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Fumigant toxicity of *Artemisia haussknechtii* essential oil and its nano-encapsulated form

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Abstract

In this study, the insecticidal activity *Artemisia Haussknechtii* essential oil, extract and encapsulated form of essential oils in nanoparticles were investigated.

Interfacial compression polymerization method was investigated for nano capsules preparation. The effects of emulsifier composition, co-emulsifier, and temperature on the properties of the nanocapsules were investigated. The scanning electron microscopy of particles proved the preparation of nanoparticles. Fumigant toxicity of oil and extract demonstrated fumigant toxicity against *Tribolium castaneum* and *Sitophilus oryzae*.

It was demonstrated a mortality of 100 percent of *Sitophilus oryzae* in the concentration of 166 ppm. The insecticidal activity varied based on insect species. With the alteration of concentrations of the oil and extracts and exposure time, the variety on fumigant toxicity had shown due to the volatility of extract. The results demonstrated 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 stored-grain insects.

Keywords: *Artemisia Haussknechtii*, Essential oil, Extract, Fumigant toxicity, Contact toxicity.

1. Introduction

The genus *Artemisia*, with the common Persian name of ‘Dermanc’ and also with the common English name of ‘Wormwood’ includes 34 species that are found wild all over Iran [1]. Many plants from the *Artemisia* genus are used throughout different cultures as traditional medicine. Wormwood is popular aromatic herb that has been used as a flavoring additive in alcoholic beverages, such as vermouth, bitters, and liqueurs and tonic [2]. The medicinal plant wormwood and the essential oils in wormwood, contain bioactive compounds such as phenolic compounds, alkaloids, vitamin A, B₁, B₂, C and various minerals. Essential oils (EOs, also called volatile, ethereal oils, essences, or absolutes) are aromatic oily liquids extracted from different plant materials. The greatest use of EOs is in food (as flavorings), perfumes and pharmaceuticals [2, 3].

The *Artemisia* species are one of the most popular plants, which are used for the management of diseases such as hepatitis, malaria, cancer, inflammation and infections by fungi, bacteria, and viruses. Furthermore, *Artemisia* species are widely used as medicinal plants in folk medicine. Some *Artemisia* species are used traditionally as a tonic, and as antihelmintic in the north of Iran [4-6]. Also, *Artemisia haussknechtii* is used in dyspepsia and other gastrointestinal disorders by local people in the western part of Iran; the province of Kermanshah. Antimicrobial effects of some endemic *Artemisia* in Iran such as *A. diffusa*, *A. oliveriana*, and *A. turanica* were reported [5, 7]. Fumigant toxicity of *A. haussknechtii* oil evaluated on *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), and *Callosobruchus maculatus* (Fab.) [8]. However, there are no reports on the insecticidal activity of essential oil of *A. haussknechtii* on larvae and adults of *T. Confusum* [9].

Aromatic plants and their volatile oils have been in use since antiquity in flavor and fragrances, in medicines, as antimicrobial and insecticidal agents, and to repel insects and stored product pests [10, 11]. Among the variety of nature’s ecosystem services, the natural pest control is an important aspect [12]. It is reported that ~99% of the crop pests are controlled by natural enemies such as spiders, birds, parasitic wasps, viral diseases and other organisms [13]. Also in recent decades, a worldwide increase in the incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungus to different fungicidal used in medicinal practice [14].

However, due to fumigant and insecticidal activity, the interest in volatile oils has increased tremendously during the last decade. Also, essential oils have been largely employed for their properties which are already observed in nature. The essential oils were well known for their antiseptic (bactericidal, virucidal and fungicidal) and medicinal properties and their fragrance, they are used in embalmment, preservation of foods, and as microbicidal, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies [4, 15, 16].

The use of fumigants is the most economical tool for managing stored grain insect pests In many storage systems. Phosphine, methyl bromide, carbon disulfide, and isothiocyanates have been investigated for stored-products protection. There are of global concerns about their negative effects on human health and environment [17].

In recent years, essential oils received a great deal of attention as pest control agents. They are characterized by a low toxicity to human and animals, high volatility, and toxicity to stored grain insect pests. According to Sahaf reported that essential oils might be apply to the protection of stored products. Also, aromatic plants and their essential oils have been used since antiquity in flavour and fragrances, as condiment or spice, in medicines, as antimicrobial/insecticidal agents and to repel insect or protect stored products [18, 19].

