TOXICITY OF GOLDEN NANOPARTICLES SYNTHESIZED BY INSECTS PATHOGEN ENTOMOPHTHRA CULICIS VERSUS MOSQUITO ANOPHELES STEPHENSI (Diptera : Culicidae)

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ABSTRACT

Bio-nanoparticles formation was noticed by the alteration in color of the solution to dark pink. This was followed by characterizing the nanoparticles using UV-Vis spectrophotometer and TEM. Gold nanoparticles were found to be Orbicular and their size was in the range of 20 - 30 nm. As well gold nanoparticles were assayed versus *Anopheles stephensi* larvae and pupae. *A. Stephensi* was exposed to varying concentrations of AgNPs synthesized from *E.culicis* under investigation (2,4,6,8 and 10 ppm) for 24 hours, which offered high toxicity with the highest mortality values of %100 against Ist instar, while both 2nd and 3nd were less susceptive to synthesized AuNPs and The lowest mortalities were against 4th instar larvae in all tested concentrations .The toxicity of AuNPs against pupae reached the highest level at 10 ppm after 24hours The results that got in this research present biosynthesized nanoparticles as novel biolarvicidal and pupicidal agent and can be used in control programs successfully.

Key words:

E.culicis, gold nanoparticles,Toxicity, *A.stephensi*

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INTRODUCTION

Causative agents of diseases such as malaria, filariasis, dengue and brain fever are particularly vectored by Mosquitoes, An immediate necessity is needed to investigate the propogation of mosquitoes for lessening diseases by exploring a convenient method to control vectors. The control of Mosquito especially in third countries earnest heed on account of absence of awareness, resistance of insects to chemical insecticides as well as economic causes(1). Malaria parasites are vectored by Anopheles species. Approximately 3.3 billion are susceptible to malaria risks. Increased prevention and control measures have led to a reduction in malaria mortality rates by more than 25% at the globe level (2). Many insecticides used to gid rid of the damage that produced by these insects, but they showed high resistance and in the same time chemicals substances are enviromenally unsafe. Microbial control by entonopathogens was used effectively ,nevertheless the cons of pathogenic agents is spending a long epoch to settle the target which lead up to considerable loses in crops and need idealistic weather circumstances to infect .In modernistic years, Gold nanoparticles (AuNPs) earned a significance as soporific carriers, seeing that their sizable surface facilitates easy functionality (3) . AuNPs that formed by Fungi are renitent to oxidation and also environmentally stabilized (4; 5; 6).The applicative aspects of AuNPs include Biology and medicine (7; 8;9; 10)

Microorganism especially entomopathogenic fungi have ability to form Bionanoparticles by metal reduction so as to clear away dissoluble metals that accumulated in environment, so that lessening its toxicity and biopresence of Microorganisms able to the reduction of metal biologically could eliminate metals that polluted environments. For instance, the growth of *Shewanella oneidensis* with the existance of sub-mM concentrations of Ag+ (11).The pros of Biosynthetic AuNPs are less costs and environmentally safer. High concentrations of extracellular redox enzymes and capping agents for AuNPs steadiness, and Its size is Smaller than nanoparticles formed by other micro-organisms (12; 13). Metal nanoparticles synthesized by insect pathogens has been used to control Medical and economic insects. Bionanoparticles especially silver nanoparticles produced by Entomofungi have been successfully used against species of mosquito such as *Tricho-derma* (14;15), *Aspergillus* (16;17) and *Fusarium* (18;19) were recounted. This is the first attempt to use Mosquito pathogenic fungus *E.culicis* to synthesize gold nanoparticles versus *Anopheles stephensi*.

MATERIAL AND METHODS

Mosquito Rearing

To rear insect, the method that described by(1) . Larvae of *A.stephensi* were brought from a water pool in AL-Diwaneyah city near AL-Qadisiyah dairy factory and put in a container and reared in laboratory at AL-Qadissiya university ,college of sciences, As insect was identified in the natural historic museum and research center in Baghdad university.

