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Preparation of Chlropyrofos Loaded Silver Nanoparticles Coated with Poly(ethylene glycol) and Chitosan and Evaluation of Termiticide Activity

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ABSTRACT

A delivery system of insecticides induces active ingredient having desirable for pest control and nanotechnology may be improve of pesticide efficiency. Chlropyrofos as an organophosphate insecticide is used to control many different type of agricultural pest such as termites, mosquitoes, cockroaches. Termites, one of the important pests, cause a lot of damages to many field of agricultutre and in the south of Iran, subterranean termite, is popularly damages many products. In this research, chlropyrofos was loaded onto poly(ethylene glycol) and chitosan decorated nanoparticles (silver/PEG/Cts NPs) and evaluated its insecticidal (termiticide) study. Silver nitrate was used as the silver source and chitosan and PEG were used as the polymeric supporter and stabilizer and chlropyrofos as insecticide. The developed Ag/PEG/Cts NPs-chlropyrofos were then characterized by a UV-VIS spectrophotometer, Fourier transform infrared, transmission electron microscope, confirmed the formation of spherical nanoparticles with the size of 15-25 nm. The insecticidal activity of nanoparticle was tested *in vitro* against termite (*Microcerotermes gabrielis* W. (Isoptera: Termitidae)). The result showed the effectiveness of the target nanoparticles after 48 h at 50 ppm on the insects.

KEYWORDS

Amino acid, Ultrasound, Aqueous media, Cavitation.

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INTRODUCTION

The use of high amounts of pesticides causes many environmental problems, and lack of full control of pesticide is reportedly responsible for insect resistance, and also being used in higher quantities raise accumulation of these compounds in environment and agricultural products. Termite is one of the important pests that damages the agricultural productions, wood structures and caused serious problems in many parts of the world in other agricultural fields. Developing the new pesticides formulations is essential to overcome commercial pesticides problems has been attractive by many researchers. Today, many pesticides were produced as nanopesticides by different method and for special targets such as slow releasing, increasing solubility and biological activity. The advantages of nanopesticides have potential ability such as the large surface area that increase accessibility to insects, which makes increasing water solubility and dispersity. They also absorbed into cuticule layer and inserted into the insect body and cause death [1]. It has also been reported that the pesticide nanoformulation showed less toxicity toward non-target organisms compared to the bulk or commercial formulations and therefore high specificity was observed [2,3]. Many studies have conducted to prepare pesticides in nanoparticles (NPs) coating on nanosilver containing polymer such as chitosan, PEG polymers [4]. For example, Navaladian *et al.* [5] used thermal decomposition as viable method for preparation of the crystalline silver nanoparticles using silver oxalate in water and in ethylene glycol, poly(vinyl alcohol) (PVA) as a capping agent. The particles were spherical in shape with size below 10 nm. The advantages of silver nanoparticles products are numerous and have long been known to have antimicrobial activities which have been used for centuries to prevent and treat diseaseses [6,7].

Silver nanoparticles have showed potential insecticide activity such as pediculocidal and larvicidal activity. The insecticide activity of silver nanoparticles has also been reported against head louse *Pediculus humanus* and fourth instar larvae of *Anopheles subpictus* and *Culex quinquefasciatus* [8,9]. Zhang *et al.* [10] used copolymer consisting chitosan and polylactic acid (Cts-PLA) to encapsulate insecticide imidichlopride in the core of micelle in 254.6-334.6 nm. Feng and Feng [6] also used chitosan derivatives C-O-carboxylated with licinoleic acid, to capsulate azadirachtin in 200-500 nm. Kumar *et al.* [11] prepared a nanocapsulated formulation of imidiclopride covered with sodium alginate, which was less cyctotoxic and better pesticidal efficiency over commercial imidoclopride. Insecticide chlopyrifos was encapsulated into the polymer PVC with a 118-140 nm in size and high biological activity was observed [12]. In other case, the pyrethroid pesticide deltamethrin was conjugated with nanosilver to form nanoparticles was effective at low concentration 9×10^{-4} M against mosquitoes after 24 h in 15 nm size. These examples show the potential vectors of nanopesticides [13].