The pesticides are formulated in the different forms such as wettable powders, emulsifiable concentrates, water solutions, powder or granules, aerosols or spray formulations. But such formulations have different degrees of health hazards ranging from respiratory exposure to penetration through the skin. Some of these forms are not suitable enough to deliver in a convenient way for field application. Considering all these facts, perhaps nanoencapsulation is the most efficient way of delivering pesticides [20-22].

Nanocapsules are solid, hollow particles ranging from 10 to 1,000 nm in diameter. They have recently been used in drug-delivery systems [23, 24]. Nanocapsules (NC) containing pesticides can result in sufficient effects on pests yet reduce the side effects of the pesticide because of the small dosage used. Because of their small size, NC may be easy to deposit on the leaves of the plants, which helps to reduce waste of the pesticide [25-27]. Generally, it is considered that an efficient pesticide formulation should not only show an effective biological activity, it must also be user-friendly and environmentally friendly. It has high insecticidal potency and relatively low side effects on birds and mammals, although it is acutely toxic to aquatic animals. Because NPs are spontaneously formed and are thermodynamically stable dispersions, they have several advantages over emulsions in complex coacervation [25, 28, 29]. The present work was carried out to determine the possible fumigant toxicity of the essential oil, extract of *A. haussknechtii* and its encapsulated form against insects. In this study, the exact fumigant toxicity of this parameters also carried out to characterize our purpose and obtaining the results.

2. Materials and Methods

2.1. Materials

Artemisia haussknechtii was collected from Oramanat area, Kermanshah, Iran; potato dextrose agar (PDA) obtained from Sigma-Aldrich (St Louis, MO). Urea and formaldehyde, tween 20, 40 and 80 obtained from Merck (Germany). Chemicals, such as *n*-heptadecane, *n*-hexadecane, anhydrous sodium sulfate and sulphuric acid with the purity higher than 99% were purchased from Merck chemical company. Other

reagents were of analytical or HPLC grade. Double-distilled water was prepared in our laboratory is located in Department of Medicinal Plant, Kermanshah Branch, Academic Center for Education, Culture and Research, Iran.

2.2. Plant Material

Artemisia haussknechtii was collected from Oramanat area, Kermanshah (western part of Iran) in June 2011 and identified in Kermanshah Research Institute of Forests and Rangelands. The fresh plants were sliced and air dried with active ventilation at ambient temperature.

2.3. Isolation of the Essential Oil

Air-dried aerial parts of *A. haussknechtii* (100g) were subjected to hydro distillation in a Clevenger – type apparatus for 4 hours period. After decanting and drying of the oil on anhydrous sodium sulfate, it was kept refrigerated until analysis.

2.4. Extract Preparation and Characterization

In order to extract, about 50g of powdered plant was treated with 100 ml ethanol at room temperature with stirring. The exact process, ferric thiocyanate (FTC) assay, Phenolic content, antifungal activity and antimicrobial activity for this compound were reported formerly with our group and reported previously [5, 7].

2.5. Nanocapsule preparation

Nanocapsules containing essential oils prepared by interfacial compression polymerization technique. For this purpose, a specific ratio of organic phase to aqueous phase was selected. By choosing an appropriate volume of aqueous phase, the amount (in the percentage of raw materials comprising polymer) is added an emulsifier. Prepared nanocapsules were frozen - dried for further evaluation and experiments. For nanocapsule preparation, the reactor instructed with ashk shishe Co. from Tehran I.R (Fig. 1).

Table 1 showed the investigated variables in NP preparation process.

For the evaluation of co-emulsifier effect 20 and 30 mL of 0.01 gr/mL of methyl cellulase, poly vinyl alcohol (PVA) with molecular weight (Mw) of 10000 and 20000 gr/mol and poly vinyl pyrrolidone (PVP) with Mw of 15000 and 25000 was added to 130 and 140 ml of aqueous phase containing 0.1 mL tween 80.

Also for evaluation of temperature, the 3 procedure were selected:

1. 60 °C from reaction beginning to reaction termination,
2. 30 °C for micelle preparation and then increasing to 60 °C for other stages,
3. 45 °C from reaction beginning to the end of the reaction.

