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GREEN SYNTHESIS OF COPPER NANOPARTICLES USING *THUJAORIENTALIS* AND ITS LARVICIDAL POTENTIAL AGAINST MALARIAL VECTOR

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Abstract

The development of nanotechnology is interesting for the synthesis of nanoparticles for the bio application. In the present study, the efficacy of copper nanoparticles was estimated against malarial vector *Anopheles stephensi*. Nanoparticles were synthesized using *Thujaorientalis* leaf extract, which act as both reducing and capping agent. When the color of the leaf extract was change with the addition of copper nitrate, it showed that the copper nanoparticles were formed. Synthesis of copper nanoparticles was detected by the help of UV- visible spectroscopy and Fourier Transform Infrared analysis (FTIR).The study confirmedthe effectiveness of that copper nanoparticles against the third and fourth instar larvae of *Anopheles stephensi* mosquito.

Keywords: Copper nanoparticles;*Anopheles stephensi*;*Thujaorientalis*; Green nanotechnology.

Introduction

In modern research nanotechnology plays an important role. This technique mostly applied in every field for example, electronics, pharmaceutical, feed, food drugs and biochemical sciences. Nanoparticles are synthesis by green synthesis. Copper nanoparticle have many properties like catalytic, optical, biomedicaland antibacterial, antifungal. In many fields nanoparticles are used as antimicrobial agents (Kaur *et al.,* 2019; Neupane *et al*., 2017; Prabhakar *et al*., 2011a) (Suttee *et al.,* 2019; Neha *et al*., 2018). For bacteria, copper is very toxic and for animal cell it is nontoxic. From a plant, nanoparticles can be formed from stem, leaf, flower and outer covering from the fruit (Attri *et al.,* 2019; Bawa *et al.,* 2019; Singh *et al.,* 2019). In plant extract alkaloid, flavonoid and fatty acid are present (Kumar *et al.,* 2019; Vyas *et al.,* 2017; Kaur *et al.,* 2016a; Kaur *et al*., 2016b; Saranyaadevi *et al*., 2014).

Nanoparticles are present in different size or in the form of cluster of atom. Nanoparticles are particles between of 1 and 100 nanometers in size.present (Mishra *et al.,* 2019a; Mishra *et al.,* 2019b; Patil *et al.,* 2019b; Mishra *et al.,* 2018a; Mishra *et al.,* 2018b; Mishra *et al.,* 2017; Kaushik, *et al*., 2011; Prabhakar *et al*., 2011b), (Ahmadi *et al*., 2019). In nanotechnology, a molecule is characterized as a little object that acts as a whole unit as for its transport and properties. Nanoparticles are also known as micro crystals (Saluja *et al.,* 2019; Prasad *et al*., 2013). Nanotechnology is almost applied to every field such as infection, cancer, allergy, diabetes, and inflammation. Among heavy metal copper is considered as human poisoning and is required by the body in small amount (Dwivedi *et al.,* 2019; Mekale *et al*., 2016 and Saranyaadevi *et al*., 2014; Devi *et al.,* 2014). Nanoparticles have selective coating for solar energy absorption and antibacterial agents. Green synthesis of copper nanoparticles provides eco-friendly, cleaner synthesis route (Mishra *et al.,* 2019c; Mishra *et al*., 2019d; Mishra *et al.,* 2019e; Mishra, 2018c; Abidin Ali *et al*., 2016; Prabhakar *et al.,* 2011b; Prabhakar *et al.,* 2009). Various method which are used for the synthesis of nanoparticles are performed in non-aqueous media (Nagpal *et al*., 2013). They are synthesized by using Thuja plant, commonly known as Morpankhi. It has genes of coniferous trees having about five different species. It belongs to the family cupressacae. Sometimes, Thuja plants are also referred as evergreen trees having length 10-200 feet tall. (Pallavi *et al*., 2016) They have needle like leaf and give fruit only one time in a year, and called evergreen scaly leaves which are present on the main shoot. Thuja is used as herbal medicinal plant. It helps in treated with fungal infection of the skin it has also many antibacterial properties. Many diseases like nervous disorder, bring down fever and insomnia is treated with the help of leaves and stem of *Thujaorientalis*. Many components are present in Thuja plant leaves like sugar, water soluble minerals, essential oils (Brijesh *et al*., 2012). These plants are commonly found in all over India having many medicinal properties.

