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Effect of nanocapsules and extract of *Metarhizium anisopliae* in inhibiting acetylcholine esterase enzyme in *Musca domestica* larvae.

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Abstract:

*M. d*omestica is the most important insect that transmit pathogens for diseases in the world. The use of nanotechnology is eco-friendly method in control pests. The study aims to investigate the feasibility of bio-manufacturing nanocapsules of fungal secondary metabolites in order to improve the efficiency of metabolite and assess their inhibitory effect on the acetylcholine esterase enzyme in housefly larvae. An equal mixture of organic solvents, ethyl acetate and dichloromethane, was used to extract the metabolic products of the fungus *M. anisopliae*, (PEG4000) and chitosan was used in the preparation of nanocapsules. The results of the DLS granular size assay showed that the size of the extract particles and the size of the chitosan and (PEG 4000) nanocapsules were 610, 217 and 188 nm, respectively. The SEM images showed that the diameter of the extract and the nanocapsules chitosan and polyethylene glycol 4000 reached a rate 547.5, 17.8 and 26.2 nm, respectively. The FTIR showed that the extract of the second products of the fungus contains functional groups like: alkynes and alkenes, amines, carboxyl and aromatic groups, while the presence of groups of phenols, alcohol, amines, alkenes, and alkyl halides was recorded for nanocapsules of chitosan and PEG. The results showed that the extract of fungal metabolic and nanocapsules has an inhibitory effect on acetylcholinesterase enzyme and reached the highest inhibition rate 53.2 ,36.3,18.2% when treated with nanocapsules PEG at a concentration 500 ppm, extract of fungal metabolites at a concentration 50,000 ppm, chitosan nanocapsules at a concentration 500 ppm respectively. It is clear that acetylcholinesterase inhibition is one of the mechanisms of fungi metabolic action and the nanocapsules prepared from them .

Keywords: Acetylcholine esterase enzyme inhibition, Housefly, *M. anisopliae*, Nanocapsules, Fungi secondary metabolites.

Introduction:

The housefly, *Musca domestica* (Diptera: Muscidae), is the fly that transmits the most diseases worldwide. Houseflies have been linked to more than 100 infectious illnesses in both people and animals, the pathogens that M. domestica transmits have been linked to cholera, anthrax, food poisoning, typhoid, diarrhea, and shigellosis [1] . Due to their vast range, strong reproductive potential, and facultative diapause, houseflies are regarded as a distinct species and a hazard to humans. They have also become a major problem since they may spread illness through polluted water and food using their vomit or excrement and legs [2]. Chemical pesticides have been extraordinarily successful against agricultural pests and arthropods that are crucial to human health, Insecticides often used to control house flies include pyrethroids, spinosad, pyrethrins, dichlorvos, imidacloprid, and cyantraniliprole [3]. Alternative methods of chemical pesticides like aqueous and alcoholic extracts of *Piper nigrum* were tested on the third instar larvae of *Culex pipiens* which achieve high mortality rates [4]. The advantages of neurotoxin insecticides include their quick action, which stops crop damage right away, their numerous highly sensitive sites of action, which make even a small disruption potentially fatal, their lipophilic nature, which makes it easy to penetrate the lipoidal sheath,

and their lack of a detoxification mechanism in nerves, which prolongs insecticidal activity. Because of these benefits, the majority of synthetic insecticides on the market today have neurotoxic properties, frequently via blocking acetylcholine esterases (carbamates) or impairing the operation of ion channels in nerve cell membranes (pyrethroids) [5]. Entomopathogenic fungi (EPF) create secondary metabolites that might be a source for the development of biopesticides and operate on other species, occasionally preventing growth, causing illness, and even death [6]. Pest management and the manufacture of pesticides both employ nanotechnology increasingly [7, 8]. Crop productivity can be increased with the use of nanotechnology, which can also be used to create novel pest control methods [9, 10]. Green synthesis techniques are one of the techniques used in nanotechnology to create nanocapsuls, nanoparticals**,** nanosuspensions and nanoemulsion[11]. These techniques have certain benefits over others since they are often easier and less expensive to carry out and don't involve harmful chemicals that might pollute the environment. Stable nanocapsules are created using green synthesis techniques using biomolecules from plants and microorganisms, such as proteins, enzymes, phenols, amines, and alkaloids [12]. Saied (Kouzegaran and Farhadi, 2017) that the toxicity of secondary metabolites against insects can be improved by adding synergist agents such as chitosan or Polyethylene glycol 4000 (PEG) [13]. Chitosan is a naturally occurring substance that is obtained from marine crustaceans and shrimp. It is a biopolymer with many uses due to its solubility, high stickiness in acetic solution, non-toxicity to mammals, reactive amino (-NH2) and hydroxyl (-OH) group composition, high biodegradability, and antimicrobial properties. Due to these qualities, it may be used in a variety of fields, including the biomedical sector, pest control, the food industry, and environmental pollution reduction [14]. Additionally, it is non-toxic to both humans and vertebrates, so it has a variety of uses [15]. Polyethylene glycol 4000 is a synthetic, hydrophilic, biocompatible polymer with widespread use in biomedical and other applications [16]. Nanoparticles (NP) prepared with chitosan and chitosan derivatives typically possess a positive surface charge and muco adhesive properties that can adhere to mucus membranes and release the drug payload in a sustained release manner [17].

This study aimed to prepare Polymeric nanocapsules of crude extract of entomopathogenic fungus *Metarhizium anisopliae* by chitosan and Polyethylene glycol 4000 and assay their inhibition effect on acetylcholine esterase enzyme in *Musca domestica* larvae.

Material and methods:

Chemicals

Materials that were used, like, Di chloromethane (Thomas Baker, India) as a solvent and as co-surfactant, tween 80 (Thomas Baker, India) as surfactant, and deionized water, chitosan, sodium phosphate buffer (Na2HPO4, NaH2PO4), fast blue B salt, acetylcholine chloride, polyethylene glycol 4000, and chitosan

Musca domestica collection and rearing

Musca domestica adults were collected from a farm in Baghdad \ Ghzallia\ Iraq by using an arial net. The adults were placed in a wooden cage with a dimension of 30×20×20 cm^3. that was covered with tulle and one lateral side modified the tulle to enter the hand for supplementing food and cleaning the cage. The adult of housefly was sent to the Natural History Museum of Iraq for identification, they were diagnosed as *Musca domestica*. The experiments were done in the laboratory of the Agricultural Research directorate ministry of science and technology in Al Twaitha/Baghdad/Iraq.

