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Cuminum cyminum Methanolic Extract – Fe_3O_4 Nanocomposite: A Novel and Efficient Insecticide against the Potato Tuber Moth (Lepidoptera: Gelechiidae) to Protect Potatoes

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Phthorimaea operculella is a significant insect pest of cultivated potatoes in tropical and subtropical regions such as Iran. Since the potato is one of the most valuable sources of human food, non-chemical control of potato pests is essential. Application of medicinal plant extracts is a low-risk alternative method to synthetic chemical insecticides. Hence, the present study was conducted to investigate a potential strategy to improve the insecticidal activity of plant extracts for efficient management of *P. operculella*. The insecticidal efficacy of pure methanolic extract (PME) and Fe_3O_4@methanolic extract (FME) of *Cuminum cyminum* were evaluated against the potato tuber moth under laboratory conditions. The morphological characteristics of Fe_3O_4@Cumin were investigated by scanning electron microscope. Magnetic properties of the samples were determined using a vibration sample magnetometer. XRD was used to prove the crystalline structure of Fe_3O_4@Cumin. SEM, FTIR, XRD, and VSM analyses confirmed that the methanolic extract was loaded on Fe_3O_4. The toxicity of PME and Fe_3O_4@Cumin were evaluated on one-day-old eggs and penetration the first instar larvae of *P. operculella* into potato tubers and leaves. The LC_{50} values of Cumin extract and Fe_3O_4@Cumin were 961.07 and 601.48 and 496.84 and 268.82 ppm for penetrating neonate larvae to tubers and leaves, and 874.90 and 595.16 ppm for eggs, respectively. Bioassays revealed that Fe_3O_4@Cumin was more toxic than non-formulated Cumin methanolic extract against eggs and neonate larvae of *P. operculella*. The formulated methanolic extract was significantly more repellent against the pest. Our results suggested that the prepared nanocomposite could be used as a new effective tool for *P. operculella* management strategies to protect potatoes.

Keywords: *Phthorimaea operculella*, Fe_3O_4@Cumin, medicinal plant extract, nanocomposite, protect potatoes.

Potato (*Solanum tuberosum* L.) is one of the most valuable foods and agricultural products. Potato tuber moth (PTM), *Phthorimaea operculella* Zeller is an economically serious and extremely invasive potato insect pest all over the world (Lacey, 2012). Be-

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cause of potato plant defense and tolerance systems against potato tuber moth damage, killing the larvae is more consequential in stored potatoes (Lacey, 2012). This pest is difficult to control and in recent years, farmers have mainly controlled potato tuber moth with broad-spectrum insecticides. Hence, the excessive application of some chemical insecticides has reduced their efficiency against PTM invasions (Lacey, 2012). Therefore, it is essential to search and find novel, effective, low-hazardous, and bio-natural methods for controlling key pests such as PTM. The use of botanical sources like medicinal plants is a considerable and safe insect pest control strategy (Downum et al., 1993; Rafiee-Dastjerdi et al., 2013). Botanical insecticides have long been regarded as satisfactory alternatives to chemical insecticides for pest management since they have low persistence in environment, no or little human toxicity, and wide public acceptance (Sampson et al., 2005; Digilio et al., 2008). Arabi et al. (2007) reported efficacy of *Cuminum cyminum* (L.) essential oil against several stored product pests. Rafiee-Dastjerdi et al. (2013) investigated efficacy of 5% methanolic extracts of *Fumaria officinalis* L., *Lavandula angustifolia* L., *Glycyrrhiza glabra* L. and *Origanum vulgare* Mill against 1st instar larval penetration of *P. operculella*. They reported the preventive effect of the mentioned extracts (except for fumitory) on 1st instar larval penetration. Therefore, we used a medicinal plant, *C. cyminum* to evaluate its efficacy against one-day-old eggs and the 1st instar larvae of PTM. Despite the potential of medicinal plant extracts, these natural control agents have some disadvantages such as low persistence in environment and sensitivity to sunlight that has limited their application under field and storage conditions. So, these problems will be overcome through recent technological advances such as nanotechnology that will permit future use of botanical extracts in crop protection systems and nanoformulation approaches will improve their efficacy and toxicity. Due to the bio-compatibility, bio-degradability, and simple surface modification of superparamagnetic iron oxide (Fe_3O_4), it is most commonly applied as drug delivery nanoparticle in biomedical applications (Wu and Jiang 2008; Arami et al., 2011). These notable advantages of Fe_3O_4 attracted our interest in utilizing it as a promising technique in plant protection and insect pest management. Cumin extract (because of the presence of hydroxyl groups) has the ability to interact with hydroxyl groups on the surface of Fe_3O_4. Consequently, we selected Fe_3O_4 as a support for Cumin extract since *C. cyminum* methanolic extract + Fe_3O_4 is a suitable and good superparamagnetic with low toxicity for environment and human health which can control potato tuber moth infestations.

