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# NANO-INSECTICIDAL ACTIVITY OF ESSENTIAL OIL FROM *CUMINUM CYMINUM* ON *TRIBOLIUM CASTANEUM*

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## ABSTRACT

In recent decades, use of botanical insecticides has been developed as alternative to synthetic pesticides. Nowadays, use of new technologies such as nano-encapsulated formulation can overcome the constraints of plant essential oils. In this study, nano-capsules of essential oil from *Cuminum cyminum* L. was prepared by in situ polymerization (O/W) emulation using poly urea-formaldehyde as wall forming material and oil as a core material. The toxic effects of nano-capsules were assessed against *Tribolium castaneum* (Herbst), as one of the most serious pest of stored products. The fumigant toxicity from nano-capsules ($LC_{50}$= 16.25 ppm) were highly effective than pure essential oil ($LC_{50}$= 32.12 ppm) with 7 days of exposure. The influences of surface morphology, wall thickness and diameter on the thermal stability of nano-capsules were investigated. In this study, the nano-capsules basically exhibited good storage stability at room temperature. Our findings show that the nano-encapsulation of *C. cyminum* oil might provide a new method for management of *T. castaneum*.

**Key words:** Essential oil, nano-capsule, nano-insecticide, *Cuminum cyminum, Tribolium castaneum,* medicinal plants, formulation, bioassay, mortality.

## INTRODUCTION

The application of various synthetic insecticides and fumigants to grain storage over the years has led to a number of problems, including the development of insecticide resistance in stored grain insect pests (Suthisut, 2011). Potential of plant essential oils as source of insecticides has been worked out and reported with references to various pests (Rajendran and Sriranjini, 2008; Zapata and Smagghe, 2010). Additionally, many plant essential oils show a broad spectrum of activity against pest insects and plant pathogenic fungi ranging from toxic, antifeedant, repellent and oviposition deterrent (Negahban et al., 2006; Sahaf and Moharramipour, 2008; Negahban et al., 2007b). These oils have also a long tradition of use in the protection of stored products (Negahban et al., 2007a; Arabi et al., 2008). Natural products are an outstanding alternative to synthetic pesticides as a means to reduce the negative impacts on human health and the environment (Vanichpakorn et al., 2010). The shift to green chemistry processes and the continuing need for developing new crop protection tools with novel function makes discovery and commercialization of natural products as green pesticides an attractive and cost-effective search that deserve attention (Koul et al., 2008). Green pesticides are more compatible with the environmental components than synthetic pesticides.

The nano-encapsulated essential oil has the advantage of overcoming the restrictions of plant essential oils usage in storage through the control release of active ingredients (Negahban et al., in press a, b). The nano-encapsulated essential oils have therefore the advantages of solubility of hydrophobic pesticides (hence no need for toxic solvents), no precipitation (therefore no need for constant mixing), increased stability (protect against oxidation), and improved uptake. However, it should be recognized that at this stage the industrialization opportunities are limited, as the precise mechanisms by which nano-encapsulated essential oils perform are still the subject of intense basic research. The present study has ascertained the potential of nano-capsule formulations of *C. cyminum* essential oil on *T. castaneum*, the widespread and critical stored-product pest in cereals and cereal products. Also chemical structure, surface morphology and thermal stability of the nano-capsules were characterized.

## MATERIALS AND METHODS

### Plant materials and preparation of essential oil formulation

Seeds of *C. cyminum* were obtained from Ferdowsi University, Mashhad, Iran. Essential oil was extracted from the seed samples using a Clevenger-type apparatus where the seeds were subjected to hydrodistillation. Conditions of extraction were: 50 g of air-dried sample; 1:10 seed material/water volume ratio, 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (4.16% w/w) was calculated on a dry weight basis. Extracted oil was stored in a refrigerator at 4ºC.