In the laboratory, plastic containers of 500 ml were filled with cotton webbed with 1:1 sugar and distilled water to nutrition adults to obtain egg , while larval grown in plastic containers of 20 ml capacity contained of (float fish diet) consisting of crude protein 28.0 % Min. , crude fat 4.0 % Min. and crude fiber 4.0 % Max in addition to amino acids, vitamins , minerals , after being crushed were taking 200 gm of (fish diet) mixed with 10 gm of dry yeast , 5gm of citric acid and dissolved in 100 ml of distilled water , the nutrient which is used to nutrition larvae and adults take care of changing it all three days [18] . The rearing cages were placed in the incubator under controlled conditions 27± 2 º C, 65± 5 RH and 10: 14 D/L photoperiod. This feeding is important for *Musca domestica* to get the energy needed for flight and other life activities.

-Crude extraction of toxin and secondary metabolism from fungi *M. anisopliae* :

The fungus *Metarhizium anisopliae* was taken from the Agricultural Research Department\ Ministry of Agriculture. To obtain the secondary metabolic compounds of the fungus Metarhizum anisopliae, a 1 cm piece of the fungus was taken from 3 day old PDA grown cultures by borer and cultured in 500 mL of PDB medium, which consisted of (200 mL potato water and water, 800 mL, 20 g sugar, and 500 g amoxylin) in conical flasks and were incubated at 25°C and humidity 80% in an incubator in an

incubate with shaking in different time for a period of 10- 12 days[19].

Classical solvent extraction method: the majority of isolation procedures still utilize simple extraction procedures with organic solvents of different polarities, water and their mixtures; The method includes maceration. Following incubation, the fungus biomass was separated from the culturing medium with gauze (soft) cloth in order to use the broth portion, which contained toxin, secondary metabolism, and spores. completing the extraction of crude secondary metabolites with Whatman filter paper No.1 separated the light-yellow color component of the fungal mat, organic phase. , 500 ml broth, 750 ml solvent 1:1 ethyl acetate and dichloromethan (DCM)) which were mixed with the fungal broth component for cold extraction with shaking for 2-3 days at 28 ^{0}C. The solvent was separated from the water by separating funel, and the solvent was filtered using a micropore filter 0.2 mM to clear it. A vacuum evaporator at 80 °C was used to separate the combined solvent from concentrated extracts [20] . Deionized water and tween 80 were used to dissolve the crud extract .

Preperation of *M. anisopliae extract* –Chitosan Nanocapsules by Ion Gelation method:

In this technique the chitosan solution positively charged is dissolved in acetic acid 0.35% w/v prepared by dissolving chitosan powder in 1.5% acetic acid solution negatively charged with a stabilizing agent such as STPP (sodium tripolyphosphate) was added as a cross linker. then, a crud dichloromethane extract of *M. anisopliae* 2 g was added to 20 mL chitosan solution, the whole mixture was then stirred at room temperature on a magnetic stirrer (Rexim RP-1DN, Agile) for homogenous. The obtained homogenous slurry was added to a screw cap vial. The change in color of the solution from colorless to yellow gradually confirmed nanocapsule synthesis, keep it at 4 ^{0}C until use. Chitosan nanoparticles are readily formed due to complexation between positive and negative charged species during mechanical stirring at room temperature, resulting in the separation of chitosan in spherical particles of different sizes and surface charges. Generally, [21,22,23]. Treated the crud extract of *M. anisopliae*, the solution *M. anisopliae*-chitosan, with an ultrasonic device (COMNI INTERNATIONAL, Homogenizer company) by inserting the device's beam into the solution and running it for 10 minutes, then repeating the process five times separately. Each treatment is stored in a dark screw container until used.

Preparations of crud extract of *M. anisopliae*– polyethylene glycol Nanocapsules

PEG 49.5g was dissolved at 65 ^{0}C, added crud dichloromethane extract of *M. anisopliae* 0.5 g with continuously stirring by using a magnetic stirrer. The heating was continued throughout the addition process to obtain the nanocapsules put the solution in the freezer until stiffened, then grind it as powder of nanocapsules. The production of nanocapsuls for crud dichloromethane extract of *M. anisopliae* – polyethene glycol 4000 was powdered and reddish in color.

Determine the characterization of M. anisopliae crud extract, M. anisopliae-chitosan Nanocapsules, and M. anisopliae-polyethylene glycol 4000 Nanocapsules

***Dynamic light scattering (DLS) analysis*:** A zeta size (Malvern, Zetasizer Nano ZS, England) was used for determining the crude extract and nanocapsuls of *M. anisopliae* size distribution by DLS technique. The means of DLS was measured in the range of 0.1–1000 μm, one milliliter of diluted crude extract *M. anisopliae*, crude extract *M. anisopliae* – chitosan, *M. anisopliae* – polyethylene glycol 4000 nanocapsules suspension was taken in zeta cell for measurement of zeta size [23].

Scanning electron microscope (SEM) analysis: To determine the structure shape and size of crude extract and nanocapsuls of *M. anisopliae* scanning electron microscopy, samples were processed and fixed by following the method of Wang et al. [24]. The images were captured under SU8010 (Hitachi, Japan) SEM operating at an accelerated voltage of 5.0 kV [25].

-Fourier transform infrared spectra (FTIR) measurements.

To determine the functional groups in the crud extract and nanocapsuls of *M. anisopliae* through FTIR analysis using MIR8035 FTIR spectrometer (Thermo Fisher, Bremen, Germany) [26].

-Bioassay of chitosane, polyethylene glycol 400 nanocapsul of crud extract *Metarhizium anisopliae* on larval acetyl cholinesterase enzyme: Treated larvae

5 g of medium that was used to rear *Musca domestica* was put in container of 50 ml , sprayed from 30 cm distance with 50.000 ppm of crud extract *Metarhizium anisopliae* , 500 ppm crud extract *M.anisopliae* – chitosane nanocapsule , and 500 ppm *M. anisopliae* – polyethylene glycol 4000 nanocapsule , then put the larval 2nd , 3rd ,4th stages without treated as 3 rep / 30 larvae, put the

experiment in an incubator at 25±5 ^{0} C / humidity 70% . for 24 – 48 h.

