

Research Article

Synthesis of *Zingiber officinale* essential oil-loaded nanofiber and its evaluation on the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae)

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Abstract: The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), is a major pest of potato, both in the field and storehouses. In this study, we have evaluated the lethal effects and persistence of *Zingiber officinale* (Roscoe) pure (PEO) and nano-formulated essential oil (NFO) on different developmental stages (egg, male and female adults) of PTM. Essential oil was extracted by hydro-distillation using a Clevenger-type apparatus. The essential oil was analyzed by gas chromatography/mass spectrometry (GC/MS). Nanofibers were produced by electrospinning technique. The morphology of nanofibers was investigated by SEM. Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the PEO, nanofiber and PEO/NFO scaffold. Bioassays were performed in 250 ml glass jars. The essential oil consisted of α -Zingiberene as the most abundant component (14.21%), followed by Ar-curcumene (12.58%), β -sesquiphellandrene (12.48%) and cis- α -bisabolene (10.29%). The results of FTIR spectra showed the establishment of the functional groups of PEO on the structure of the nanofiber. The images of SEM also demonstrated the establishment of PEO in the structure of the nanofiber. LC₅₀ values of PEO and NFO were estimated 75.44 and 30.24 μ l/l air for eggs, 19.08 and 10.28 μ l/l air for female adults, and 17.76 and 9.56 μ l/l air for male adults, respectively. Persistence data showed that nano-formulated essential oil (49 days) in comparison with pure essential oil (15 day) had longer persistence. The results demonstrated that *Z. officinale* PEO and its nano-formulation could play an important role as natural pesticides for the management of PTM.

Keywords: Fumigant bioassay, ginger essential oil, nanotechnology, persistence, potato tuber moth

Introduction

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae),

is an oligophagous pest on agricultural crops of the Solanaceae family, especially potato *Solanum tuberosum* L. (Westedt *et al.*, 1998, Flanders *et al.*, 1999; Kroschel and Schaub, 2012). Larvae feed on potato leaves, stems, and more importantly potato tubers both in the field and in storage. The newly hatched larvae produce mines on leaves by feeding on leaf tissue. Typical damage results from larvae

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boring tunnels into the tubers. Severe infestations result in yield and quality losses during storage where previously infested tubers are stored with healthy potato tubers (Rondon, 2010).

Since the larvae feed inside the tubers and they are not exposed to the insecticides, pesticide application in order to control this insect won't be successful (Rondon, 2010). An alternative strategy was the development of fumigants. However, synthetic fumigants have adverse effects on the environment, and insects may develop resistance against them (Nakakita and Winks, 1981). In recent years, researchers are more interested in using natural compounds as pesticides in insect pest management programs. Plant essential oils may be an alternative source for PTM control, because they constitute a rich source of bioactive compounds and are commonly used as fragrances and flavoring agents in foods and beverages. Because of this, much effort has been focused on plant essential oils (EOs) or phytochemicals as potential sources of commercial insect control materials. Several plant species, from different families with fumigant potential, have been tested for PTM control (Naghizadeh *et al.*, 2016; Tayoub *et al.*, 2016; Hannour *et al.*, 2017). The family of Zingiberaceae represents a key source of herbal preparations and phytoconstituents of interest for current pharmacology, parasitology, and entomology (Bendjeddou *et al.*, 2003; Jirovetz *et al.*, 2003; Patricia *et al.*, 2003; Nguefack *et al.*, 2004; Govindarajan *et al.*, 2016). *Zingiber officinale* Roscoe (Zingiberaceae) is a species native to tropical Asia. The rhizome (ginger) is one of the best known spices in the world and has been used since ancient times for its health benefits (Varakumar *et al.*, 2017). There has not been much study so far on the effect of ginger essential oil on insect pests (Rajeswary *et al.*, 2017).