Chitosan a naturally occurring polymer has been extensively investigated as a natural cationic biopolymer with excellent biocompatibility, biodegradability, non-toxicity, bioactivity, interesting structural and functional properties, cationic exchange and hydrogen bonding ability, the high hydrophilicity and other interesting chemical properties due to the presence of amino and hydroxyl groups [14]. Chlorpyrifos, *o*,*o*-diethyl *o*-3,5,6-trichloropyridin-2-yl phosphorothioate (Fig. 1) , is one of the organophosphate insecticides that are used for controlling different insect pests such as termites and mosquites in agricultural, domestic and industry, and highly toxic to *Formosan subterranean* termite and *Coptotermes formosanus* Shiraki [15,16].

$$AgNO_3 + \text{PLG/Cts (aq)} + NaBH_4 \longrightarrow Ag^0\text{PEG/Cts (aq)}$$

$$Ag^0\text{PEG/Cts (aq)} + \text{Chlropyrofos (a.i.)} \longrightarrow Ag^0\text{PEG/Cts/chlropyrofos}$$

Chlropyrofos

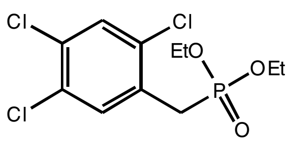

Fig. 1. Schematic representation of Ag/PEG/Cts NPs Chlorpyrifos synthesized from $AgNO_3$ by reduction route

In this study, an efficient one-step synthesis of nanopesticide was performed to prepare a stable aqueous colloids of Ag/PEG/Cts NPs chlropyrifos. The nanoparticles of silver coated with poly(ethylene glycol) (PEG) and chitosan (Cts) which can efficiently protect from aggregation [2,17] and incubated with chlropyrifos insecticide. The efficacy was evaluated in vitro tests by contact treatment against termites. This study would be useful to develop a practical controlling method for termites which is a serious pest of wooden structures and agricultural crops.

EXPERIMENTAL

Silver nitrate (98 %) and PEG was obtained from Merck. High-purity deionized water was obtained from milli-Q. Chlropyrofos as powder was procured from Aldrich-Sigma as technical 98 %, water solubale chitosan from Aldrich-Sigma. Other chemicals were analytical grade supplied from Aldrich-Sigma, Merk and Acros and used as received.

FT-IR spectra of nanoparticles were determined by using a Shimadzu IR prestige-21 FT-IR. Size distributions of the nanoparticles were determined by dynamic light scattering using Malvern Zetasizer Nano ZS (Malvern Instrument Ltd., UK) at a scattering angle of 173º and a temperature of 25 ºC. Samples were diluted by a factor of 100 prior to the measurements to avoid multiple scattering effects. The UV-visible spectra were acquired with Perkin Elmer Lambada 28 and visible absorption spectra were collected over a wavelength range of 300 to 500 nm. The morphology of nanoparticles was characterized by depositing the suspension (2 μL) onto Formvar-coated copper grids and excess water was evaporated. The obtained samples were left to dry under ambient air, andthen visualized *via* TEM (Zeiss-EM10C-100 KV). Size of nanoparticles was analyzed using iTEM software (version 3.2, Soft Imaging System GmbH) equipped with TEM. Structure of Ag/PEG/Cts NPs was determined using selected area electron diffraction (SAED) technique *via* high-resolution.

Synthesis: For synthesis of Ag/Cts/PEG/chlropyrofos NPs, chitosan solution was prepared by addition of a solution of chitosan (20 mg in 1.0 wt % of acetic acid (10 mL) in 10 mL deionized water and PEG-6000 solution was prepared by addition of PEG-6000 (20 mg) in deionized water (10 ml). These two solutions were mixed in 100 mL conical flask and stirred at room temperature for 15 min, then 40 mg of silver nitrate was added under nitrogen atmosphere to prevent oxidation reaction during silver nanoparticles formation. Sodium borohydride (80 mg) was added in portion to the latter solution followed by addition of a solution of chlropyrofos (100 mg) in acetone (1 mL) and stirred for 4 h to incubate pesticide on AgNPs. The colour of the solution converted from pale brown to dark brown indicating the formation of AgNPs. Finally, the solution was centrifuged and washed four times with deionized water, dried at 40 ºC in dark place under nitrogen atmosphere. The precipitate was centrifuged three times and washed with distilled water and EtOAc (to remove unabsorbed chlropyrofos). The black grey precipitate was dissolved in 20 mL of H_2O and four concentrate solutions (100, 50, 10 and 5 ppm) was provided for contact biological test on termites. Chlropyrofos sample as technical grade (98 %,100 ppm) was prepared and