Enzyme assay

Preparation of whole-body homogenate for enzymatic estimation, the larvae of *M. domestica* were washed thoroughly with distilled water and the extra water adhering to the larvae was cleared by using filter paper. Then, treated larvae were subjected to homogenization utilizing ice-cold sodium phosphate buffer pH 7.0, 20 mM with the help of Teflon hand homogenizer then centrifugation was done at 4500 run and 4 ° C for 30 min and resultant supernatant was used for the estimation of acetylcholine esterase. Solutions and glassware used for homogenization were kept at 4 °C before use, and homogenates were also placed on ice until utilized for enzyme assays [27].

Estimation of acetylcholine esterase activity:

In the 50 ml of enzyme solution, 50 ml of acetylcholine chloride 2.6 mM as a substrate and 1 ml of SBP (sodium phosphate buffer, pH 7.0, 20 mM) were added. It was incubator at 25 ° C for 5 mints. Then 400 ml of 0.3% Fast blue B salt was mixed to stop the reaction. Blank and sample were run through a spectrophotometer. Optical density OD was checked at 405 nm. The percentage inhibition of the enzyme activity by the test extracts was calculated as follows**:**

% Enzyme inhibition = OD of Control larvae _ OD of treated larvae / OD of Control larvae x100 [28]**.**

Statistical analysis

The experiments were carried out according to factorial experiments using a completely randomized design (CRD), and the differences between the means of the treatments were tested according to the value of the least significant difference at the probability level of 0.05. The results were analyzed by the statistical program Genstat 10 [29].

Result and discussion:

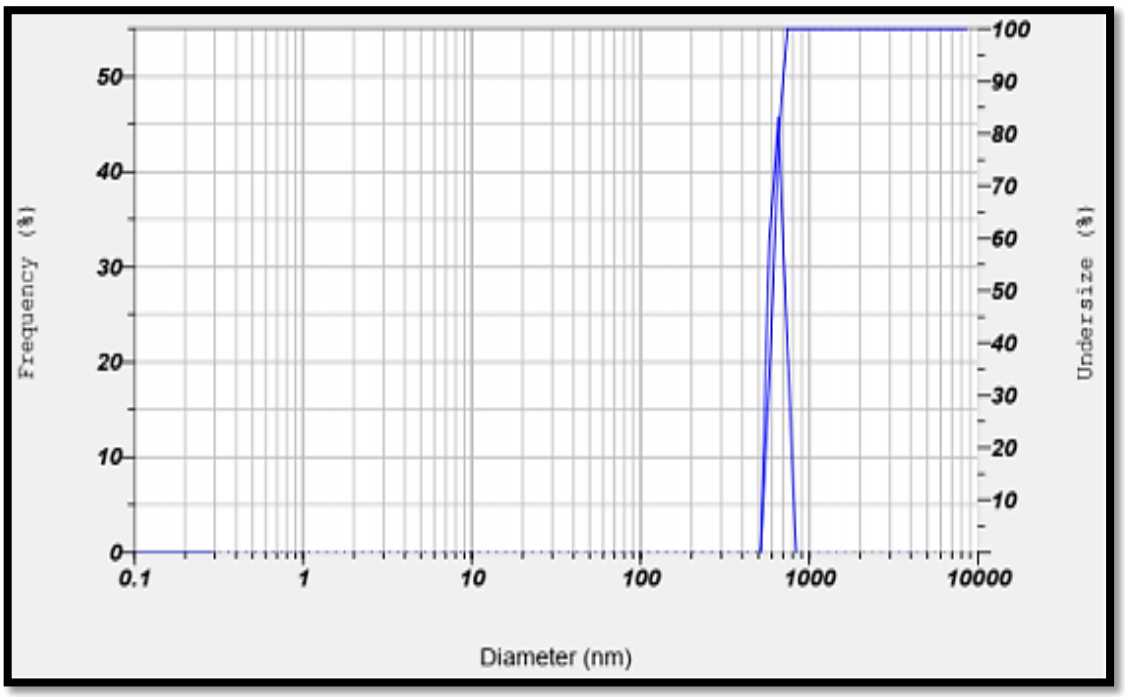
Crude extraction of toxin and secondary metabolism from fungi *M. anisopliae*

The result of extraction showed After 10–12 days, M. anisopliae mass growth was observed, and a reddish-yellow extract was obtained from the biomass after extracting it with a mixture of solvents ethyl acetate: dichloromethan (DCM) representing the fungus metabolic secondary products

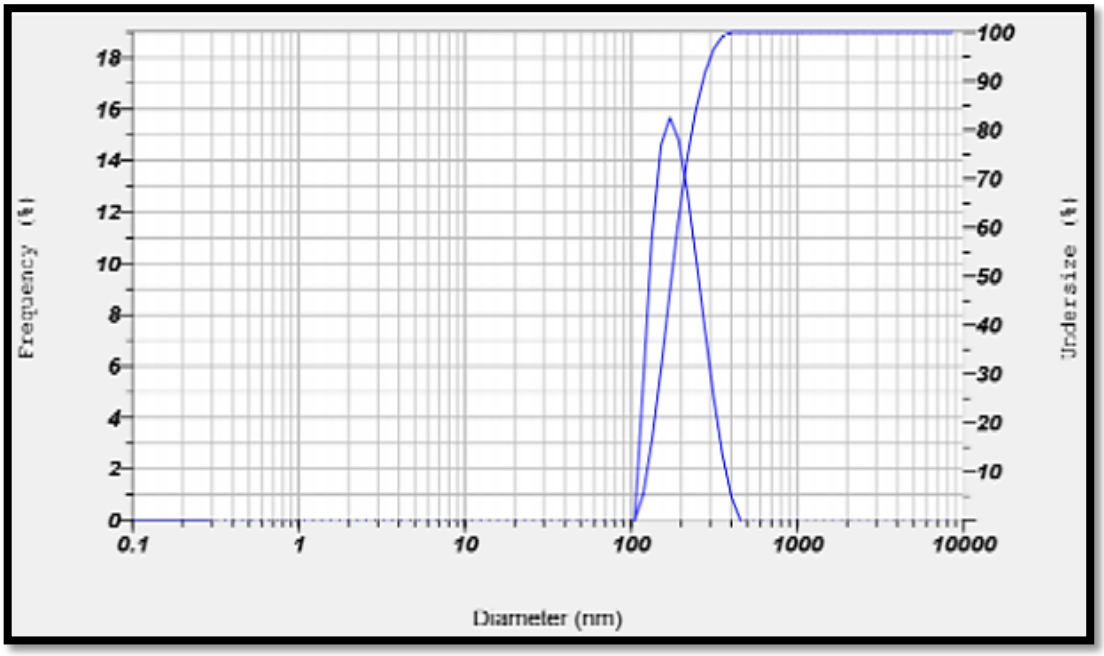
Characterization of crud dichloromethane (DCM) extract *M. anisopliae*, and nanocapsules:

Dynamic light scattering analysis : The DLS analysis revealed that the size of the crud DCM extract of *M. anisoplia* , *M. anisopliae* –Chitosan Nanocapsules , *M. anisopliae*– polyethelen glycol 4000 nanocapsules, as it was 610 nm in crud DCM extract and 217nm in *M. anisopliae* –Chitosan Nanocapsules , while was 188nm in *M. anisopliae*– polyethelen glycol 4000 nanocapsules as in Fig.1.

A



B



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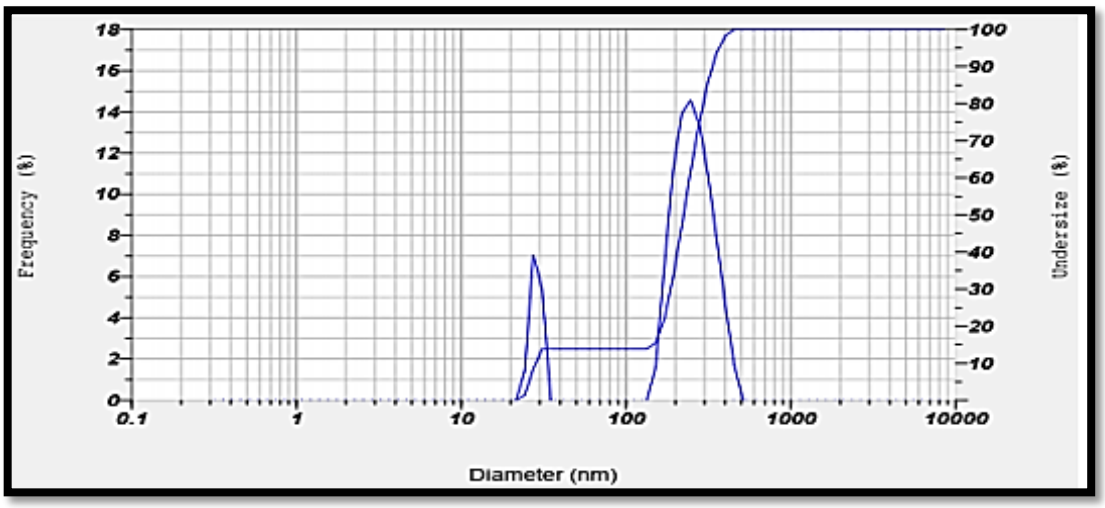

Figure 1. Average size of A- DCM crude extract of *M. anisopliae* , B- nanocapsules of *M. anisopliae*- polyethylene glycol 4000 , and C- nanocapsules of *M. anisopliae*- chitosan determined by Dynamic light scattering .

Scanning Electron Microscopy (SEM) analysis : In Fig.2 SEM analysis showed the diameter and shapes of crude extraction of *M. anisopliae* , *M. anisopliae* –chitosan , and *M. anisopliae* – polyethylene glycol 4000 nanocapsules. The diameter of DCM crude extraction of *M. anisopliae* was 547.5 nm with aggregate irregular shape of granular structure. The diameter of *M. anisopliae* – chitosan nanocapsules was 17.8 nm ,with spherical irregular shape rather than granular . The diameter of *M. anisopliae* – polyethylene glycol 4000 nanocapsules 26.2 nm .