On the other hand, the use of essential oils has some limitations. Due to the low molecular weight of their compounds, EOs are highly volatile and therefore have low persistence in the environment (Campos *et al.*, 2015). Nano-formulation of essential oils is an appropriate

method to overcome the problem of volatility (Yang *et al.*, 2009). Few studies have been done regarding the nano-formulation of plant essential oils in pest control. Ziaee *et al.* (2014) studied the toxicity of *Carum copticum* L. (Apiaceae) essential oil-loaded nanogel against *Sitophilus granarius* L. and *Tribolium confusum* Jacquelin du Val and reported that the oil-loaded nanogels were 8.9- and 3.7-fold more toxic than the pure essential oil against *S. granarius* and *T. confusum*, respectively.

The present work was carried out to determine the possible fumigant toxicity of the pure and nano-formulated ginger essential oil, and their persistence, against PTM.

Materials and Methods

Insect rearing

Potato tubers *Solanum tuberosum* L. cv. Agria, infested with PTM were obtained from a storage warehouse in the Ardabil province (38° 15' 53" N, 48° 16' 18" E), Iran. The insect rearing was carried out in the rearing room set at a temperature 26 ± 2 °C, $60 \pm 5\%$ RH, and a photoperiod of 14:10 (L: D) h for five generations. PTM adults were transferred into transparent plastic containers to lay eggs. The moths laid eggs on the lower surface of the filter paper. After 10-12h, the filter paper was removed and eggs were used in the experiments.

Plant material and extraction of essential oil

Ginger rhizomes *Z. officinale* was obtained from a local market in Ardabil, Iran. The essential oils were obtained by hydro-distillation in a Clevenger type apparatus. The extraction condition was as follows: 60g of dry rhizomes (in powder form); 700ml distilled water, and 3 h distillation. The oils obtained were dried over anhydrous sodium sulphate to extract the oil. Extracted oil was stored in a refrigerator at 4 °C for required studies.

GC-MS analysis

The ginger essential oil was analyzed by using gas chromatography-mass spectrometry (GC-MS) to determine its chemical composition.

Gas chromatographic analysis of *Z. officinale* essential oil was performed on an Agilent 6890N Gas Chromatograph (Agilent, USA) with a 5973N mass spectrometer with a HP5ms capillary column (30m × 0.25mm, 0.25µm in film thickness). Chromatography conditions were as follows: Column pressure: 8.75 psi, injector temperature: 150 °C, carrier gas: Helium at 1 ml min⁻¹ and the column temperature program 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. Component identification was determined through comparisons between their calculated Kratz retention index (RI) and those found in the literature (Adams, 2001) and through direct similarity searches of mass spectra databases (Adams, 2001).

Fabrication of nanofibers loaded with EO by electrospinning method

An electrospinning apparatus (Fannavaran Nano-Meghyas Co., Iran) was used for the electrospinning process (Sill and Recum, 2008). In the next step, the desired concentration of essential oil was loaded on nanofibers produced.

Characteristics of nanofibers

Surface morphology of the fibers before and after loading of EO was studied with the scanning electron microscopy (SEM) (LEO 1430VP, Germany) at an accelerating voltage of 15 kV. Specimens were sputter coated with gold for 120s. The structural characterization of the chemical blend was carried out using Fourier transform infrared (FTIR) spectroscopy (PerkinElmer, Spectrum RXI, USA). The wave number region for the analysis was 4000-400cm⁻¹ (in the mid-infrared range).

Efficiency percentage

The essential oil content in the nanofibers was quantified using ultraviolet-visible (UV/VIS) spectroscopy (Rayleigh, model UV-2100, Beijing, China) at 329nm. The efficiency

percentage of NFO was determined using the following equation (Natrajan *et al.*, 2015):

Efficiency percentage = [(Total amount of oil-Free oil)/Total amount of oil] × 100.