Materials and Methods

Insect rearing

A colony of potato tuber moth was obtained from the University of Mohaghegh Ardabili, Ardabil, Iran. The colony was continuously reared on potato cultivar Agria. Experiments were carried out under laboratory conditions at 26 ± 1 ºC, 60 ± 5% RH and a photoperiod of 8: 16 (L: D). To achieve cohort eggs of *P. operculella*, 30 male–female pairs of newly emerged moths were kept in cylindrical containers that were covered with fine mesh netting on the heads. Filter papers placed on the head of containers provided an oviposition site for female moths (Golizadeh and Zalucki, 2012). The adults were fed using a solution of 10% honey-water.

Preparation of methanolic extract

Methanolic extract was obtained from seeds of *C. cyminum* by using a Soxhlet extractor. 40 g powdered plants were placed in a Soxhlet apparatus. The apparatus was charged with 300 ml methanol. Soxhlet was heated to the boiling point of the solvent and allowed to cycle for 10 h. Excessive methanol was evaporated in a rotary evaporator.

Instruments

The particle morphology was determined by a scanning electron microscope (SEM) (Day Petronic Company, Tehran, Iran) using FESEM-TESCAN MIRA3 scanning electron microscope. Fourier transform infrared (FT-IR) spectra were recorded using KBr pellets on a Nexus 670 FT-IR spectrometer (Medical Sciences of Urmia University, Urmia, Iran). Powder XRD was performed on an X'Pert Pro Panalytical diffractometer (Holland) at 40 kV and 30 mA with a CuKα radiation (λ = 1.5418 Å) and diffraction patterns were recorded in 2θ range (10°–80°) (Kurdistan University, Sanandaj, Iran). Magnetic properties of the samples were determined by a vibration sample magnetometer (VSM, Meghnatis Daghigh Kavir Co., Iran) under magnetic field up to 20 kOe.

Preparation of nanocomposite (Fe_3O_4@Cumin methanolic extract)

Generally, solutions of $FeCl_3{\cdot}6H_2O$ (2 g) and $FeCl_2{:}4H_2O$ (0.8 g) were separately prepared in 20 ml distilled water under inert gas atmosphere for 10 min. Then, the above solutions were slowly added to 20 ml Cumin methanolic extract solutions at determined concentrations. The mixture was stirred at 50 °C under inert gas atmosphere for 1 h, and then, aqueous ammonia was added to the solution until pH value of above 10 was obtained. Then, the solution was kept at 50 °C under rigorous stirring for further 20 min. The precipitate was collected with an exterior magnet and washed with water several times and finally, dried in vacuum for 24 h.

Bioassays
Larval penetration
Potato-dipping

Each potato was separately dipped in different concentrations of pure Cumin and Fe_3O_4@Cumin methanolic extracts that were determined by preliminary dose setting experiments. Concentrations of PME (pure methanolic extract) and Fe_3O_4@Cumin methanolic extract were 500–1500 and 300–1000 ppm, respectively. When tubers were dried, they were transferred into plastic containers with ventilated lids and were kept at 26 ± 1 °C, 60 ± 5% RH and photoperiod of 8 L: 16 D. Then 20 newly larvae (< 5-h old) were placed on each tuber by a soft hair brush. Criterion of larval penetration was the number of adult emergence in experiments. Each trial was replicated three times. In all Fe_3O_4@Cumin methanolic extract experiments, 0.1 g nanocomposites were dispersed in 100 ml distilled water + 0.02% Tween-80 until water absorption was stabilized. After shaking and product dispersion, potato tubers were dipped in the solutions for 15 sec.

Leaf-dipping

The fresh leaves of potato used in leaf-dipping under laboratory conditions. Cumin and Fe_3O_4@Cumin methanolic extract were applied to fresh leaves of potato in laboratory conditions at 250–750 and 150–400 ppm, respectively. For each trial, the fresh leaves were dipped in the solutions. The leaves were then dried and placed in Petri dishes. To supply humidity, wet filter papers were placed on the bottom of the Petri dishes. Then 20 new larvae (< 5-h old) were placed on each leaf. Mortality was recorded after pupae and adult emergence. Each treatment and the untreated control had three replications per experiment.