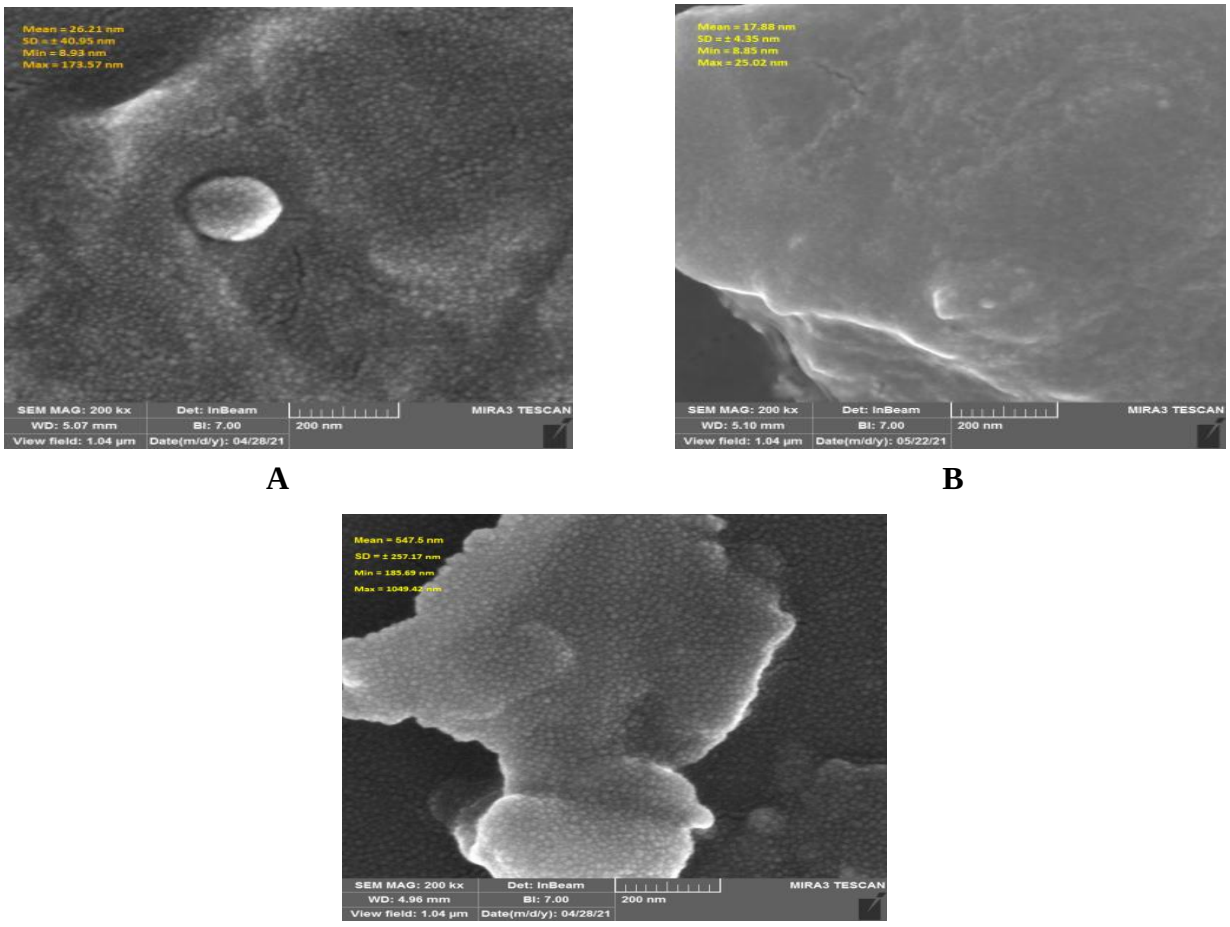

Figure 2. SEM images of formulations (A) PEG *M. anisopliae* nanocapsules (B) *M. anisopliae*- chitosan nanocapsules(C) DCM crude extract M. anisopliae

Fourier transform infrared spectra (FTIR) analysis

The FTIR analysis revealed functional groups in the crude extraction of *M. anisopliae* , *M. anisopliae* – chitosan , and *M. anisopliae* – polyethylene glycol 4000 glycan nanocapsules, as shown in Figs. 3,4,5. The results in Fig.3 showed the main functional groups in crud extract of *M. anisopliae* observed by FTIR includes , N-H strrech at 2922.34 cm^{-1} Amine salt , C-H stretch medium bending at 2853.72 cm^{-1} Alkane , C≡C weak at 2149.53 cm^{-1} owing Alkyne , C-O stretch at 1717.54 cm^{-1} Carboxylic acid , C=C medium at 1651.25 cm^{-1} Alken , C=C medium at 1456,85 cm^{-1} Aromatics compound , C=C medium at 1375.27cm^{-1} Alkan , C-O stretch at 1259.41 cm^{-1} Ether , C-F bending at 1167.77 cm^{-1} Alkyl aryl halid , C-f stretch at 1091.43 cm^{-1} Halid alkyl , C-F stretch at 1022.33 cm^{-1}, Alkyl aryl halides.

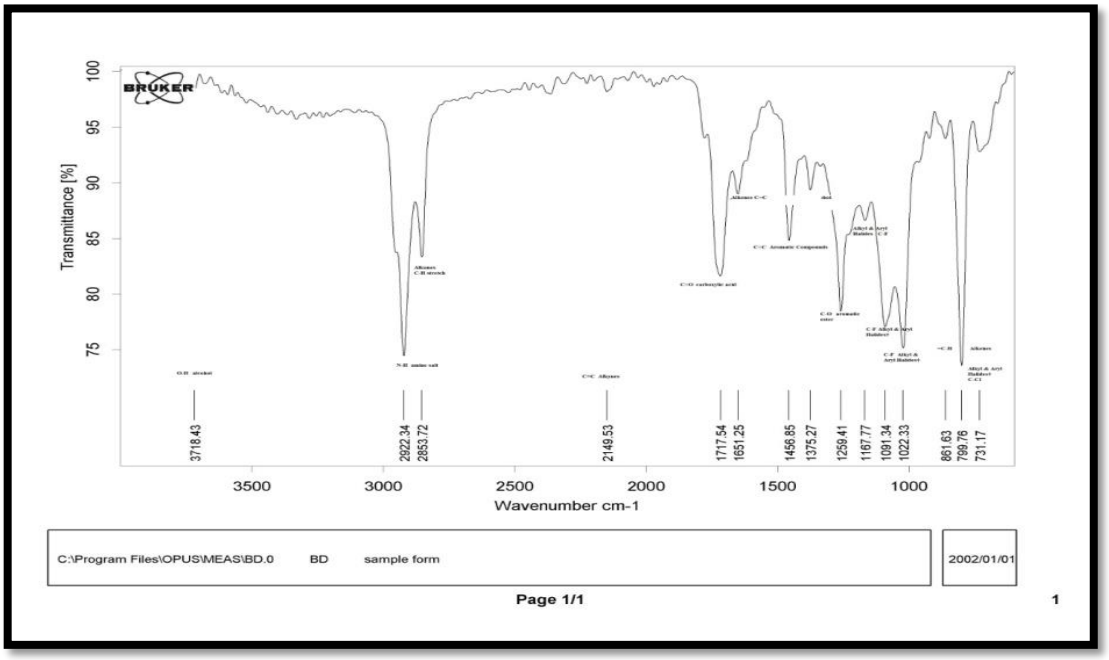

Figure 3. Functional group in crude extraction of *M. anisopliae*.

in Fig. 4 the functional groups of *M. anisopliae* –chitosan nanocapsules observed O-H strong broad at 3296.27 cm $^{-1}$ which found in Phenol , alcohol, -C≡C strong broad at 2153.65 cm $^{-1}$ alkyne , N-H at 1636.90 cm $^{-1}$ Amin complex , and C-F strong broad at 1018.82 cm $^{-1}$ Halid alkel, in Fig.5 the functional groups of *M. anisopliae* –PEG nanocapsules observed were , O-H strong broad at 3286.08 cm $^{-1}$ which found in Phenol , alcohol , -C≡C at 2136.70 cm $^{-1}$ Alkyne , N-H strong broad at 1637.38 cm $^{-1}$ amine complex , and C-CL strong broad at 1000 cm $^{-1}$ Alkyl halides