Fumigant toxicity

The fumigant toxicity of the essential oil against different developmental stages of PTM was examined using a method described by Negahban *et al.* (2007). Fumigant toxicity on eggs, male and female adults were investigated. Glass containers (250mL) were used as a fumigation chamber. 25 individuals of selected developmental stages of insects were placed in the glass vials. To determine the fumigant toxicity of the PEO and NFO, filter papers (Whatman No. 1), and non-loaded nanofibers were impregnated with different concentration of essential oils. Estimated concentrations of PEO and NFO were 52.4-98.8 and 23.2-37.2µl/l air for egg stage, 12.8-25.6 and 5.6-16.4µl/l air for female adult stage, and 12.4-22.8 and 6.8-12.4µl/l air for male adult stage, respectively. The control followed the same protocol, but without exposing the insects to the essential oils. All experiments were replicated four times. The impregnated filter papers and non-loaded nanofibers were then attached to the screw caps of glass jars. Mortality was recorded 24 h after treatment in the adult stages and after hatching in the egg stage.

Persistence experiments

LC₉₉ values obtained from previous experiment were used to determine persistence of the PEO and NFO. At 2-day intervals after the commencement of experiments, 25 individuals of egg stage were introduced to each jar. Subsequently, the mortality was counted 5 days after exposure. The period times used in the experiments were 1, 3, 5, 7, 9, 11, 13, 15 days for PEO treatment and continued to 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47 days in the NFO treatment. Four replications were made for each exposure. The persistence effect

experiment was continued until the treatments lost their insecticidal effect.

Data analysis

Experiments were tested for lack of fit by using PROC GENMOD (SAS Institute, 2002; Robertson *et al.*, 2007), and data were analyzed using PROC PROBIT to compute LC₅₀ and LC₉₉ (Lethal Concentrations) values on a standard and log scale with associated 95% fiducial limit by SAS program (SAS Institute, 2002). Graph was plotted using Excel software (2010 version).

Results

Chemical composition of the essential oil

The hydro-distillation of ginger rhizomes gave oil yield of 2%. Gas Chromatography-mass spectrometry analysis of the plant essential oil led to the identification of 71 components (Table 1) which accounted for 99.18% of the total oil. α -zingiberene (14.21%), Ar-curcumene (12.58%), β -sesquiphellandrene (12.48%) and *cis*- α -bisabolene (10.29%) were the most abundant components.

Table 1 Chemical compounds of essential oil of *Zingiber officinale*.

Compounds	Retention time (min)	Content (%)
α -Pinene	4.67	0.05
Camphene	4.95	0.14
Sabinene	5.39	0.52
Linalool L	7.58	0.07
Borneol L	8.83	0.35
Terpinen-4-ol	9.04	0.08
α -Terpineol	9.32	0.30
α -Terpinene	9.34	0.09
β -Citronellol	10.06	0.11
Z-Citral	10.42	0.29
Geraniol	10.67	0.19
E-Citral	11.07	0.46
(-)-Bornyl acetate	11.42	0.16
2-Undecanone	11.51	0.33
Carvacrol	11.68	0.09
<i>cis</i> -2,6-Dimethyl-2,6-octadiene	12.64	0.44
(+)-Cyclosativene	13.03	0.38
Copaene	13.41	1.05
β -Elemene	13.47	0.38
(-)-Zingiberene	13.68	0.31
<i>trans</i> -Caryophyllene	14.27	0.21
E-Farnesene	14.78	1.02
Viridiflorene	14.98	1.19
Ar-Curcumene	15.63	12.58
β -Curcumene	15.88	7.13
α -Zingiberene	16.17	14.21
<i>cis</i> - α -Bisabolene	16.37	10.29
β -Sesquiphellandrene	16.81	12.48
α -Elemene	16.97	0.35
Epizonarene	17.07	1.14
Nerolidol	17.29	0.92
Germacrene B	17.34	0.86
Dihydrocurcumene	17.58	0.15
<i>cis</i> - α -Copaene-8-ol	17.80	0.34
α -ongipinene	17.91	0.14
(-)-Campherenone	18.07	0.79

Continued in the next page.

Table 1 Continued.