The nano-encapsulation procedure was conducted 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 used to control the pH of emulsion and tween 80 (Polysorbate 80), used as emulsifier (Merck Germany). After the UF pre-polymer solution was obtained, aqueous solution of tween 80 was added to the prepared pre-polymer solution. Then the prepared oil was added to form oil in water (O/W) emulsion. The pH of the emulsion was adjusted slowly to 3 while the solution was slowly heated to the target temperature of 60-65$^{o}$C. After 4 h, the reaction was stopped. The obtained suspension of nano-capsules was cooled down to ambient temperature, rinsed with deionized water, filtered and finally dehydrated by freeze-drying. Chemical structure of samples was identified using Fourier transform infrared spectroscopy (FTIR) (BRUKER EQUINOX 55). Transmission electron microscope (TEM, Philips CM120) were used to observe surface morphology of the nano-capsules. The thermal properties were analyzed by differential scanning calorimetry (NETZSCH DSC, 200 F3) at a heating rate of 10°C/min from 25 up to 400°C in nitrogen atmosphere. Thermal stability and overall quality of the prepared capsules was assessed by thermogravimetric analysis (TGA) at a heating rate of 10°C/min.

### Test insects

*T. castaneum* was reared on wheat flour mixed with yeast (10:1 w/w), Adult insects, 1–3 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 of the essential oil was investigated to determine lethal concentration for 50% mortality ($LC_{50}$) (Negahban *et al*., 2006). A series of concentrations ranging from 6 to 28 ppm for nano-capsules and 13-15 ppm for pure oil were used with logarithmic distance. Then, 20 adults (1–3 days old) were placed 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. Control insects were kept under the same conditions without any oil. Each concentration 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.

# RESULTS

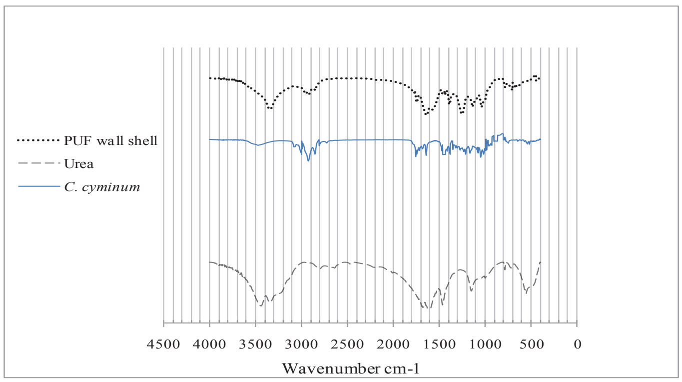


Fig. 1- Fourier transform infrared spectroscopy (FTIR) of urea, *Cuminum cyminum* oil and nano-capsules containing oil with PUF shell wall material.

Fig. 1 indicates FTIR spectra of urea, *C. cyminum* and nano-capsules containing disperse *C. cyminum* oil. N-H and C-H stretching vibration at 3348, 2923 $cm^{-1}$ are presented by the FTIR spectrum of urea and formaldehyde, respectively. As it can be seen, poly condensation reaction between urea and formaldehyde were proved by the absence of absorption band owing to urea at 3450, 2640, 1490 and 580 $cm^{-1}$ and manifestation of absorption peak of poly (urea formaldehyde), which is assigned at 3507-3050 (NH and OH), 1635 (—NH—C(=O)—NH—), 1568 (—C(=O)—NH—) and 1037 (—CH—O—CH—) $cm^{-1}$. The absorption peaks of 1568, 1037, and 646 $cm^{-1}$ of poly urea and the absorption peaks of 2850, 2905, 1751, 1248 and 1105 of *C. cyminum* appeared in nano-capsules containing *C. cyminum* spectra which indicate that the core content has been embraced with poly urea formaldehyde.

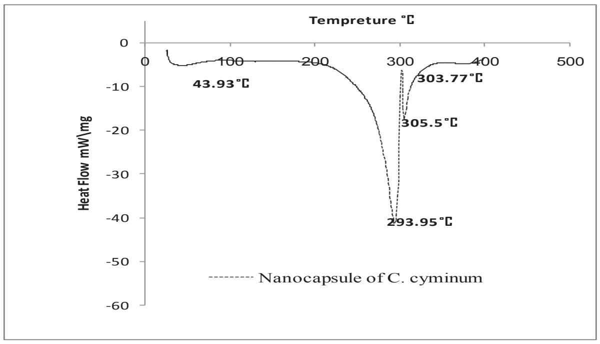


Fig. 2- The differential scanning calorimetry (DSC) of nano-capsules containing *Cuminum cyminum* with PUF shell wall material.

