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FUMIGANT PROPERTIES OF NANO-ENCAPSULATED ESSENTIAL OIL FROM *ARTEMISIA SIEBERI* ON *TRIBOLIUM CASTANEUM*

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ABSTRACT

Various policies and initiatives exist to reduce the effects of chemical pesticides in the environment. Recent research has focused on insecticidal properties of plant essential oils in biological control of insects. Controlled release by nano-encapsulated formulations allows the quantities of the oils to be used more effectively over a given time interval, enhances the suitability to mode of application and minimizes the environmental damage. In this research, the essential oil of *Artemisia sieberi* Besser was encapsulated by in-situ polymerization of oil/water emulsion in nano-scale. Then fumigant toxicity and persistence of produced nano-encapsuled essential oil (NEO) were examined against *Tribolium castaneum* (Herbst) and compared with pure essential oil (PEO) (not encapsulated). The fumigant toxicity of nano-capsules (LC_{50}= 11.24 ppm) was significantly higher than that of PEO (LC_{50}= 15.68 ppm). At sub-lethal doses after 7 days exposure, allowed the essential oil to be entrapped without any changes in their composition and control release. Also, the half-life time of the NEO (LT_{50}= 28.73 days) was significantly much longer than that of the PEO (LT_{50}= 4.27 days). Overall, it seems that the findings of present study could be promising to make practical use of plant essential oils. As the new technology in NEO allows the control release of active ingredients, it could be overcome the restrictions of plant essential oils usage in storage and farms.

Key words: Nano-capsule, plant essential oil, *Artemisia sieberi, Tribolium castaneum*, fumigant toxicity, stored product insects, formulation

INTRODUCTION

Several studies have focused on the potential use of botanicals applications in biological control of different insect pests since some are selective, biodegrade to nontoxic products, and have few effects on non-target organisms and the environment (Singh and Upadhyay, 1993; Isman, 2000; Kim et al., 2010). In the past few years, several studies have focused on the potential use of essential oil applications in biological control of different insect pests. The essential oils may be more rapidly degraded in the environment than synthetic compounds, and some have increased specificity that favors beneficial insects. Their action against stored-product insects has been extensively studied (Negahban et al., 2007a; Sahaf and Moharramipour, 2008).

In spite of the fact that essential oils have most promising properties, problems related to their volatility, poor water solubility, and potential for oxidation have to be resolved before used as alternative pest control means (Moretti et al., 2002). In this endeavor the use of encapsulated formulations seem to be useful tools to answer those problems (Clancy et al., 1992; Passino et al., 2004).The propagation of poly urea-formaldehyde (PUF) nano-encapsulation techniques would supply insecticide formulations of essential oils without widespread destruction effects on their major compounds. Urea formaldehyde is used in agriculture as a controlled release source of nitrogen fertilizer. Urea formaldehyde's rate of decomposition into CO_2 and NH_3 is determined by the action of microbes found naturally in most soils (Martin and Trenkel, 1997). Studies relating to the activity of PUF nano-capsules of *Artemisia sieberi* as an insecticide have not been carried out. The purpose of this study was to test the fumigant toxicity and persistence of produced nano-encapsulated essential oil (NEO) against *T. castaneum* (the most economically deleterious pest of stored grain throughout the world) and compared with pure essential oil (PEO).

MATERIALS AND METHODS

Plant materials and preparation of essential oil formulation

Aerial parts of *A. sieberi* were collected at full-flowering stage in October, 2011 from Qom province in Iran. The plant material was dried naturally on laboratory benches at room temperature (23–24 ºC) for 5 days until crisp. The dried material was stored at 24 ºC until needed and then hydrodistilled to extract its essential oil. Essential oil was extracted from the plant samples using a Clevenger-type apparatus where the plant material was subjected to hydrodistillation. Conditions of extraction were: 50 g of air-dried sample; 1:10 plant material/water volume ratio, 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (2.86%w/w) was calculated on a dry weight basis. Extracted essential oil was stored in a refrigerator at 4 ºC.

The nano-encapsulation process was carried out by polymerization technology. Essential oil was used as a core material, and Urea (U) and formaldehyde (F) as shell materials. Sulphuric acid solution (10 % w/w) was prepared in our laboratory to control the pH of emulsion and tween 80 (Polysorbate 80) was used as emulsifier (Merck, Germany). The obtained suspension of nano-capsules was cooled down to ambient temperature, rinsed with deionized water, filtered and finally dehydrated by freeze-drying using a LIO-5P apparatus (CinquePascal, Trezzano SN, Milan, Italy). Scanning and transmission electron microscopy were used to observe surface morphology of the nano-capsules.

Test insects

T. castaneum was reared on wheat flour mixed with brewer yeast (10:1, w/w). Adult insects, 1 to 3 days old, were used for toxicity tests. The cultures were maintained in dark in a growth chamber set at 27±1°C and 65±5% r.h. All experiments were carried out under the same environmental conditions.

Bioassay

Fumigant toxicity bioassay was deliberated (Negahban et al., 2006) to assess 50% lethal doses of its essential oil content. At first, concentration ranges of fumigant toxicity of nano-encapsulated oil (NEO) and pure essential oil (PEO) were determined by using a preliminary experiment and logarithmic distance. Then, 20 adults (1 to 3 days old) were put into 280 mL glass bottles with screw lids, the experimental apparatus was designed in order to obtain *T. castaneum* kept 10 cm away from the oil formulation. A series of concentrations from 3 to 25 ppm of the NEO and PEO was tested on *T. castaneum* adults. Control insects were kept under the same conditions without any oil. Each dose was replicated five times. The number of dead and live insects in each bottle was counted 7 days after initial exposure to the essential oil. Probit analysis (Finney, 1971) was used to estimate LC_{50} values.