2.6. Scanning electron microscopy

In order to verify the result, the morphology of the triamcinolone acetonide-solid lipid nanoparticles (SLNs) was studied by scanning electron microscopy using a Philips XL30 microscope at an accelerating voltage of 10 KV. One droplet of SLN nanosuspension was placed on an aluminum specimen stub. After oven-drying for 12 hours, the sample was coated with a platinum layer using an SCDOOS sputter coater (BAL-TEC, Sweden) in an argon atmosphere. Subsequently, the sample was scanned and photomicrographs were obtained.

2.7. Fumigation of extracts and essential oil on the *Sitophilus oryzae* and *Tribolium castaneum*

The fumigant toxicity of the essential oil and extracts against adults of *Sitophilus oryzae* and *Tribolium castaneum* was examined using a modified fumigant toxicity assay as described by Keita *et al* [30].

To determine the fumigant toxicity of the essential oil and extracts, filter papers (Whatman No. 1, cut into 2 cm diameter pieces) were impregnated with different concentration of essential oils calculated to give equivalent fumigant concentrations of 3.3, 16 and 166 ppm in air. The impregnated filter papers were then attached to the screw caps of glass vials with volumes of 27 ml. Caps were screwed tightly on the vials, each of which contained separately 10 adults (1–7 days old) of each species of insect. For control, non-treated filter paper was used. All treatments were replicated three times. Mortality was determined after 3, 6, 9, 12 and 24 h from the commencement of exposure. Insects were considered to be dead if appendages did not move when they were prodded with fine pins. Percentage of insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls [30].

Mortality

$$\% \text{ test mortality} - \% \text{ control mortality}$$

$$(\%) = \frac{100 - \% \text{ control mortality}}{\% \text{ control mortality}} \times 100$$

Another experiment was designed to assess the exact fumigation of extracts on *Sitophilus oryzae* and *Tribolium castaneum*. In this case to determine the fumigant toxicity of the extracts of *A. haussknechtii*, filter papers (Whatman No. 1, cut into 2 cm diameter pieces) were impregnated with oil at doses calculated to give equivalent fumigant concentrations of 37, 185, 370, 444, 556, 741, and 926 ppm in air. The impregnated filter papers were then attached to the screw caps of glass vials with volumes of 27 ml. Caps were screwed tightly on the vials, each of which contained separately 10 adults (1–7 days old) of each species of insect. Each concentration and control were replicated three times. Mortality was determined after 3, 6, 9, 12 and 24 hr from the commencement of exposure. When no leg or antennal movements were observed, insects were considered dead. This experiment designed with five replicates in control conditions at 27 °C, relative humidity was 65 ± 5% and in the dark condition [31].

2.8. Contact toxicity testing of oils and extracts

For this experiment in 14 cm diameter petri dish sprayed with different concentrations of *A. haussknechtii* extracts and essential oils along with the usual control and evenly placed. A number of adults twenty male and female insect poured into a petri dish, and after a period of time extract giving containers doors open and are counting the number of dead insects in the treatment and control containers. Insects each dish separately transferred to another container, then again in 72 hours measured insect mortality and mortality is calculated.

2.9. Stability evaluation of NP

For stability evaluation of the prepared NPs investigated with extract and control on *Sitophilus oryzae* and *Tribolium castaneum*. For this experiment in 7 cm diameter petri dish sprayed with different concentrations of *A. haussknechtii* extracts or nano-encapsulated product. After three days the amount of 20 insects inserted in a petri dish and after 24 hours

the number of dead insects obtained. The number of insects done after two days for several times until 47 days.

2.10. Data analysis and LC₅₀ investigation

Mortality characterization showed for Fumigant toxicity and contact toxicity for insects. For Fumigant toxicity, the phosphine used as a witness for further results. Acute toxicity of compounds was made after 24 hours exposure to 27±1 °C and relative humidity of 65±5. Mean percent adult mortality data were subjected to analysis of variance and compared with Duncan's multiple range tests to determine any differences between plant species and within species and concentration (SPSS 13.0 and SAS 5.01).

3. Results and Discussion

3.1. Nanocapsules characterization

The effect of emulsifier on the particles sphericity and stability evaluated with optical microscope is given in Table 1. The obtained data showed tween 80 had a positive effect on the size and stability of NPs. Table 2 showed the effect of polymerization and micelle preparation temperature on the stability of NPs showed the 45 °C as a suitable temperature for NPs fabrication. The rough morphology of the NPs that obtained with optimum formulation offers a brilliant insecticidal activity for the synthesized NPs.