Egg-dipping

PME and Fe_3O_4@Cumin methanolic extracts were applied at 650–2000 and 350–1500 ppm, respectively. For treatments, each filter paper contained 20 one-day-old eggs separately dipped in solutions of PME and Fe_3O_4@Cumin methanolic extracts. When filter papers were dried, they were transferred into plastic containers with ventilated lids containing potato tubers to penetrate into the potatoes. The mentioned plastic containers were kept under laboratory conditions. Egg hatch was investigated using a light microscope after eight days. Each experiment was replicated three times. The trials were carried out same as the previous one.

Repellency

Repellency trials were carried out following Talukder and Howse (1994). Potato leaves were used. One half of the potato leaf was treated with 100 ppm of Cumin/ Fe_3O_4@Cumin methanolic extracts, respectively and the other half of the leaf was treated with methanol/water only (as control). After 30 min and evaporation of the solvent/ water, they put on the bottom of Petri dishes (8 cm diameter and 2 cm height). Next, 10 numbers of neonate larvae were released on the centre of potato leaf. The number of insects was accounted in each side after 72 h. Percentage of repellency was accounted by $PR=\frac{Nc-Nt}{Nc+Nt}$. In this equivalent, Nc = the number of insects on control surface and Nt = the number of insects on treated surface (Obeng-Ofori, 1995). Each treatment was replicated three times.

Data analysis

In order to determine LC_{50} values, the data were analyzed by probit procedures with SPSS for Windows® release 16. Mean comparisons were examined by *T*-test.

Results

By comparing FT-IR spectra of Fe_3O_4@Cumin nanocomposite and Cumin, the presence of Cumin surrounding Fe_3O_4 can be clearly observed by related peaks (Fig. 1). Both samples showed typical absorption bands of Cumin: ~1180 cm^{-1}, C-O and C-OH stretching, ~1426, 1664 cm^{-1} aromatic C = C stretch, ~2809–2940, vinylic-CH and ~3394, and -OH stretch; therefore, it was shown that biopolymer chains maintained their chemical features when in contact with Fe_3O_4. Magnetic hysteresis loop measurements of Fe_3O_4@Cumin indicate that its saturation magnetization value (Ms) is 52.65 emµ/g^1 (Fig. 2). SEM images show the morphology of Fe_3O_4@Cumin nanocomposite is homogeneous (Fig. 3). However, the calculated average crystal size based on SEM was below 20 nm. This shows that Fe_3O_4 nanoparticles have been synthesized and embedded inside Cumin network. Therefore, Fe_3O_4 nanoparticles are surrounded by Cumin and have generated Fe_3O_4@Cumin nanocomposite. Additionally, X-ray diffraction (XRD) peaks verifies the formation of Fe_3O_4 (Fig. 4).

LC_{50} values of *C. cyminum* methanolic extract and Fe_3O_4@Cumin on the 1st in-star larval penetration of potato tuber moth into potato tubers and leaves were 961.07

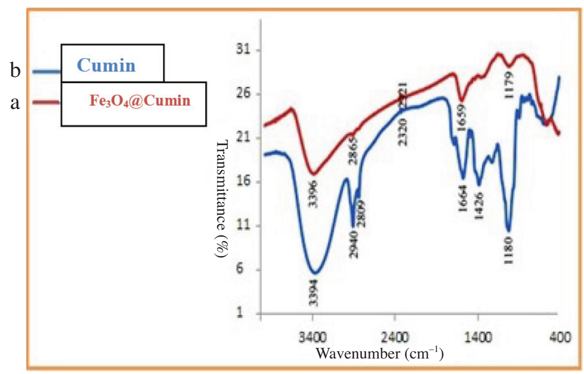

Fig. 1. FT-IR spectra of Fe_3O_4@Cumin (a) and Cumin (b)