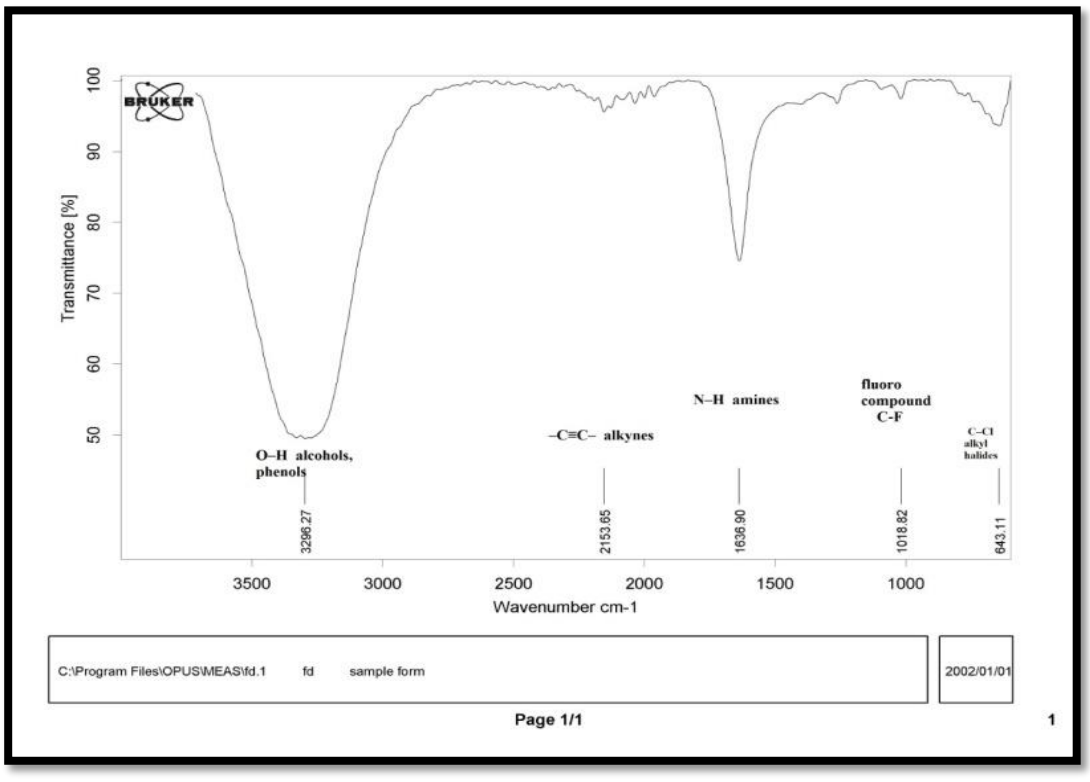

Figure 4. The functional groups of *M. anisopliae* –chitosan nanocapsules

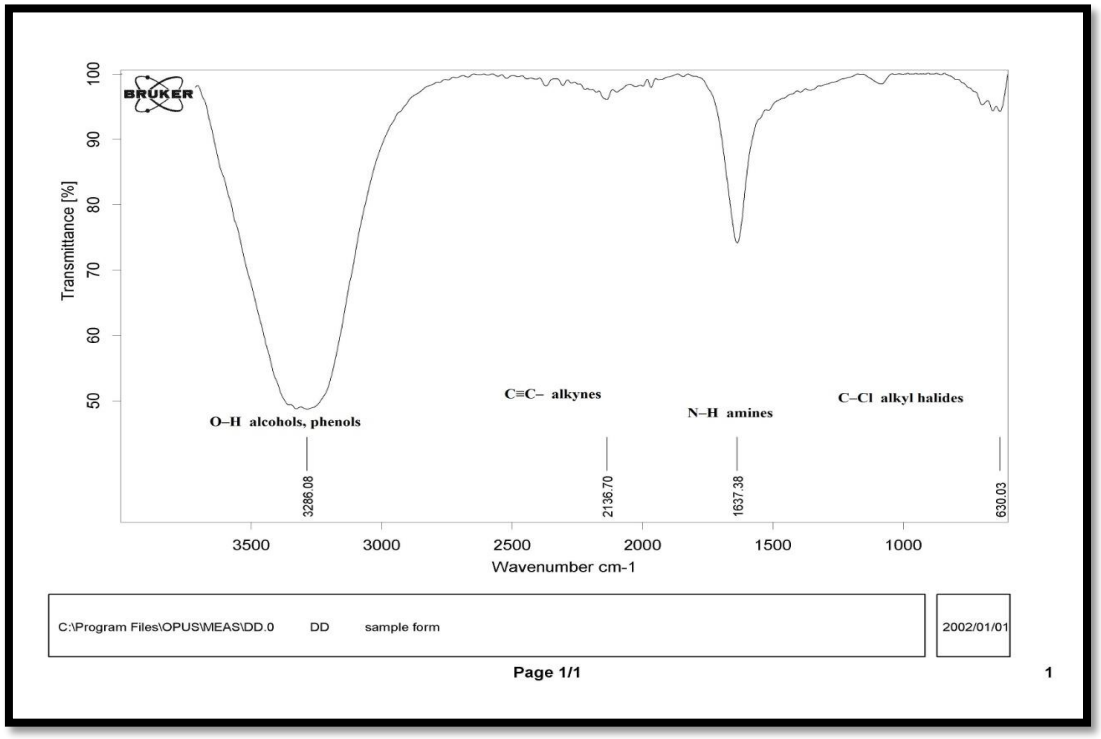

Figure 5. The functional groups of *M. anisopliae* – polyethylene glycol 4000 nanocapsules. Bioassay of nanocapsules types on larval acetyl cholinesterase enzyme.