The SEM micrographs of synthesized NPs by tween 20, 40 and PVA as an emulsifier and co-emulsifier respectively are shown in Fig. 2. As shown from this figure, aggregation of NPs occurred (Fig. 2. a). For optimized NPs by using tween 80 and PVP as an emulsifier and co-emulsifier and the polymerization temperature of 45 °C the NPs with 40 – 50 nm size, granular and spherical shape achieved (Fig. 2. b).

3.2. Contact toxicity and Fumigant toxicity characterization and comparison between encapsulated and free form

The oil from some kind of *Artemisia* showed contact toxicity against insects [32]. For *A. haussknechtii* regarding obtained mortality on investigated insects after 72 hours no contact toxicity was observed (Fig. 3). This is due to the higher volatility of active ingredient in samples.

Artemisia plant showed fumigant activity against several stored-product pests and herbs [6, 33]. The insecticidal constituents of these essential oils are monoterpenoids. Due to their high volatility, they have fumigant activity that might be of importance for controlling stored-product insects [6].

In all cases, considerable differences in mortality of insects between essential oils and extract vapors were observed with different concentrations and times. It has been shown that essential oils and extracts were relatively more toxic to *Tribolium castaneum* and *Sitophilus oryzae*. This is clear that the toxicity of oil is due to the active ingredient in it.

Another experiment was designed to assess the exact cytotoxicity of extracts on *Sitophilus oryzae* and *Tribolium castaneum* (Fig. 4). From this Figure, it was shown that extract has effective influence in mortality of insects. The minimum concentration of extract was 444 ppm that after that 100% of insects death.

From fumigant toxicity investigation it was clear that the more toxicity of *A. haussknechtii* comes from extracts of essential oil that similar to Abad *et al.*, results [4].

According to Elek prepared and investigated the NPs of novaluron, a water-insoluble insecticide. In this study *in vivo* experiments carried out with Egyptian cotton leafworm *Spodoptera littoralis* larvae and indicated that the toxicity of

NPs of novaluron resembled that of the commercial formulation [26].

In another study Boehm *et al.*, prepared stable polymeric polycaprolactone and polylactic acid nanospheres (135 nm), encapsulating 3.5% of ethiprole. Initial biological testing for aphid control on cotton plants showed the controlled release of nanosphere formulations and the chemical application of pesticide after release without change [34].

Yang and coworkers investigated the garlic essential oil loaded on polymer NPs (240 nm) that coated with polyethylene glycol (PEG) to evaluate their insecticidal activity against adult *Tribolium castaneum* [35]. In this study, the oil loading efficiency reached 80% at the optimum ratio of essential oil to PEG (10%).

Nevertheless, the nanosphere formulation showed enhanced systemicity of the active ingredients and improved its penetration through the plant, due to their small size.

3.3. Stability evaluation

Fig. 5 showed the stability results on insects. As shown in the figure the stability of oil was lower than NPs. The stability of *A. Haussknechti* extraction in 444 µg on *Sitophilus oryzae* and *Tribolium castaneum* were 29 and 23 days respectively. However, this amounts for encapsulated extracts were 59 and 45 days. As shown in Fig. 5, the percent of mortality was decreased with time, but the mortality for NPs was constant that's due to controlled release and evaporation of extracts from particles [36].

3.4. LC₅₀ characterization

As indicated from Table 3 the mortality in contact toxicity was low for investigating NPs (LC₅₀ of nano-encapsulated

extract in contact toxicity were 0.08 and 0.09 for *Sitophilus oryzae* and *Tribolium castaneum* respectively). Table 4 showed the LC₅₀ of nano-encapsulated extract and phosphine on insects. As shown in Table 4, the LC₅₀ decreased for investigating insects from phosphine to NPs. So its obvious that the mortality of insects represented in fewer concentrations of nano-encapsulated extract as an insecticidal agent.

Many plant extracts have shown potential insecticidal activity against insects [37-42]. Earlier authors reported the insecticidal activity of *Artemisia* species extracts and essential oil [43, 44].