100 mL of each sample was applied on filter paper for the biological test. The obtained black grey precipitate Ag/Cts/PEG NPs-chlropyrofos were also used for further characterization.

***in vivo* Study of contact treatment against termites:** Termites were collected from the local gardens of Khozestan province in Iran. Termites (*Microcerotermes gabrielis* W. (Isoptera: Termitidae)) were put in petri-dishes containing moist filter paper. They were put in dark place. Six samples including water, chlropyrofos loaded Ag/PEG/Cts NPs with four different concentration (100, 50, 10 and 5 ppm) were used in the contact treatment. Water was used as control, all six samples were used in four replicates. For the contact treatment, termites were placed in a group of 10 termites in a petri-dish (92 mm × 16 mm). 100 µL of each of the samples was applied on filter paper (90 mm, Whatman) in petri-dishes and then termites were then transferred to petri-dishes. The petri-dishes were placed into a controlled environment chamber containing a thermometer, a lid and a metal container with water and the heater to maintain the temperature at 28 ± 1 ºC and high humidity and total darkness (except during observation). At different time intervals, the petri-dishes were removed from the chamber and the lids were opened with the least possible disturbance in order to examine termite response (mortality).

Statistical analysis: The average termite mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95 % confidence limits of upper confidence limit and lower confidence limit and Chi-square values were calculated using the statistical package of Polo-PC. Results with $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

In the present study, polymer modified silver nanoparticles were prepared to load the insecticides *in situ via* stabilization by PEG/Cts (Fig. 1).The process of the reaction is proposed as follow: First, chitosan solution was mixed with PEG solution to form Cts/PEG solution in deionized water, then this aqueous solution was treated with Ag^+ ion to form silver polymer $[Ag/PEG/Cts]^+$. Reduction of this silver/polymer with $NaBH_4$ at room temperature afforded Ag/PEG/Cts NPs, in which Ag^+ was easily reduced to Ag^0 forming AgNPs. The chemical reduction method is commonly used to prepare AgNPs because of its great advantages in generating high yields and easy reaction method [18,19]. Huang *et al*. [20] reported the synthesis of different Ag-chitosan nanoparticles in aqueous solution by the reduction of corresponding salts with $NaBH_4$ [21,22]. In other study, Wei *et al*. [23] also carried out research on chitosan-based silver nanoparticles by reducing silver nitrate salts in the presence of chitosan.

In the next step as shown in Fig. 1, the incubation of nanoparticles with chlropyrofos was conducted by addition of pesticides onto the colloidal solution on silver nanoparticles, then the active ingredient (chlorpyrifos) gets included/sorbed to the matrix surfaces. It is well reported that the reduction of silver ions in aqueous solution to silver nanoparticles is accompanied by the colour change. The colour change of the solution to dark brown indicate the successfully formation of Ag/PEG/Cts NPs chlorpyrifos. The formation of silver nanoparticles in the nanocomposite was also confirmed by UV-visible spectroscopy and TEM (Figs. 2 and 4). The product nanoparticles were not aggregated during incubation and the colloidal stability was confirmed by the absence of precipitate after centrifugation. In similar study, silver-impregnated chitosan films prepared from silver nitrate and chitosan as stabilizer and ascorbic acid as reducing agent *via* thermal reaction [19,23]. In the process of synthesizing nanoparticles, a stabilizer is used to control the formation and dispersion stability of metal nanoparticles. For this purpose, polymers of chitosan and PEG have been widely used as a particle stabilizer to control the particle growth, stabilize the metal dispersions and limit the oxidation of the particle [24,25]. Notably, chitosan is a natural cationic biopolymer obtained from deacetylation of natural chitin, which consists of polymeric β-(1,4)-2-amino-2-deoxy-D-glucose [26]. Due to its excellent biocompatibility, biodegradability, non-toxicity and bioactivity properties, chitosan has gained much attention due to it's a potential polysaccharide resource makes its use preferable [20].