Compounds	Retention time (min)	Content (%)
Zingiberenol	18.26	1.96
Cadinene	18.47	0.36
8-Himachalene	18.61	2.26
1-formyl-2,2-dimethyl-3-trans-(3-methyl-2-buten-1-yl)-	18.89	2.59
Murolol	19.15	1.77
α -Humulene	19.37	0.55
cis-Z- α -Bisabolene epoxide	19.43	0.33
Phenol-2,4-bis(1,1-dimethylethyl)-	19.73	1.26
Farnesol	19.85	1.13
Spiro [4.5]dec-8-en-7-one, 1,8-dimethyl-4-(1-methylethenyl)-	20.02	0.34
trans- Carveol	20.19	1.99
(-)-Caryophyllene oxide	20.42	1.41
Caryophyllene oxide	20.53	1.21
(-)-(E)-Myrtanyl acetate	20.77	1.99
trans-Z- α -Bisabolene epoxide	20.95	0.22
8-Elementene	21.00	0.72
2-Bromo-1-adamantanole	21.24	0.64
Farnesyl acetone C	21.48	1.21
7-(1,3-Dimethylbuta-1,3-dimethyl)-...	21.63	0.10
Geranyl acetone	21.81	1.58
6. β .Bicyclo [4.3.0] nonane, 55. β -iodomethyl-1	21.99	0.41
Alloaromadendrene oxide – (1)	22.40	0.18
7-Pentadecyne	22.60	0.11
Cyclododecanecarbonitrile	22.78	1.57
2,4,7,9,-Tetramethyl-5-decyn-4,7-diol	23.15	0.93
Nerylacetone	23.26	0.17
4-(3,4-Dimethoxybenzyl)- gamma-...	23.41	0.96
Cyclopentanecarboxaldehyde, 2-methyl-3-...	23.61	0.24
Capnellene-5. α -ol-8-one	23.78	0.12
1,2,3,4-tetrahydroisoquinoline	23.98	0.47
9-Methyl (7) (2,4) thiophenophane	24.22	0.32
4-(2,2-Dimethyl-6-methylenecyclohexyl) butanal	24.36	0.16
4-(1,3-Dimethyl-3-cyclohexenyl)-...	24.52	0.07
(E,E,E)-3,7,11,15-Tetramethylhexadecene	26.70	0.06
Ethanone, 1- (1,4-dimethyl-3-cyclohexen-1-yl)-	32.49	0.20

FTIR and SEM

To support the results of the establishment of essential oil on the PVA scaffold, further studies were carried out by FTIR analysis to determine the complex formation and interaction of major functional groups involved in the nano-formulation of EO (Figs. 1a, 1b and 1c). These results helped us in confirming the presence of EO in our nanofibers.

Figure 1a reveals the peaks associated with PVA scaffold. These include 3327cm^{-1} (-OH), 1735cm^{-1} (-C = O), 1376cm^{-1} (-CH₂) and 1098cm^{-1} (-CO). The FTIR spectrum of the

pure essential oil of ginger, showing the expected characteristic C-H (~ 3023 and 2872cm^{-1}), C = C (~ 1642 , 1515 and 1451cm^{-1}) and -C = C ($\sim 729\text{cm}^{-1}$) (Fig. 1b). The FTIR spectrum of the PVA-EO (Fig. 1c) showed absorption bands at 3022 , 2871 , 1734 , 1642 , 1515 , 1451 , 1377 , 1098 and 729cm^{-1} .

Figure 2 shows SEM images of the electrospun nanofibers obtained to investigate the effect of loading EO on nanofiber morphology. Images obtained of SEM show that the ginger essential oil is trapped among the PVA nanofibers. The entrapment efficiency was found to be 97.50% for the formulated essential oil.

Fumigant toxicity and persistence

The results of fumigant tests for the EO and NFO of ginger against different developmental stages of PTM are presented in Table 2. The LC₅₀ values of PEO and NFO were 75.44 and 30.24 µl/l air on the eggs, 19.08 and 10.28 µl/l air on the female and 17.76 and 9.56 µl/l air on the male adult stages, respectively. At the all growth stages of PTM, there was a difference between

PEO and NFO treatments, as inferred by the confidence intervals of LC₅₀.

