamaldehyde, α-copaene), Duarte et al. (2015) (α-pinene, cinnamaldehyde), and Kazemi and Mokhtariniya (2016) (curcumene, α-pinene, naphtalene-1,2,3,4,4a-7-hexahydro).

Our results indicated that nanofibers loaded with EOs are more durable in comparison to PEOs. Thus, the residual effect of nanofibers formulated with EOs was more than 40 days, while that of PEOs was 15 days. Thus, it can be concluded that nano-fiber structures protect the oil and lead to controlled slow release. Adel et al. (2014) studied the biological activity and field persistence of EOs of *Pelargonium graveolens* L. (Geraniales: Geraniaceae) loaded on solid lipid nanoparticles (SLNs) on *P. operculella*. They found that geranium essential oil loaded on solid lipid nanoparticles is stable. A possible reason for the high durability of the nano-formulated constituents is found in oxygen. Oxygen molecules, by linking the present molecules with the PVA structure, allow greater stability of the constituents in the environment. Our GC/MS analysis showed that the main constituent in the structure of cinnamon EO is cinnamaldehyde with oxygen in its structure. Other studies shown that EOs, with oxygenated monoterpenes, have a higher durability than oils containing hydrocarbonated monoterpenes (Ngamo et al. 2007; Ilboudo et al. 2010). The results of the current study show that the insecticidal efficacy of EOs decreases over time. This may be correlated to high volatility and rapid decomposition of the chemical constituents of the essential oils (Ndomo et al. 2010). The PT_{50} (half-life time) of EO and NFO were 4.60 and 18.27 days, respectively, indicating the higher stability of NFO in comparison to the EO. These results are consistent with the results of Ziaee et al. (2014). They reported that the PT_{50} values in EOs formulated in the form of a nanogel was lower than that of PEOs. The nano-gel containing EOs had greater stability than PEOs. Our study showed that the formulation of essential oils with nanofibers lowers the LC_{50} values. The low LC_{50} value will reduce the amount of essential oil that will be consumed in terms of the amount applied. Also, our persistence study showed that the nano-formulated EOs are more persistent indicating that its release is slower. Slow release will result in control of the target pest over a longer time.