The DSC thermogram is shown in Fig. 2 two endothermic peaks at 43.69 and 295°C appear in the DSC thermogram of nano-capsules. The weak endothermic peak below 43°C is related to the evaporation of free formaldehyde. The second one at temperatures about 262-295°C is due to the decomposition of poly urea formaldehyde as shell materials and the weak exothermic peak at about 303°C on DSC curves may be due to the continuous polymerization reaction of core material and the weak endothermic peak at approximately 305°C is due to the further decomposition of the residue. TG curves for nano-capsules of *C. cyminum* is shown in Fig. 3 indicates that the weight loss near 100-127ºC is mainly due to the removal of entrapped residual water and the elimination of free formaldehyde and the weight loss at temperatures between 235 and 351ºC is mainly due to the decomposition of the PUF wall shell.

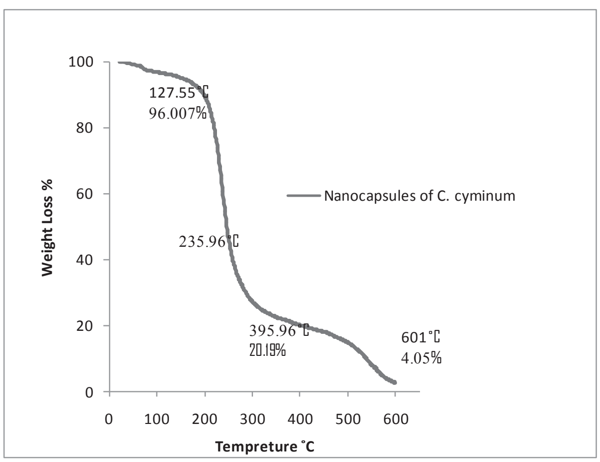


Fig. 3- Thermogravimetric analysis (TGA) of nano-capsules containing *Cuminum cyminum* with PUF shell wall material.

The weight loss of nano-capsules was in the range of 351– 600ºC. In Fig. 4, transmission electron microscopy (TEM) shows that the nano-particles are composed of a core phase entrapped in a shell material of a fairly constant thickness. Nano-capsules appear to be made up of spherical particles of about 30 nm in diameter. The external surface of each particle is almost regular and smooth, showing that poly urea-formaldehyde forms a continuous film surrounding the essential oil droplets.

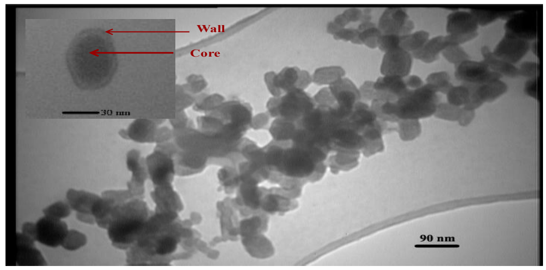


Fig. 4- Transmission electron microscopy (TEM) image of nano-capsules of *Cuminum cyminum* oil shows the core–shell-structured poly urea formaldehyde nano-capsules.

Lower $LC_{50}$ value of nano-capsule (16.25 ppm) indicates higher toxicity than pure essential oil (32.12 ppm) (Table 1).

Table 1. Fumigant toxicity of nano-encapsuled essential oil and pure essential oil of *Cuminum cyminum* against *Tribolium castaneum* 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 ($\chi^2$)** |
|---|---|---|---|---|---|---|
| Nano-capsule | 600 | 16.25 (14.84 - 17.63 ) | 2.83 ± 1.10 | 4 | 0.86 | 1.05 |
| Pure oil | 600 | 32.12 (30.34 - 35.18 ) | 6. 7 ± 0.43 | 4 | 0.96 | 0.87 |

## DISCUSSION

Several studies have been undertaken to explore the potential use of essential oils and their constituents as insect fumigants (Nikooei et al., 2011; Ghasemi et al, 2011). For taking into account the limitations and the physicochemical characteristics of the essential oils, nano-encapsulated formulations seem to be the best choice. In this study, high fumigant toxicity of nano-encapsulated *C. cyminum* essential oil have been demonstrated as a new formulation against *T. castaneum* as a result of controlled-release formulations allowing smaller quantities of essential oil over a given time interval. Also, the cross-linked polymer yielded by the core material and the weight loss of nano-capsules in the range of 351– 600ºC indicate higher thermal stability. Moreover, the thermal degradation of PUF nano-capsule containing oil is complicated and indicating that the prepared nano-capsules with PUF wall shell material has a good thermal stability. Therefore, it is time to focus the consideration of the researchers on the way to the expansion and application of known essential oils and their constituents by highly developed formulation technologies.

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