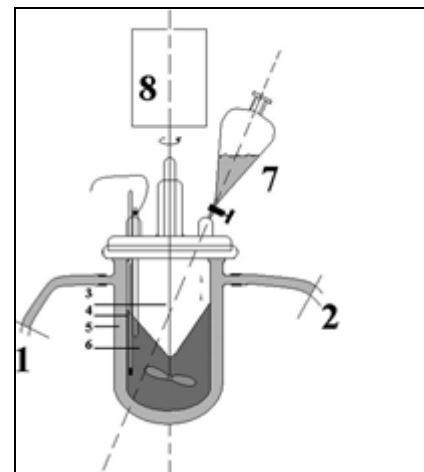


Fig 1: The schematic image of using reactor. (1 and 2: for entering and exiting of water; 3: stirrer; 4: thermometer; 5: Balloon; 6: continuous phase; 7: Hopper and 8: electrical stirrer engine)

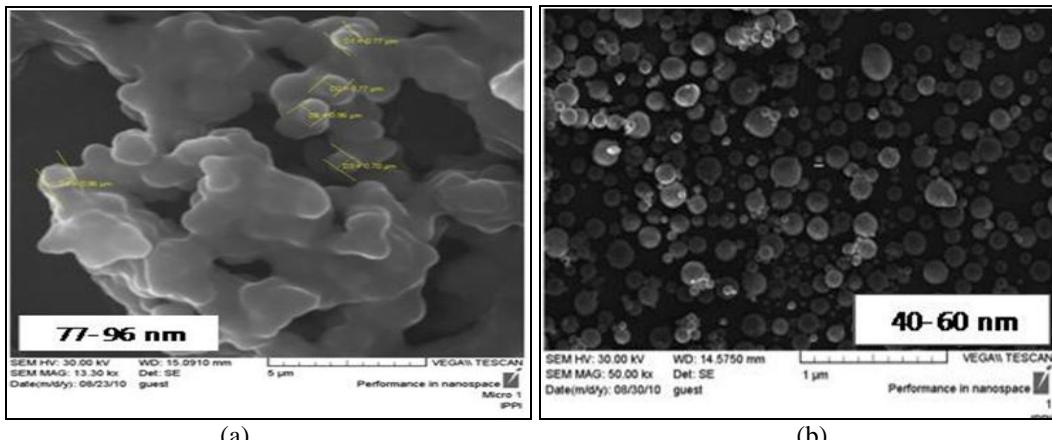
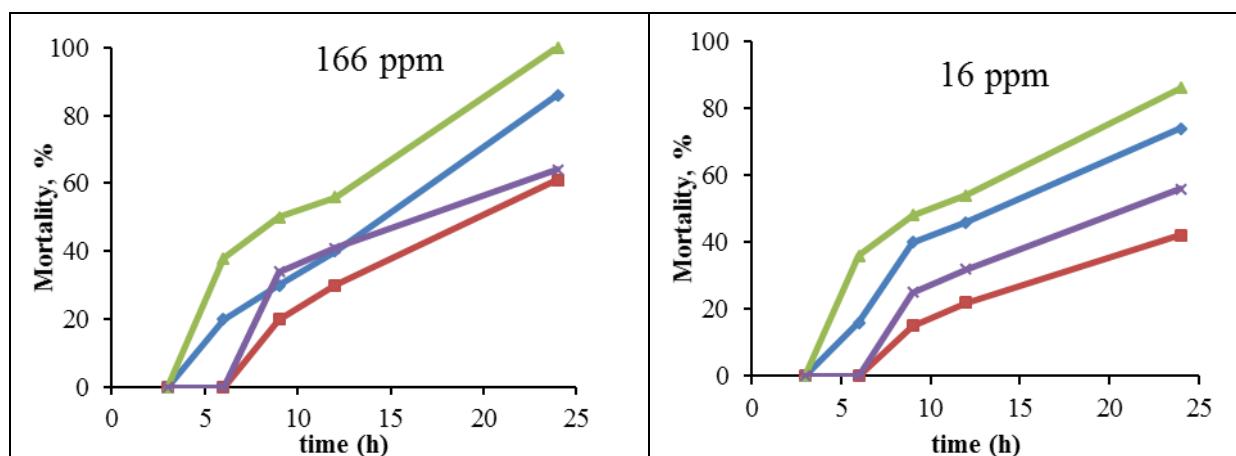