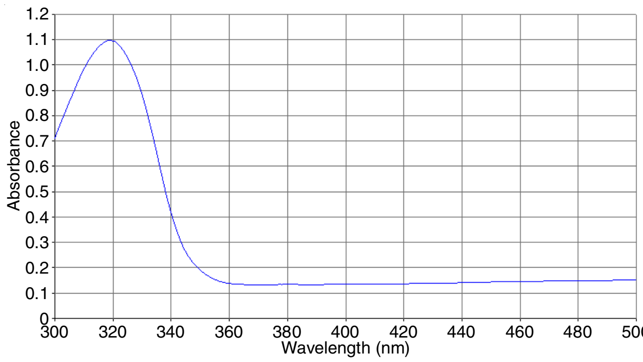

Fig. 2. UV-visible spectrum of Ag/PEG/Cts NPs-Chlropyrofos

The TEM images of Ag/PEG/Cts NPs chlorpyrifos (Fig. 3) revealed the spherical particles of average size range 23-30 nm and majority of the spheres are in this size range. It is obvious that these nanoparticles are monodisperse. TEM micrographs are the best method of determining the morphology of nanoparticles [27,28]. The Ag/PEG/Cts NPs chlorpyrifos had a shape size distribution and a mean diameter of 240 nm, this was due to the fact that big large particles distribution corresponds (Fig. 3). UV-visible spectroscopy of obtained NPs-chlorpyrifos is shown in Fig. 2, where broad peak at 315-330 nm strongly suggested that AgNPs were spherical, in addition, chlorpyrifos absorption bands was at 609 nm and 660 nm, which proved it incubated onto Ag/PEG/Cts NPs. Sharma *et al*. [29] and Shameli *et al*. [30] explained that spherical AgNPs contribute to the absorption bands at around 400 nm in the UV-visible spectrum.

In FT-IR spectrum of Ag/ PEG/Cts NPs chlorpyrifos (Fig. 4), the peaks at 2901, 1650 and 1606 cm^{-1} were due to N-H bending, while the peaks at 1436 and 1385 cm^{-1} were due to C-H bending, 1335 cm^{-1} as due to C-N stretching and finally, the deformation vibration of amine group in chitosan at 1080 and 1053 cm^{-1}. The appearance of medium peak at 1346 cm^{-1} in Ag/PEG/Cts spectrum is attributed due to the complexation between Cts/PEG and $AgNO_3$ to form metallopolymer (Ag/Cts/PEG). Additional peaks at 1404 and 842 cm^{-1} corressponds to chlropyrofos adsorption on the silver surface of AgNPs [31].

TABLE-1
INSECTICIDAL ACTIVITY OF Ag/Cts NANOPARTICLES AGAINST

Insect	Concentration (ppm)	48 h Mortality (%) ± SD	LC_{50} 72 h (μg/mL) (LCL-UCL)	LC^{90} 72 h (μg/mL) (LCL- UCL)	X^2
Termite	100	97.5 ± 0.43	8.97 (5.78-12.57)	58.61 (37.56-122.87)	5.7
	50	85.0 ± 0.50			
	10	50.0 ± 0.70			
	5	37.5 ± 0.82			
	Control	0.0 ± 0.0			

*$p < 0.05$, level of significance, [a]Values are mean = SD of five replicates, df = 14 SD = Standard deviation, LCL = Lower confidence limits, UCL = Upper confidence limits, X^2 = Chi-square test