Isolation and identification of *E.culicis*

The insect pathogen fungus cultures that was exploited in the formation of gold nanoparticles was isolated from adult mosquitos ,where the cadaver placed on Entomophthora complete medium (ECM) and incubated for 7 days at 25C .Pure culture was prepared from subculturing of the fungus on new prepared ECM. Slide culture was used to identify fungus with observing under microscope and was confirmed by Dr.Sulaf Hmid Taimoz, College of Agriculture , AL-Qadisiya university. Pure isolate were kept at 4C in refrigerator for more experiments.

Synthesis of gold nanoparticles

A liquid medium composed of glucose, yeast extract, peptone and malt extract was used to culture the fungus in flasks. After incubation at 27°C and for 7 days. Plastic sieves were used to obtain the culture supernatant by segregation the biomass . 10 mL of the culture filtrate was challenged with 20 mL of HAuCl4 and incubated under dark conditions at room temperature for 48 hr.

Gold nanoparticles characterization

The steps that followed to characterize the formation of golden nanoparticles are change in color of fungal filterate solution that is an important clue for Bionanoparticles synthesis. Uv-Vis spectroscopy to confirm the existence of nanoparticles and Transmission electron microscope(TEM)

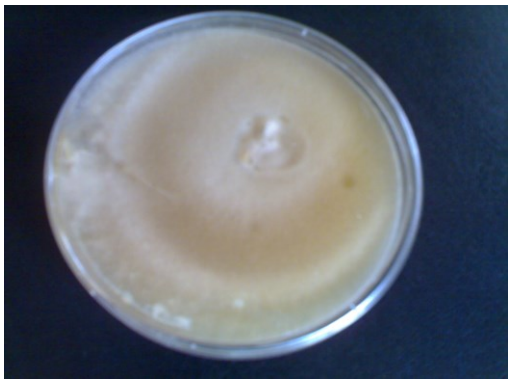
Toxicity AuNPs Versus *A.stephensi*

The standard method (21) was used to evaluate toxicity of synthesized AgNPs against the *An. stephensi* . Bioassay was achieved singly at five various concentrations (2, 4, 6, 8, and 10) ppm of AuNPs. The toxic activity of formed AuNPs were evaluated, where 25 larva and pupa of *An. stephensi* were singly sprayed with 100 ml of aforesaid concentration, As the control (without AuNPs) was left without treatment to compare mortalities. Then, mortalities examined after 24 hours of treatment with thrice replications to support the outcomes.

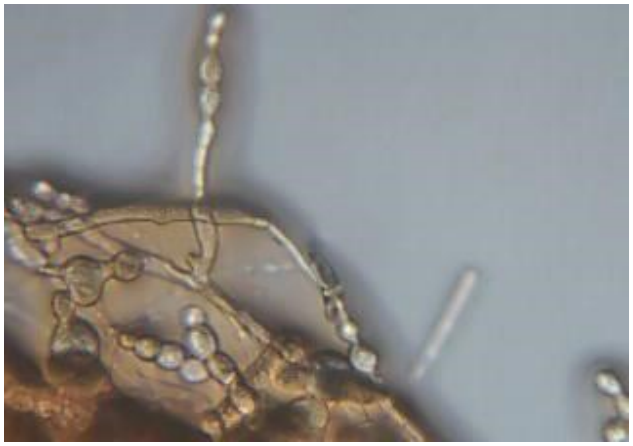
RESULTS AND DISCUSSION

Insect pathogen *E.culicis* fungus

In the current investigation , *E.culicis* was invitro isolated for the first time in Iraq. The fungus was identified relying on colony properties and Microscopic appearance as show in figures(1and 2).