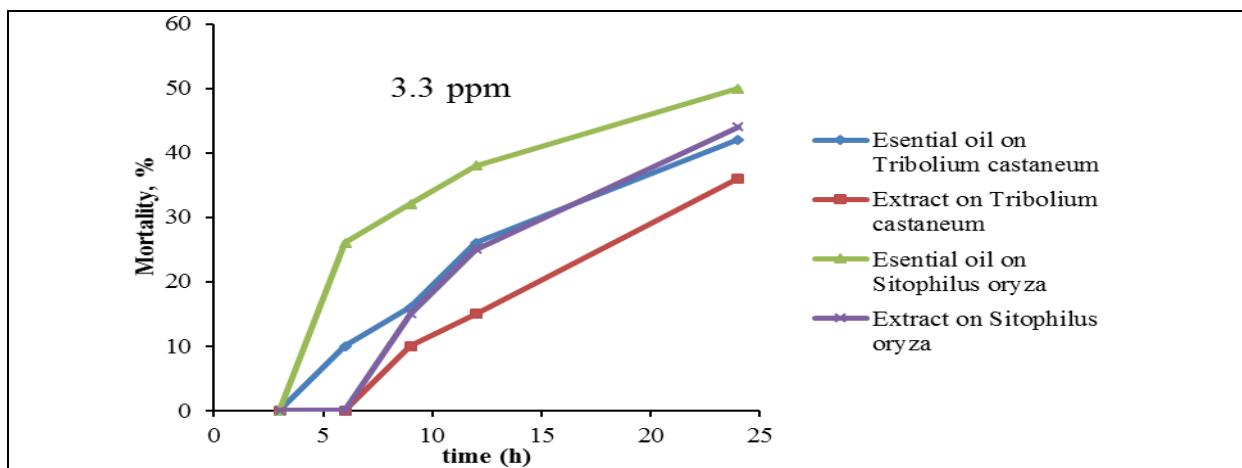
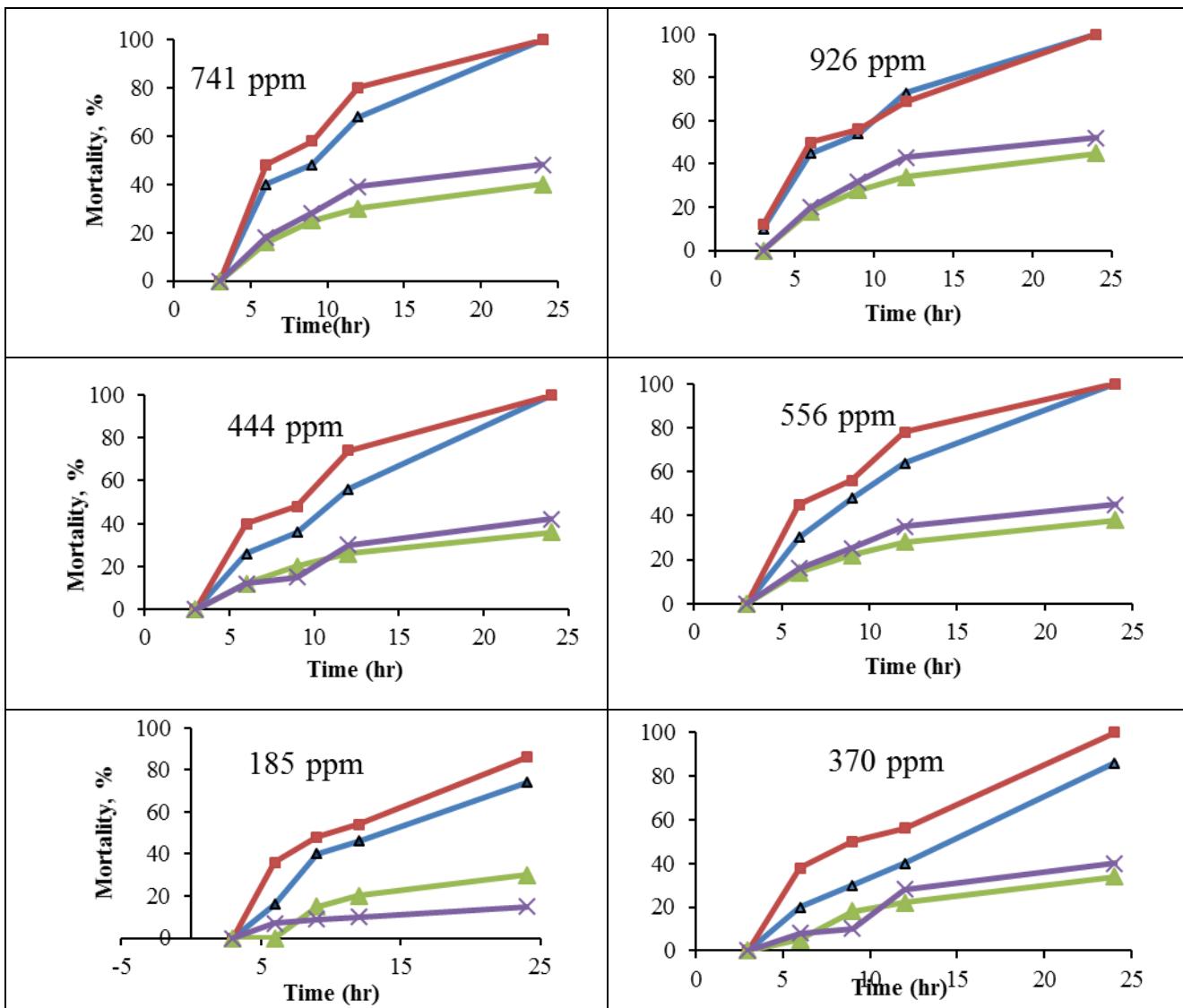
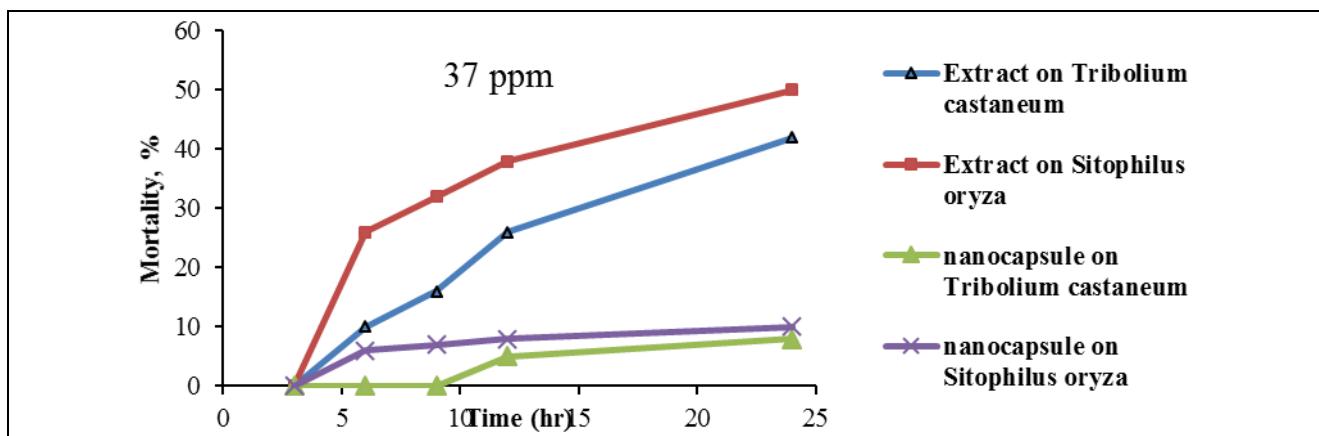
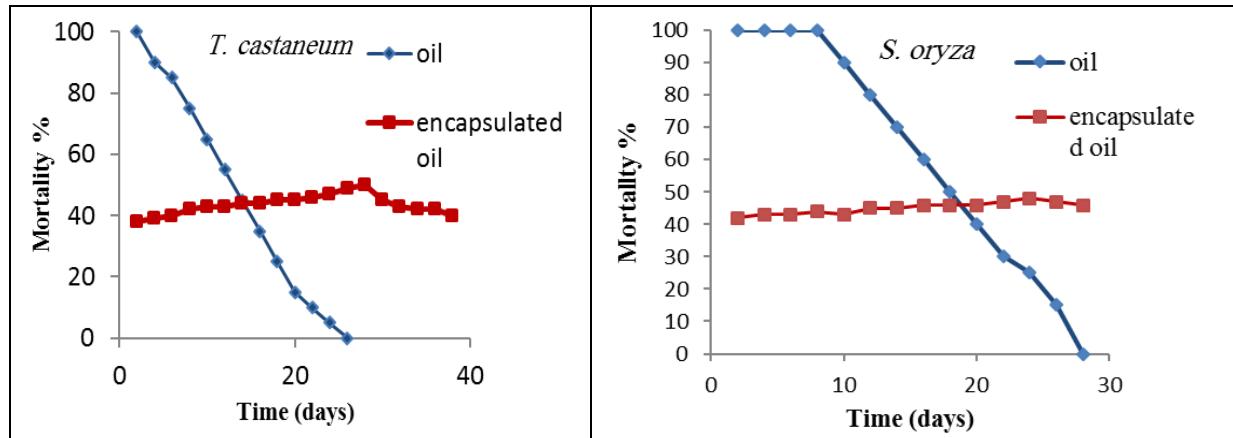