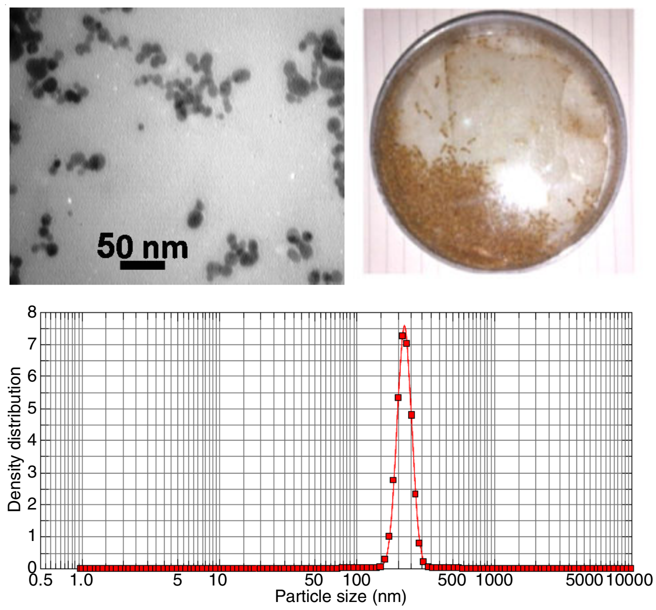

Fig. 3. Representative transmission electron microscopy (TEM) images of Ag/PEG/Cts NPs-Chlropyrofos (above) and DLS picture: particle size distribution/ nm of nanocapsules and termite: *M. gabrielis* (below)

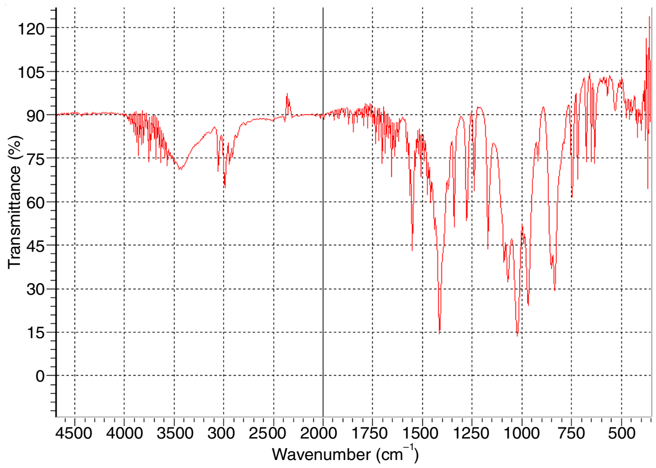

Fig. 4. FT-IR spectra of Ag/ PEG/Cts NPs-Chlropyrofos

As shown in Table-1, the result of biological study of four dose of Ag/PEG/Cts NPs chlorpyrifos (100, 50, 10, 5 ppm), water control. Increasing the nanoparticles concentration from 5 to 100 ppm, increases the termites mortality. The lowest average dose was 5 ppm gave 33 % mortality in 48 h but higher concentrate 10 ppm after 48 h gave 50 % mortality. In the highest dose of 100 ppm, the mortality was 97.5 %. However, the mortality value of 97.5 % for LC_{50} and LC_{90} at 48 h were 8.97 and 58.61 ppm, respectively. At 50 ppm, the nanoparticles showed 85 % mortality of the termite groups in 48 days after the application. There is a different distinguish in comparing mortality on LC_{90} with 95% confidence between four doses. The assay on biological study showed for four concentrate with control was evaluated for LC_{50} and LC_{90} after 48 h. The control rate was varied by changing dose. Other studies showed the same correlation with concentrate and mortality percentages such as Veerakumar [32] the study activity of aqueous leaf extract and silver nanoparticles synthesized using *Helitropium indicum* plant leaves against late third instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The synthesized AgNPs from *H. indicum* were highly toxic than crude leaf aqueous extract in three important vector mosquito species. Ihegwuagu *et al.* [33] also showed the presence of nanosilver in the starch matrix making diffusion and release of the active ingredient easier and faster.

The biological study revealed that Ag/PEG/Cts NPs chlorpyrifos could achieve highly effective and this novel organophosphate pesticide formulation, therefore, presents safer, efficacious, lower dose, environmental friendly and economical choice.

Conclusion

The silver/Cts/PEG/chloropyrofos nanoparticles synthesized in this study has a potential for insecticide activity, prepare *via* a facile, cheap and reproducible method, characterized them as well as evaluated their ability to be effectively deliver the insecticide active ingredient. The pesticide-delivery system studies presented in this paper can be developed for other pesticide delivery system.

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