The mortality half-life of the NEO and PEO, as opposed to chemical half-life, involves determining the mortality caused by a pesticide over time (Stark and Wennergren, 1995; Negahban et al., 2007b). Determination of chemical half-life involves quantization of actual residue levels over time. Our approach involved exposing 20 adults (1 to 3 days old) to glass vial (27 mL) treated with the essential oil at 30 ppm. Thereafter, new adults were introduced in vials every 2 days. The following time steps were used in this study: 3, 5, 7, 9 and 30 days (5 replicates per time step). For each time step (2 days), the adults were removed after 24 h and the mortality was recorded after 48 h later. The mortality half-life data for each experiment were analyzed by the method of Finney (1971), indicating the loss of essential oil activity over time.

RESULTS

As shown in Fig. 1, nano-capsules appeared to be made up of single spherical units of about 80 nm diameter. The external surface of each unit was almost regular and smooth, showing that the Poly (urea-formaldehyde) forms a continuous film surrounding the essential oil droplets.

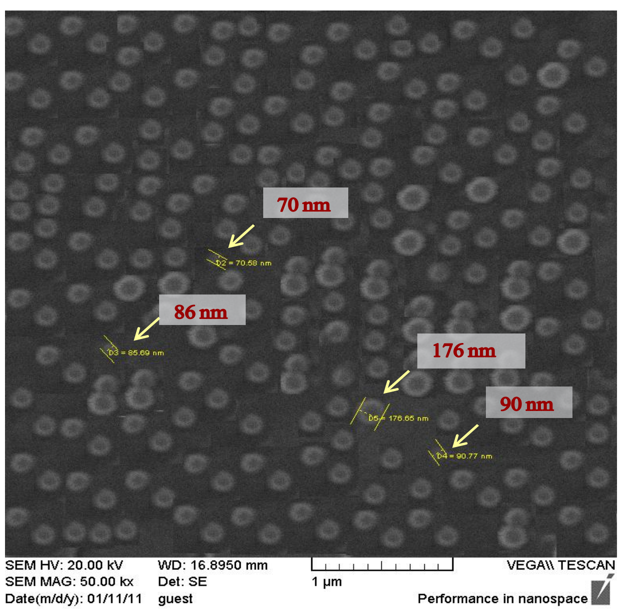

Fig. 1- The scanning electron micrograph shows the external surface of each unit of nano-capsule.

On the basis of the LC_{50}s, NEO shows higher mortality than PEO as a case in point LC_{50} values were 11.24 ppm for NEO and 15.68 ppm for PEO for adult insect after 7 days exposure time (Table 1).

Table 1. Fumigant toxicity of nano-encapsuled essential oil (NEO) and pure essential oil (PEO) against *Tribolium castaneum* adults after 7 days exposure at 27°C and 65% r.h.

Type	N	LC_{50} (ppm) (95% fiducial limits)	Slope ± SE	df	P-value	Chi square (χ^2)
NEO	600	11.24 (10.92 - 11.58)	1.73 ± 1.11	4	0.86	1.15
PEO	600	15.68 (15.26 - 16.13)	7. 3 ± 0.78	4	0.96	0.67

The estimate of LT_{50}s for *T. castaneum* showed that the half-life time of the NEO was significantly longer than that of PEO (Table 2).

Table 2. LT_{50} values expressing persistence of nano-encapsuled essential oil (NEO) and pure essential oil (PEO) on *Tribolium castaneum* adults exposed to 30 ppm of each essential oil at 27°C and 65% r.h.

Type	n	LT_{50} (h)[1] (95% fiducial limits)	Slope ± SE	df	P-value	Chi square (χ^2)
NEO	700	28.73 (28.40 - 29.95)	1.61 ± 0.39	5	0.88	1.15
PEO	700	4.27 (4.47 - 6.42)	-7. 21 ± 1.04	5	0.99	0.67

[1] LT_{50} values indicate half-life time of the essential oil

DISCUSSION

Essential oil contains compounds that show ovicidal, repellent, antifeedant and toxic effects in insects (Arabi et al., 2008). The toxicity may act by fumigant action (Negahban et al., 2007a). Focus on the insecticidal toxicity of essential oils of plants and their constituents have sharpened since the 1980s specifically on essential oils (Rajendran and Sriranjini, 2008). There are many reviews dealing with the use of plant products in general, against insect pests (Isman, 2006; Rajendran and Sriranjini, 2008). Therefore, it was considered appropriate to look into the status of research on plant essential oils and their constituents as insecticides. The present study examines the work conducted and addresses the prospects and problems of the use of plant products as fumigants within these parameters. The nano-capsule of *Artemisia* oil was shown here to possess fumigant toxicity, as well as its longer persistence compared to *Artemisia* oil before formulation. In consistence with studies of Moretti et al. (2002) and Passino et al. (2004), our findings showed higher mortality rates in nano-capsule than in pure essential oil due to controlled-release formulations allowing smaller quantities of essential oil to be used more effectively over a given time interval.

The reasons for nano-encapsulating the essential oil have been to improve its stability to reduce side effects or to reduce dosing frequency and total dosing amount, to obtain better toxicity activity, and for long-lasting release (Huang et al., 2006). Generally, the modifications of nano-capsules prepared by Poly (urea-formaldehyde) (PUF) are required in order to improve their insecticidal toxicity stability, strength or sustained release.

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