Fig. 2: SEM image of prepared NPs in different condition: (a) emulsifier tween 20, Co-emulsifier PVA and temp. 45 °C is showing aggregated particles; (b) emulsifier tween 40, Co-emulsifier CM and temp. 45 °C.



Fig 3: Fumigant toxicity of essential oil and its extract against *Tribolium castaneum* and *Sitophilus oryza*

**Fig 4:** Exact fumigant toxicity of extracts and its nanocapsulated form against *Tribolium castaneum* and *Sitophilus oryza***Fig 5:** The stability investigation of nanoparticles and extract for 444 µl**Table 1:** Evaluated variables in NPs preparation

sample	aqueous solution		organic solution		stability	sphericity
	water (ml)	Emulsifier 0.1 ml	solvent (ml)	Emulsifier 0.1 ml		
1*	160	tween 40	12	-----	weak	medium
2*	160	tween 20	12	-----	weak	weak
3*	160	tween 20	12	tween 40	weak	medium
4*	160	tween 20	12	tween 80	medium	medium
5*	160	tween 20	12	tween 20	weak	medium
6*	160	tween 40	12	tween 40	medium	medium
7*	160	tween 80	12	-----	medium	good
8*	160	tween 80	12	tween 80	good	good

Table 2: The effect of polymerization and micelle preparation temperature on the stability and shape of NPs.

micelle preparation temperature	Polymerization temperature	stability	sphericity
60	60	medium	medium
30	60	medium	medium
45	45	good	good

Table 3: LC₅₀ of nanoencapsulated extract in contact toxicity evaluation against *Sitophilus oryzae* and *Tribolium castaneum* (number of insects: 500).

Insect	Test material	p-value	χ^2 (df=4)	LC ₅₀ (ppm) (Confidence 95%)
<i>Sitophilus oryzae</i>	Nanoencapsulated extract	0.89	0.64	0.08
<i>Tribolium castaneum</i>	Nanoencapsulated extract	0.86	0.74	0.09

Table 4: LC₅₀ of nano-encapsulated extract and phosphine as potent pesticide in fumigant toxicity evaluation against *Sitophilus oryzae* and *Tribolium castaneum* (number of insects: 500).

Insect	Test material	p-value	χ^2 (df=4)	Intercept±SE	Slope±SE	LC ₅₀ (ppm) (Confidence 95%)
<i>Sitophilus oryzae</i>	phosphine	0.99	0.73	-7.06±1.01	2.58±0.36	550.11
	nanoencapsulated	0.89	0.64	-5.59±0.99	3.73±0.61	30.29
<i>Tribolium castaneum</i>	phosphine	0.98	0.72	-7.74±1.11	2.71±0.38	713.24
	nanoencapsulated	0.86	0.75	-8.37±1.27	4.97±0.82	45.82

4. Conclusion

Until now studies have not been reported previously concerning the activity of *A. haussknechtii* as a fumigant for insect pest control. The fumigant activity of essential oils from other *Artemisia* species has been evaluated. Through the extraction-evaporation of the dissolvent, *A. haussknechtii* oil has been encapsulated in a polymer. In the continue, the fumigant toxicity of oil, extract and encapsulated oil characterized that demonstrated fumigant toxicity against *Tribolium castaneum* and *Sitophilus oryzae*. The observations indicated that the insecticidal activity of the tested essential oils varied with insect species.

The control efficacy of oil loaded NPs against adult *Tribolium castaneum* and *Sitophilus oryzae* remained nearly 50 percent after 45 dayes, due to the controlled slow release of the active components, in comparison to free essential oil.

The limitations of present study were that there are the associated problems with these compounds for the practical application of essential oils, such as rapid degradation and high cost must be resolved. To overcome the rapid degradation of essential oils for using as pesticides, new formulation and techniques such as nanoencapsulation oil and controlled release technique were applied. Large quantity harvesting of plants may be the answer to the high-cost problem. Further, field trials of plant essential oils should be done for their practical application.

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