Figure(1) *E.culicis* on culture medium



Figure(2) Microscopic view of *E.culicis*

Synthesis of gold nanoparticles

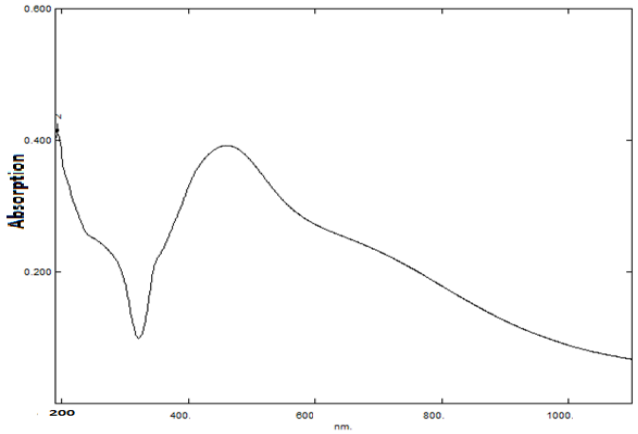
The alteration in color(yellow to pink) as illustrated in figure3(A and B) was Seeing that the collective coherent oscillation of conduction electrons at the surface of the gold nanoparticles when The interaction between particles and oscillating electric field of the incident light happened , a phenomenon is said to be surface plasmon resonance (SPR). Alteration in color points out the reduced amount of HAuCl4 to nanogold which is the initiative in the AuNPs formation (15; 16). Gold nanoparticles formation using biological resources is realized to be a type of bottom-up preface , where in the key reaction is reduction/oxidation of substrates, herewith the formation of colloidal compositions. It was as well revealed that micro-organisms enzymes with antioxidant or reduction specifications are in charge of the reduction of metal compounds into their particular nanoparticles (12).

Figure(3A) fungal filtrate before addition of AuNo3

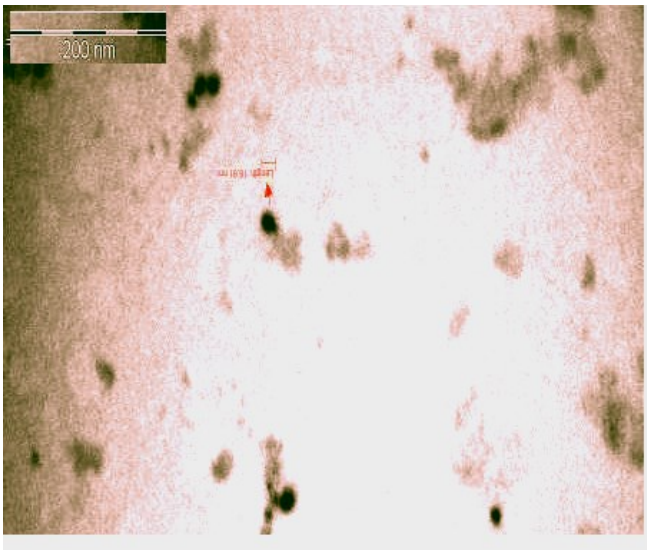
Figur(3B) AuNPs synthesized by *E.culicis*

AuNPs characterization

Alter in color in the interaction medium (from yellow to dark pink) is a decisive proof for synthesis of golden nanoparticles .For extra information concern the existence of AuNPs ,UV-Vis spectroscopy which is the most common method for affirming the formation of nanoparticles .350-600 nm Uv –visible absorption may provide sight to surface ,allotment ,size and optical characteristics of the nanosized Au particles. In aquatic solution, The band of the goldnanoparticles ordinarly align relying on the their morphology role, seeing that the high sensitivity of plasmon bands to the sharpness and length of the nanomaterials tips. The fungus isolate showed apeak at 510 nm(figure4) which proofs the spherical nature of AuNPs according to standard peak (SPR) wavelength as well as the existence of AuNPs in the solution . As substantiated by (22), A strong absorption of the orbicular nanoparticles was at 520 nm and often no absorption at 600 nm. The shape of AuNPs formed by *E.culicis* were orbicular and their size 20 to 30 nm according to TEM microscopic view (figure5)