The persistence of essential oil of ginger and nano-formulated essential oil over time is presented in Figure 3. The EO effectiveness decreased with increasing the storage time. Persistence test results showed that insecticidal efficacy of NFO lasted up to 49 days, whereas in the case of PEO it lasted only 15 days.

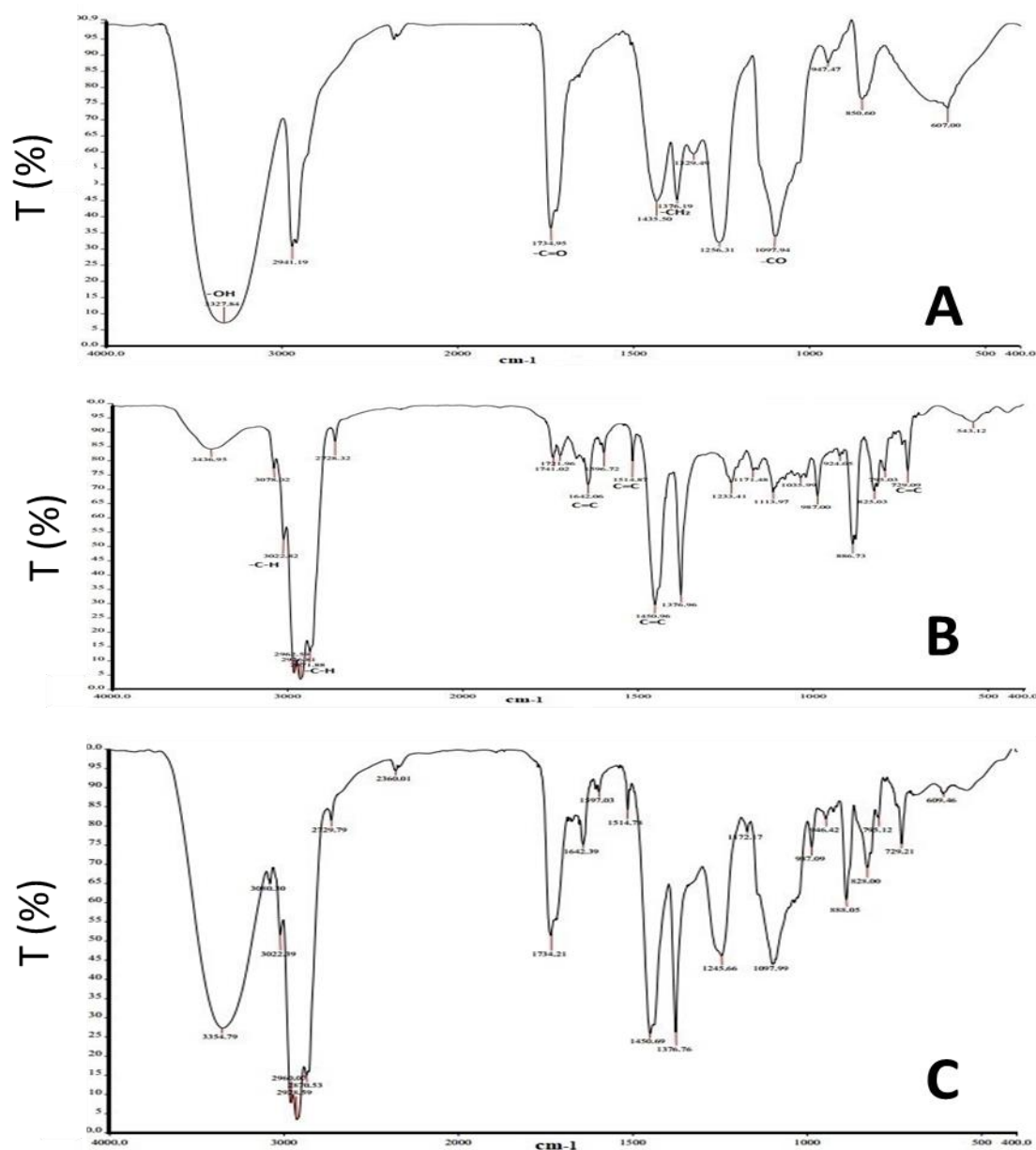


Figure 1 FTIR spectra for (A): PVA nanofiber, (B): *Zingiber officinale* EO and (C) NFO.