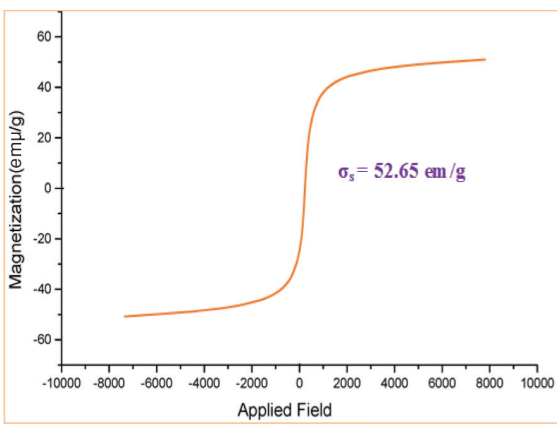

Fig. 2. VSM of Fe_3O_4@Cumin at 300 K

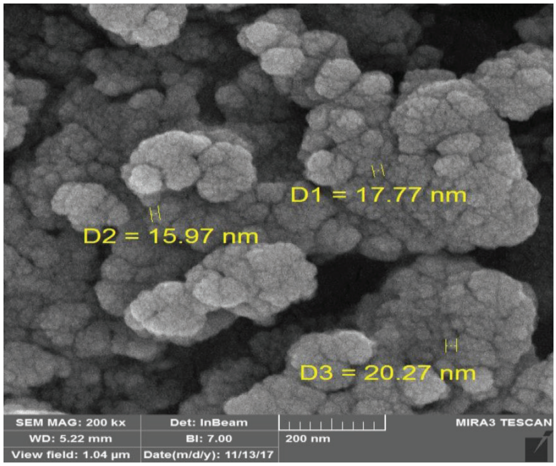

Fig. 3. SEM image of synthesized Fe_3O_4@Cumin

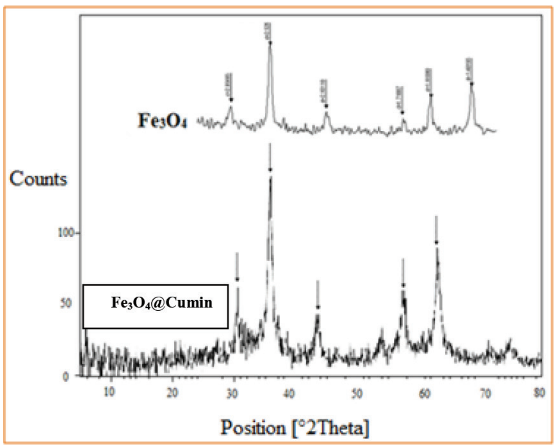

Fig. 4. XRD pattern of Fe_3O_4@Cumin

and 601.48 and 496.84 and 268.82 ppm, respectively (Table 1). LC_{50} values of PME and Fe_3O_4@Cumin at the egg stage of *P. operculella* were 874.90 and 595.16 ppm, respectively (Table 1). A statistically significant difference in the toxicity of PME and Fe_3O_4@Cumin was observed on the 1st instar larval penetration and the eggs of *P. operculella*, as inferred by the lack of overlap in the LC_{50} confidence intervals. Additionally, the LC_{90} values of PME and Fe_3O_4@Cumin were 2886.10 and 1938.92 and 365.75 and 1385.60 and 694.17 ppm for neonate larval penetrations into potatoes and leaves, respectively. These values were 3686.35 and 3386.04 ppm for eggs of PTM, respectively (Table 1). It can be concluded that the insecticidal activity of pure Cumin extract and Fe_3O_4@Cumin depended on their concentrations. According to Table 2, there is a significant difference between % repellency of formulated and non-formulated methanolic extract so the Fe_3O_4@Cumin was more repellent against neonate larval penetration into leaves. In this investigation, Fe_3O_4 was synthesized in the presence of Cumin which led to the synthesis of Fe_3O_4@Cumin.

Table 1

Toxicity of *C. cyminum* methanolic extract and Fe_3O_4@Cumin to 1st instar larvae and eggs of *Phthorimaea operculella*

Growth stages	Treatments	Slope ± S. E.	χ^2 (df)	LC_{50} (ppm)	LC_{90} (ppm)
Egg	PME	4.90 ± 0.44	2.47 (3)	1210.50 (1062.14–1396.82)	3686.35 (2701.69–6710.83)
	Fe_3O_4@Cumin	5.97 ± 0.35	1.68 (3)	827.92 (702.87–998.12)	3386.04 (2274.45–5721.07)
Neonate larvae (Penetrate into potatoes)	PME	4.77 ± 0.46	2.40 (3)	961.07 (845.71–1111.76)	2886.10 (2104.85–5364.64)
	Fe_3O_4@Cumin	5.04 ± 0.42	1.30 (3)	601.48 (524.97–702.58)	1938.92 (1394.31–3634.27)
Neonate larvae (Penetrate into leaves)	PME	5.01 ± 0.47	2.29 (3)	496.84 (441.18–571.79)	1385.60 (1035.60–2397.19)
	Fe_3O_4@Cumin	5.96 ± 0.52	1.78 (3)	268.82 (240.75–304.89)	694.17 (530.86–1160.52)