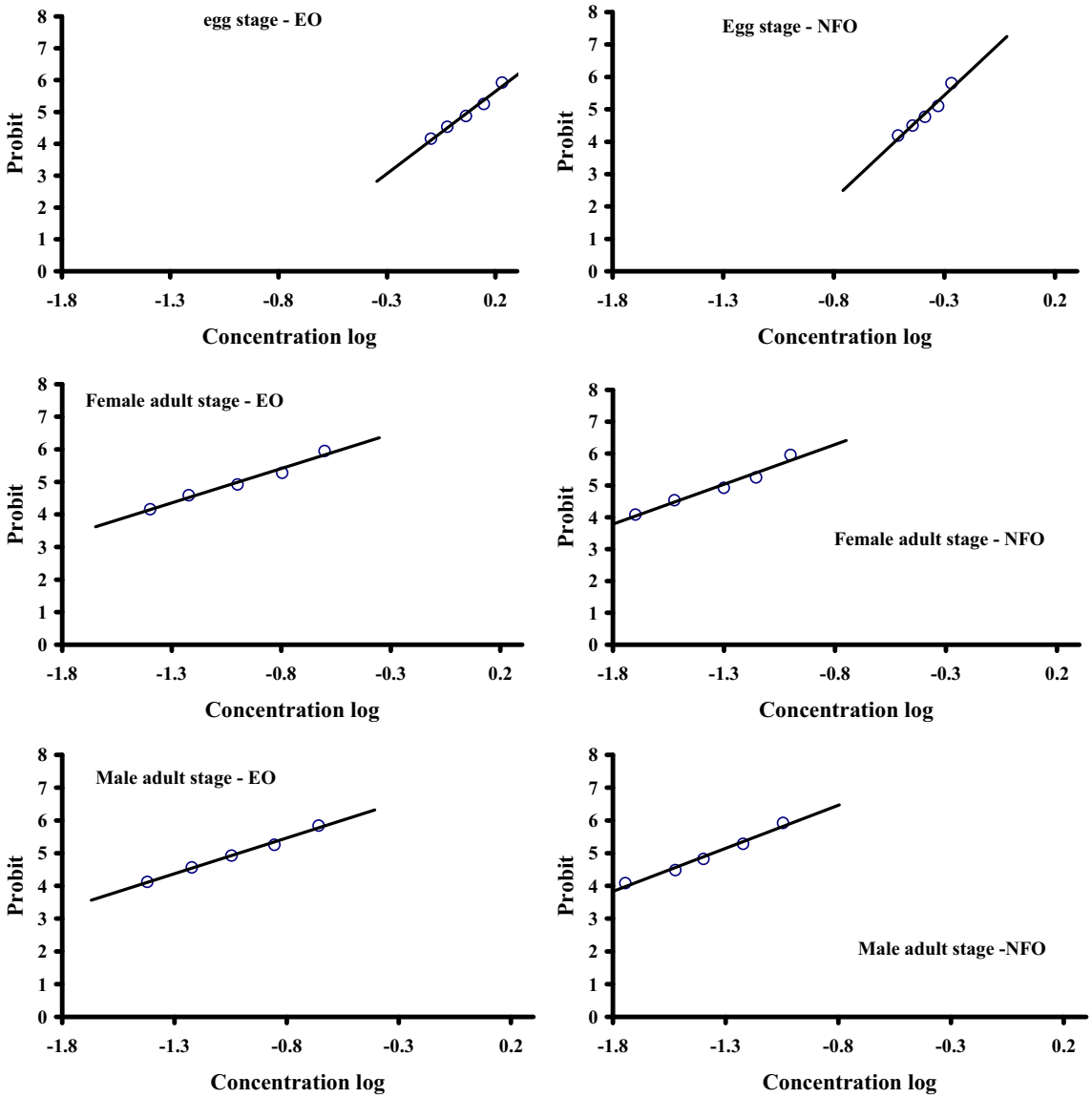

Fig. 3 Concentration-mortality response lines for growth different stages of *Phthorimaea operculella* exposed to different concentrations of *C. zelanicum* EO and NFO

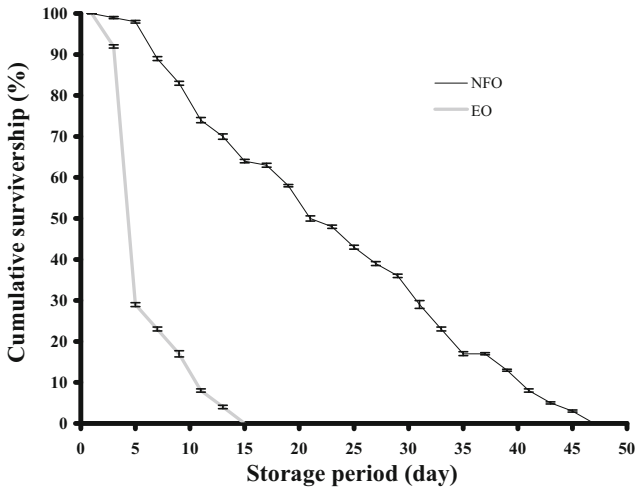

Fig. 4 Residual effects of *C. zelanicum* PEO and NFO against *P. operculella*

Conclusion

The use of nanotechnology in pest management has recently attracted the attention of agricultural scientists. In this regard, the use of insecticides formulated at the nano-scale show improved insecticidal efficacy and efficiency. These nano-scale formulations reduce lethal concentrations and increase the stability of the insecticidal compound. The strategy employed in this study to formulate essential oils of cinnamon with polyvinyl nanofibers led to a reduction in the minimum concentration of active ingredient needed to retain insecticidal and residual activities. Specifically, the use of cinnamon EO formulated in the form of nanofibers showed effectivce PTM management. If these results are confirmed under typical storage conditions in the field, these nano compounds could be

applied widely. We also suggest, for the first time, that this type of nano-scale formulation of horticultural extracts is highly effective for use in stored product pest control in general.

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