Discussion

Increasing concerns for the detrimental impacts of chemical pesticides to human health and the environment have lead the researchers to look for alternatives, especially plant-derived pesticides. In this study, we have evaluated the lethal toxicity and persistence of *Z. officinale* essential oil (pure and nano-formulated) for PTM control.

In order to confirm the effect of superfine grinding on ginger chemical components, FTIR spectra of the PEO, PVA and PEO-PVA are compared in Fig. 1. We focused on the spectral region from 4,000 and 400 cm^{-1} , which contained more relevant information on the main chemical functional groups (Fig. 1). The results showed that Spectrum of PEO-PVA contains spectra of PVA and PEO. These bands include: 3022, 2871, 1734, 1642, 1515, 1451, 1377, 1098 and 729 cm^{-1} . Our results are

consistent with the results of Purnomo *et al.* (2010). They studied the IR spectrum of the ginger *Z. officinale* essential oils and their results confirmed the existence of functional groups of (-O-H) [3436 cm^{-1}], (-C-H) [2872 cm^{-1}] and (-C = C) [1642, 1515 and 1450 cm^{-1}] in the structure of ginger. The results obtained from the study of the features of the scaffold surface also confirmed the deposition of EO on the scaffold structure (Figure 2).

GC-MS analysis of the essential oil revealed that the main composition in the EO of ginger included: α -zingiberene, Ar-curcumen, β -sesquiphellandrene and *cis*- α - bisabolene. The compounds identified in ginger essential oil are consistent with previous studies. Azhari *et al.* (2017) studied the chemical compositions of ginger *Z. officinale* essential oil and reported that the α -zingiberene is the main component of the essential oil in its rhizomes.

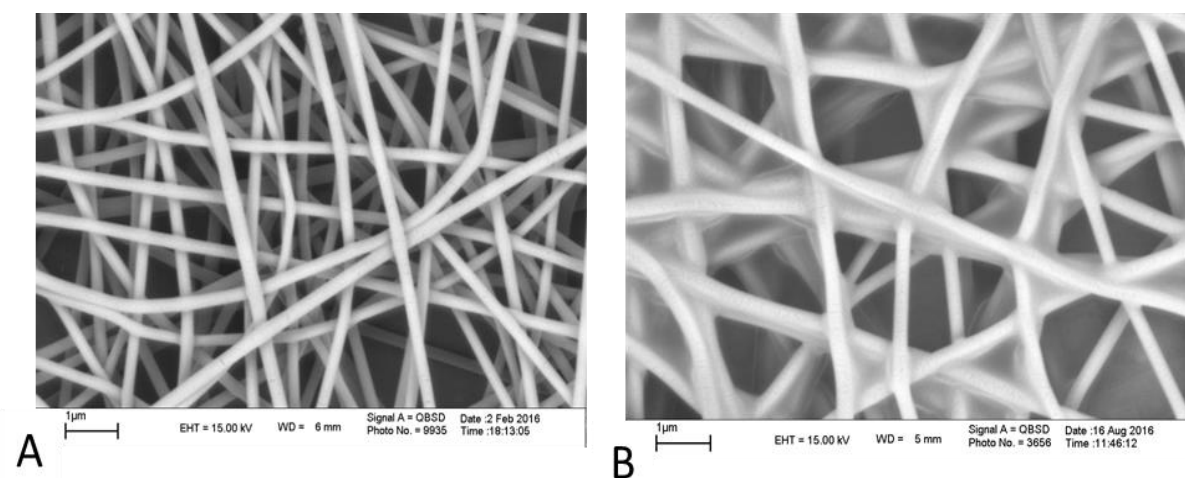


Figure 2 (A): Scanning electron microscopy (SEM) image of nano-fiber PVA without oil and (B): nano-fiber PVA with oil (b).