Figure(4) UV-vis spectra of synthesized AuNPs from fungal liquid of *E.culicis*

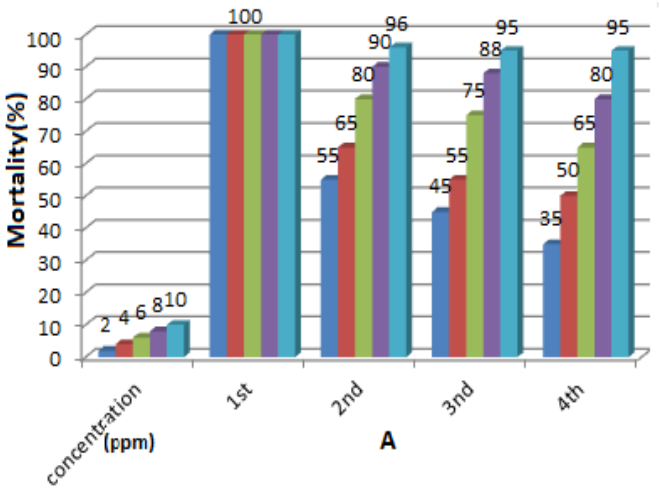


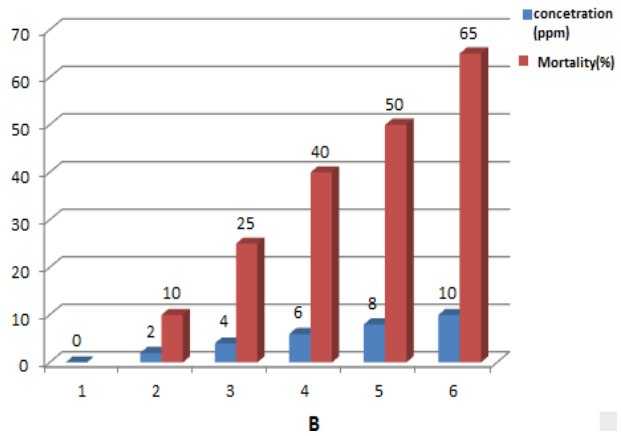
Figure(5)TEM im-age of *E.culicis* synthesized AuNPs

Toxicity of BioAuNPs versus *E.culicis*

The current investigation illustrated the activity of Entomopathogens *E.culicis* mycellial extract that has approach for gold nanoparticles formation as biolarvicidal and pupicidal agent for controlling the most important insect in health .This is in all probability the first report with nanogold using entomopathogenic fungus *E.culicis*. The first instar of *An.stphensi* was very sensitive to AuNPs ,where the mortality reached 100% at an hour of treatment with all tested concentrations ,As the lowest death rates were versus fourth instar ,Where mortalities amounted to (35,50,65,80and 95)% at (2,4,6,8and10)ppm respectively in the same term. The percent mortality of the third and fourth instar larvae of *An.stphensi* was (65,65,80,90 and 96)% and (45,55,75,88and 95)% sequentially after an hour with respect to the above ppm concentrations Figur6(A).Pupae were the less effect than larvae with AuNPs ,where the mortalities reached (10 ,25,40.50 and 65)%after 2 hours when treated with all the above mentioned concentration as show in Figure6(B)

Many scientists focused on the importance of nanoparticles in insects control (23). The toxicity of silver nanoparticles formed by fungus *Cochliobolus lunatus* versus *Ae. aegypti* and *Anopheles stephensi* have been noticed (24). (25) reported that silver nanoparticles synthesized via *Aspergillus niger* have high toxicity versus larvae of some mosquitos species, where the mortality amounted to 100 percent against *Cx. quinquefasciatus* larvae to AgNPs through an hour of treatment, As the effect of AgNPs on *An. stephensi* and *Ae. Aegypti* larvae was less.The activity of silver nanoparticles formed by entomopathogen *Beauveria bassiana* to control dengue vector, *Aedes aegypti* was used for the first time by (26) whom pointed out that death rates of the first and second instar larvae of *Ae. aegypti* reached cent percent ,As 86.6 and 83.3%mortality was scored against third and fourth instars at 24 hr accordingly. It is proposed that the formation bio-nanoparticles by the insect pathogenic fungi will be suitable for environmentally safer and greener program in the control of pathogen vectors strategy through a biological process.





Figure(6) effect of AuNPs synthesized by *E.culicis* against *A.stephensi* (A) larvae and (B) pupa

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