PME = pure methanolic extract (non-formulated extract)
95% fiducial limit (FL) is shown in parenthesis

Table 2

Percentage of repellencies caused by Cumin and Fe_3O_4@Cumin against neonate larvae of *Phthorimaea operculella* after 72 h

Compound	Concentration (ppm)	% Mean repellency ± S.E
Cumin	100	60.00 ± 2.17
Fe_3O_4@Cumin	100	83.33 ± 3.02*

*Showed significant difference ($P < 0.05$).

Discussion

During the synthesis, Cumin acted as a stabilizer and after the synthesis, Fe_3O_4 nanoparticles were functionalized by Cumin. Hydroxyl groups of Cumin can act as a reaction mediator to proceed with the reaction. Fe_3O_4 can be functionalized by hydroxyl groups. Fe_3O_4 nanoparticles have numerous hydroxyl groups on their surface and therefore they are naturally hydrophilic (Safaei-Ghomi and Zahedi, 2015). Surface coating or modification of iron oxide nanoparticles is very important in many applications due to their aggregation and difficulty in dispersion in organic media (Safaei-Ghomi and Zahedi, 2015). Several studies are available on the application of non-formulated and nano-formulated *C. cyminum* seed essential oils in plant protection against pests (Arabi et al., 2007; Bashiri et al., 2016). Similarly, the results of Khorrami et al. (2018) showed that methanolic extract of cumin exhibited the highest toxicity to 2nd instar larvae of *Pieris brassicae* (L.) and acceptable efficacy for *Brevicoryne brassicae* L. adults. Karakas (2017) demonstrated that Fennel ethanolic extract had moderate-low efficacy against *Sitophilus granarius* L. (%mortality = 26.4). Ethanolic extracts of *Foeniculum vulgare* and *Ocimum basilicum* L. are potential agents to be used in termite control (Aihetasham et al., 2017). As plant extracts are sensitive to environmental conditions and their stability is affected by several factors, we used Fe_3O_4 as a Cumin methanolic extract support. Nakhjiri et al. (2017) used a high effective biologically method for the reduction of Ag^+ to Ag NPs using *Echinops* extract as a stabilizer and reducing agent. They synthesized AgNPs which exhibited potential antibacterial activity against pathogenic bacteria. AgNPs have a key function in nanotechnology and nanomedicine.

Based on LC_{50} values, a significant reduction was seen in the amount of Cumin extract found in the formulated one. Our results were consistent with those reported by Loha et al. (2012). They investigated the efficacy of β-cyfluthrin formulation synthesized from poly (ethylene glycols) based on amphiphilic copolymers against *Callosobruchus maculatus* F. Their results showed that the formulations demonstrated greater efficiency compared to control. Green synthesis of AgNPs using *Euphoria prostrate* Aiton was applied to control the adults of *Sitophilus oryzae* L. (Zahir et al., 2012). Silver and lead nanocomposites synthesized with *Avicennia marina* (Forssk.) Vierh extract displayed pesticidal activity against *S. oryzae* and the results exhibited that treatment with the mentioned nanocomposite caused 100% mortality within four days after treatment (Sankar and Abideen, 2015). Zahir et al. (2012) reported insecticidal activity of AgNPs by using aqueous extracts of *E. prostrate* leaves against *S. oryzae* adults. LD_{50} values of aqueous extract, $AgNO_3$ solution, and synthesized AgNPs were 213.32, 247.90 and 44.69 mg/kg, respectively. Their bioassays revealed that AgNPs was more toxic than aqueous extract against adult pests which was consistent with our results.

In general, nanomaterials can be advantageous in agricultural research and productions due to their sizes which are similar to most biological molecules allowing them to spread through cell membranes to act on the target. These nano-scaled formulations reduce lethal concentrations and increase the stability of insecticide compounds. The strategy applied in this investigation to formulate methanolic extract of Cumin with Fe_3O_4 led to a reduction in the concentration of methanolic extract needed to provide insecticidal activity. Fe_3O_4@Cumin was more toxic than *C. cyminum* methanolic extract against eggs and neonate larvae of *P. operculella.* Finally, we concluded that this type of nano-scaled formulation of Cumin extract was highly effective to be used in stored product pest management.

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