Table 2 Toxicity of *Zingiber officinale* PEO and NFO to different developmental stages of *Phthorimaea operculella*.

Developmental stages	Treatments	n	Slope \pm SE	χ^2 (df)	Lethal concentrations ($\mu\text{l/l}$ air)	
					LC ₅₀ (95% FL)	LC ₉₉ (95% FL)
Egg	PEO	600	7.02 \pm 0.74	2.92 ^{ns} (18)	75.44 (72.32-19.71)	161.84 (140.88-199.24)
	NFO	600	8.86 \pm 0.96	2.14 ^{ns} (18)	30.24 (29.24-31.28)	55.32 (49.48-65.52)
Female adult	PEO	600	6.45 \pm 0.67	2.89 ^{ns} (18)	19.08 (18.2-20.00)	43.72 (37.64-54.68)
	NFO	600	4.21 \pm 0.43	3.46 ^{ns} (18)	10.28 (9.56-11.04)	36.68 (29.24-51.48)
Male adult	PEO	600	7.35 \pm 0.77	4.56 ^{ns} (18)	17.76 (17.08-18.56)	36.84 (32.24-44.92)
	NFO	600	7.46 \pm 0.78	2.75 ^{ns} (18)	9.56 (9.2-9.96)	19.60 (17.24-23.76)

FL: 95% fiducial limits (FL), ns: non-significant, PEO: Pure essential oil, NFO: Nano-formulation essential oil.

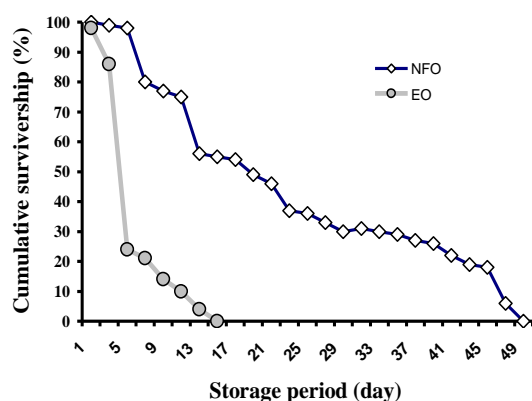


Figure 3 Persistence of *Zingiber officinale* pure essential oil (PEO) and nano-formulation essential oil (NFO) against *Phthorimaea operculella*.

The LC_{50} values showed a significant decrease in the amount of essential oil needed in the form of nanoformulation. This item shows that ginger NFO compared to PEO is effective at lower doses. Kim et al. (2003) stated that insecticidal activity of essential oils is according to their fumigant mode of action, and they may be toxic by penetrating via respiratory system of insect body. The small size of the oil-loaded nanofibers can lead to their better penetration to the insect body via respiratory system than the oil and thereby increase their efficiency. These results were consistent with the results of Sabbour and Abd El-Aziz (2016). They studied the role of Purslane, Mustard and Castor essential oils and their nano-formulations against *Ephestia cautella* (Lepidoptera; Pyralidae) under laboratory and storage conditions and reported that the nano oils were more effective than the bulk phase against this pest. Khanahmadi et al. (2017) also studied the fumigant toxicity of *Artemisia haussknechtii* essential oil and its nano-encapsulated formulation against *Tribolium castaneum* and *Sitophilus oryzae*. They observed that the mortality of insects occurred at lower concentrations of nano-encapsulated extract and concluded that *A. haussknechtii* essential oil and its nano-encapsulated form could play a significant role in the formulation of essential oil-based insecticides for the management of insect pests.