The results in Table 1 and Fig.6 showed the effectiveness of crude extract *M. anisopliae* , nanoparticals of crude extract *M. anisopliae* - chitosane , and nanoparticals crude extract *M. anisopliae* – polyethylene glycol 4000 nanocapsuls on larval acetyl cholinesterase enzyme . enzyme inhibition was 53.2 % with nano capsuls of *M. anisopliae* – polyethylene glycol 4000 in concentration 500 ppm , 18.2 % with *M. anisopliae*- chitosane nanocapsuls in a concentration 500 ppm , and 36.3 % in crude dichloromethane extract of *M. anisopliae* in a concentration 50.000 ppm With the significance different in % enzyme inhibition at the level of probability 0 .05 . as in shape 4 the treatment with crude extract of *M. anisopliae* - polyethylene glycol 4000 nanocapsuls was the higher inhibitor to enzyme acetylcholine esterase in larvae *Musca domestica* with significant effect. While the lowest inhibitor was with treatment by crude extract of *M. anisopliae* - chitosan nanocapsuls.

Recently, Nanotechnology is increasingly being used in areas of pesticide production and pest management. fungi target their hosts by producing toxins and anti-insect secondary metabolites resulting in an array of effects ranging from paralysis to immune-suppression [30] . The fungus gradually weakened the enzyme activities as time progressed, indicating that the physiological attributes of the host were adversely affected [17]. FTIR results of synthesized crud extract of *M. anisopliae* – polyethelenglycol nanocapsules showed the presence of O-H , C≡C , N-H , and C-CL theses found in Alcohol / phenol , Alkyne, Amine complex, and Alkyl halides, which is similar to the FTIR profile of *M.anisopliea*–chitosan nanocapsules, explained by Namasivayam et al [31]. The changes in C≡C , C-F , and N-H stretching in both formats of nanocapsule green synthesis structural changes in response to the polyethylene glycol 4000, chitosan coating of secondary metabolites of fungal extraction [32]. FT-IR spectral investigations revealed the presence of carboxylic acid, amides, and phenol groups, Aromatics compound, Alkane , Alken , Alkyne , Ether , and Halid alkyl all of which could be involved in inhibition enzyme activity , this agrees with Vivekanandhan et al . [33]. which reported that The *M. anisopliae* derived chemical constituents are effective on targeted pests, pollution-free, target-specific, and are an alternative chemical insecticide [34]**.** The average size of nanocapsules format plays a major role in effect speed effect to inhibit enzyme activity, which includes the average size of *M. anisopliae* –chitosan nanocapsules was 17.8 nm with spherical irregular shape not granular . The size of *M. anisopliae* – polyethylene glycol 4000 nanocapsules 26.2 nm . *M. anisopliae* –chitosan nanocapsules was smallest , spherical , crude extraction of *M. anisopliae* was 547.5 nm with aggregate irregular shape of granular structure. DlS

and SEM analysis explain the difference between crud extract and nano format of green synthesis.

The smallest average size of *M. anisopliae* – polyethylene glycol 4000 nanocapsules and nanocapsules *M.anisopliae* – chitosan gave it the ability to enter the body and rapidly reach the target sit. the enzyme Acetylcholinesterase (AChE) found in the synaptic cleft of insects, is responsible for the deactivation of the principal neurotransmitter acetylcholine (ACh) that transmits nerve impulses from one nerve cell to another or to involuntary muscles , it conceder as the target site for neurotoxic insecticides [35] . Similarly, previous studies showed that the metabolites of entomopathogenic fungi are founded in several chemical classes (phenols, alcohols, misc, carboxylic acids, aromatics, phosphor amide, and disulfides), which may be engaged in the mosquitocidal effects [33]. The decrease in AchE activities can be related to the mode of action of both agents, phenol, an alkaloid extracted from *M. anisopliae* which targets insect Ach receptors that turn off AchE production [36]. Due to the accumulation of ACh in the synaptic cleft because of inhibition AChE enzyme production leads to continuous nerve signaling, and finally to paralysis and death of the insect [37].

The neurotoxic activity of crude extract and Nano DEPA treated mosquito larvae was determined by AChE assay [38]. Also, secondary metabolites may cause membrane depolarization Yadav *et al.*[39] . Opening calcium channel caused tetanic paralysis in the insects Samuels et al. [40]. Alkenes are excellent starting materials for organic synthesis as a result of the changeable reactivity of **C=C** bonds and the easy availability of many unfunctionalized alkenes. Direct regio- and/or enantio selective conversion of alkenes into functionalized compounds has immense potential for industrial applications [41]. Due to PEG characteristics often selected for adsorption or grafting applications to surfaces of nanocapsule, including electrical neutrality, significant spatial repulsion, and hydrophilicity. After PEGylation, a hydrophilic protective layer is formed around the nanocapsule, which increases several times for the blood circulation half-life of the particles through spatial repulsion rejection, Also, it is easy for nanocapsule to use PEG as a bridge to conjugate targeted ligands or peptides onto its hydroxy terminal, which can bind the corresponding over-expressed receptors on the surface of cells, to realize targeted drug delivery. PEG can improve the targeting delivery capability of nanocapsules, inhibit the removal of nanocapsule by the mononuclear phagocytic system MPS, and tune some physicochemical properties of nanocapsule , such as the mechanical properties of membranes, stability, and drug loading and release behavior , Molecular weight MW and PEG density are the key factors affecting the physicochemical and biological properties of nanocapsule [42] . Many natural products trigger very specific physiological responses in other organisms and in many cases bind to the receptors which have a remarkable complement, which means that the natural products may aid in an organism's survival in the absence of an immune system. AChE enzyme has a large but flexible structure consisting of 535 amino acids with a 20 Å deep and 5 Å wide narrow tunnel leading up to its active site , The tunnel, also known as the active-site gorge, consists of 14 flexible aromatic amino acid residues, These aromatic residues play essential roles in binding to the substrate. Cholinesterase inhibitors enhance cholinergic activity by inhibiting enzymes AChE and/or BuChE that hydrolyze ACh following synaptic release and, hence, prolong the activity of ACh molecules [43].

Table 1. The percent inhibition of acetylcholinesterase enzymatic activity in *M. domestica* L. under treated with chitosan and ethylene glycol nanocapsul of *Metarhizium anisopliae* extract.

Type of nanoformulation	Concentration	% enzyme inhibition
M. anisopliae – polyethylene glycol4000 nanocapsules	500	53.2 a
M. anisopliae –chitosan nanocapsules	500	18.2 c
Crude *M. anisopliae extract*	50.000	36.3 b
LSD 0.05		5.99 *

* SIG.DIFF.