Persistence experiments showed that NFOs in comparison with PEO persist longer periods of time. Our results showed that nano-formulation of essential oils increases their persistence. A possible reason for the high durability of the nano-formulated constituents could be explained by the presence of oxygen. Oxygen molecules, by linking the OH molecules with the PVA structure, allow greater stability of the constituents in the environment. The present results are consistent with results of González et al. (2014) and Ziaee et al. (2014).

Previous studies on EO nanoparticles have shown that essential oils nanoparticles appear to be promising candidates for controlling the major pests of plants, due to their high volatility and stability. The aim of this study was to obtain and characterize polymeric nanofiber containing ginger essential oil and to evaluate its lethal effects and persistence against PTM. Our results showed that the effective components of EO loaded on nanofibers become more efficient due to their slow and constant release. Finally, we can conclude that the integration of essential oils into a planned release nano-formulation stops fast vaporization and degradation, enhances persistence, and maintains an effective low concentration.

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ساخت اسانس زنجبیل *Zingiber officinale* بارگذاری شده روی نانوالیاف و ارزیابی آن روی بید سیب زمینی *Phthorimaea operculella* (Lepidoptera: Gelechiidae)

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چکیده: بید سیب زمینی (*Phthorimaea operculella* (Zeller)، از آفات مهم سیب زمینی در مزرعه و انبار می باشد. در این مطالعه اثرات کشندگی و دوام اسانس زنجبیل (*Zingiber officinale* (Roscoe) (به صورت خالص و فرموله شده) روی مراحل مختلف نشوونمایی بید سیب زمینی (تخم، حشرات کامل نر و ماده) ارزیابی شد. اسانس گیاهی به روش تقطیر با بخار آب توسط دستگاه کلونجر استخراج گردید. تجزیه ی اسانس گیاهی توسط دستگاه کروماتوگرافی متصل به طیف سنج جرمی انجام گرفت. مورفولوژی نانوالیاف با استفاده از دستگاه میکروسکوب الکترونی روبشی مشاهده گردید. دستگاه FTIR برای شناسایی گروه های عاملی در اسانس خالص، نانوالیاف و اسانس بارگذاری شده روی نانوالیاف استفاده گردید. آزمایش های زیست سنجی در ظروف شیشه ای ۲۵۰ میلی لیتری انجام شدند. ترکیب اصلی اسانس زنجبیل، α -zingiberene (۱۴/۲۱ درصد) بود. سایر ترکیبات شامل Ar-curcumene (۱۲/۵۸ درصد)، β -sesquiphellandrene (۱۲/۴۸ درصد) و *cis*- α -bisabolene (۱۰/۲۹ درصد) بودند. نتایج طیف های FTIR استقرار گروه های عاملی اسانس زنجبیل را روی ساختار نانوالیاف نشان دادند. تصاویر میکروسکوب الکترونی روبشی نیز استقرار اسانس را روی ساختار نانوالیاف به اثبات رساندند. مقادیر LC₅₀ اسانس خالص و نانوفرموله زنجبیل به ترتیب برای مرحله ی تخم، ۷۵/۴۴ و ۳۰/۲۴ میکرولیتر بر لیتر هوا، برای حشرات ماده ۱۹/۰۸ و ۱۰/۲۸ میکرولیتر بر لیتر هوا و برای حشرات نر ۱۷/۷۶ و ۹/۵۶ میکرولیتر بر لیتر هوا برآورد گردیدند. نتایج آزمایش دوام نشان داد که اسانس نانوفرموله شده (۴۹ روز) در مقایسه با اسانس خالص (۱۵ روز) دوام بیش تری داشت. طبق نتایج، اسانس خالص و نانوفرموله شده ی زنجبیل می تواند نقش مهمی در مدیریت بید سیب زمینی به عنوان آفت کش های طبیعی داشته باشد.

واژگان کلیدی: اسانس زنجبیل، بید سیب زمینی، دوام، زیست سنجی تدخینی، نانوتکنولوژی