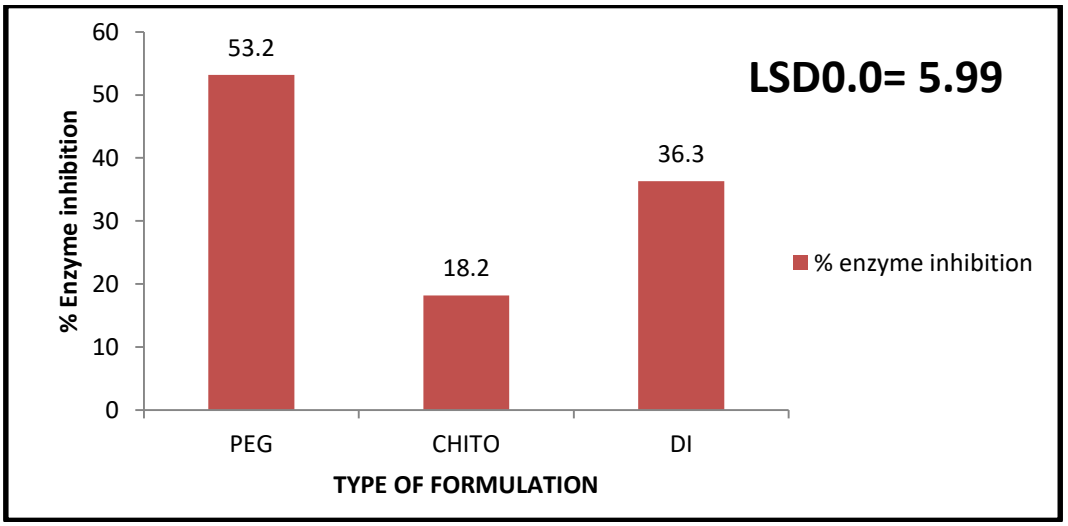

Figure 6. Effect of different nanocapsuls of *M. anisopliae* extract in the percentage inhibition of acetylcholine esterase enzymatic activity in *M. domestica* (L.).

Conclusion:

It is believed that *M. anisopliae* – polyethylene glycol4000 nanocapsules of DCM crude extract (secondary metabolites and toxin) of fungus *Metarhizium* anisopliae has a natural inhibition activity of acetylcholine esterase enzymatic activity in *M. domestica* (L.). It can be concluded that *M. anisopliae* – polyethylene glycol4000 nanocapsules of DCM crude extract (secondary metabolites and toxin) of fungus *Metarhizium* anisopliae can be used as a safe and effective alternative in the control of vector-borne diseases caused by Mucidae larvae.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Author's contributions:

H I A S and S A K conceived of the presented idea and supervised the findings of this work. While, A A-A H did all the expermints and verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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فاعلية الكبسولات النانوية لمستخلص الفطر *Metarhizium anisopliae* في تثبيط انزيم acetylcholine esterase في يرقات الذباب المنزلي *Musca domestica*

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الخلاصة:
يعد الذباب المنزلي (***Musca domestica***) من اهم الحشرات الناقلة للمسببات المرضية للعديد من الامراض في العالم .ان استخدم تقنية النانوتكنلوجي في ادارة ومكافحة الافات يعد من الطرائق الحديثة والصديقة للبيئة . يهدف البحث الى امكانية التصنيع الحيوي للكبسولات النانوية للمنتجات الايضية للفطرو تحسين كفاءة المنتجات الايضية الثانوية وتقييم تاثيرها المثبط لانزيم استيل كولين استريز في يرقات الذباب المنزلي .استخدم مزيج المتساوي من المذيبات العضوية الاثيل استيت وثنائي كلوروميثان في استخلاص المنتجات الايضية للفطر *Metarhizium anisopliae* واستخدم البوليمر الصناعي(PEG 4000) Polyethylene glycol 4000 و البوليمر الطبيعي الكيتوسان في تحضير الكبسولات النانوية لمستخلص المنتجات الايضية للفطر . اظهرت نتائج فحص الحجم الحبيبي DLS ان حجم جزيئات المستخلص الخام و حجم الكبسولات النانوية النانوية المغلفة بالكيتوسان والمغلفة بالبولي اثلين كلايكول 4000 بلغ 610 و 217 و 188 نانومتر على التوالي . اظهرت صور المجهر الالكتروني الماسح ان قطر المستخلص الخام و الكبسولات النانوية المغلفة بالكيتوسان والمغلفة بالبولي اثلين كلايكول 4000 بلغ معدلا قدره 547.5 و17.8 و 26.2 نانومتر على التوالي . كما اظهر اختبار FTIR ان المستخلص الخام للمنتجات الثانية للفطر يحتوي على العديد من المجاميع الوظيفية اهمها وجود الالكاينات والالكينات والامينات والكاربوكسيل والاروماتية فيما سجل وجود مجاميع الفينولات والكحول والامين والالكين وهاليد الالكيل للكبسولات النانوية المغلفة بالكيتوسان والبولي اثلين كلايكول . بينت النتائج ان المستخلص الخام للمنتجات الايضية للفطر والكبسولات النانوية ذات تاثير تثبيطي لانزيم الاستل كولين استريز بلغ اعلى نسبة تثبيط 53.2 % عند المعاملة بالكبسولات النانوية المغلفة بالبولي اثلين كلايكول بتركيز500 جزء بالمليون يليه المستخلص الخام للمنتجات الايضية للفطر بنسبة تثبيط بلغت 36.3 % بتركيز 50.000 جزء بالمليون ثم المعاملة بالكبسولات النانوية المغلفة بالكيتوسان بنسبة تثبيط 18.2 % بتركيز 500 جزء بالمليون . يتضح من النتائج ان وجود نسب من تثبيط انزيم الاستيل كولين استيريز هو احد ميكانيكيات التاثير المحتملة للمنتجات الايضية للفطر والكبسولات النانوية المحضرة منها.

الكلمات المفتاحية: تثبيط الانزيم استيل كولاين استيريز, الكبسولات النانوية, المركبات الايضية الثانوية الفطرية, *M. anisopliea*، الذبابة المنزلية.