Malaria is caused by the Plasmodium falciparum. Large number of death are caused by malaria in every year which is cause by rapid spread of Plasmodium falciparum(Collin *et al*., 2000).There are 300-500 million cases of clinical malaria found per year according to world health organization by which 1.4-2.6 million death found in African children. The malarial parasite spread many type of disease all over the world i.e. acute respiratory infection, diarrhea, and tuberculosis. These disease cause 25% infant mortality, 30% childhood mortality and 11% maternal death (Samid, 2012).

Material and Methods

All the materials used in this process were made in India. Double distilled water used in this process and filtration done by Whatman no.1 filter paper. Theglassware's used for experiment were rinsed in distilled water and dried in hot air oven. Copper nitrate (Cupric nitrate) used in this process was made up by Thomas Baker and fresh leaves of *Thuja* plant used which was washed with distilled water. Third and fourth instar larvae used for the testing. Centrifugation donein the centrifuge machine and pellets dried in hot air oven. Detection of nanoparticle done by the UV- visible spectroscopy and FTIR.

Preparation of *Thujaorientalis* leaf extract

Fresh leaves of *Thujaorientalis* were collected from the University Park in LPU (Fig.1).The leaves (10 grams) were weigh using weighing machine after that all the leaves were washed with distilled water. Then all the leaves were cut into small pieces with the help of cutter and then boiled in 100 ml

distilled water at 80º temperature for 10 minutes. Heating process was done into heating mantle and after boiling all the extract filtered out from the leaves with the help of whatman no. 1 filter paper. Then the leaf extract was stored inrefrigeratorwith all the precaution.

Fig. 1: *Thujaorientalis* leaf

Preparation of copper nanoparticles

For the preparation of copper nanoparticles,10 gram of leaf extract was added into 100 ml of copper nitrate solution (0.1mol). After the addition of both the solution, the color of the leaf extract was changed from light green to dark brownish green which indicate that the nanoparticle was present into that solution. After that stirring process was done with magnetic stirrer for 3 – 4 hours. Then for overnight the solution was stored into the refrigerator without any disturbance. Next day the, solution was centrifuged into centrifuge machine at 12,000 rpm for 30 minutes by which the supernatant and pellet was separate out from each other and two layers were formed in centrifuge tube. Then the pellet was take out from the tube. Pellet was taken out into petri dish which was present in wet form and in lesser amount. For drying of pellets put the petri dish into hot air oven at 50ºC for 4–5 hours. After drying, pellets were converted into powder form which have very small size. The nanoparticle was stored into Eppendorf tube.

Characterization

Many biomolecules are present in leaf extract which are determined using FTIR analysis which is responsible for the reduction of copper ion at spectral range 500–4000cm^{-1}. For FTIR process, 99% KBr added into 1% copper nanoparticles. The synthesis of copper nanoparticles was detected by using UV – visible spectroscopy. Size of nanoparticles was detected by UV – visible spectroscopy at range between 300–700nm.

Collection of larvae for setup

Mosquito larvae basically found in fresh water, rice field, small rain pool and pond water. For the sampling, *Anopheles*larvae were collected from pond water at Guru Nanak Nagari, near Lovely Professional University, Phagwara, Punjab.The larvae were collected for testing then for experimental setup, and 15 larvae were poured in each beaker for testing the nanoparticle effect on them. Setup was prepared in triplicate form.

Bioassay

Firstly, many different doses were tested and standardization, afterward these 4 doses i.e. 150, 200, 250, 300 ppm were selected for the final treatment of larvae. For the treatment set each dose was taken and poured in 250ml of dechlorinated water having 15 larvae. The same procedure has been repeated for all doses. Whereas for the control set only 250ml of dechlorinated water and 15 larvae were taken. The final readings were taken after 3, 6, 12, 24, 48, and 72 hours, respectively.

Result and Discussion

Color change of copper nanoparticles

When copper nitrate wasadded into leaf extract at that time the color of the leaf extract was changed from light green to dark green, it showed, that the synthesis of copper nanoparticles take place.To confirm the synthesis of nanoparticles UV – visible spectroscopy and FTIR was also done (Fig. 2).

Fig. 2: The formation of *Thuja* nanoparticles confirmation with the change in colour of extract after addition of copper nitrate

(a) *Thuja* leaf extract before addition of copper nitrate

(b) *Thuja* leaf extract after the addition of copper nitrate

UV – visible spectroscope

The reduction of copper ion to copper nanoparticles was observed by UV-visible spectroscopy. Synthesis of copper nanoparticles was observed at wavelength 300 – 700 nm. (Rajeshkumar *et al*., 2019)

FTIR study

FTIR commonly used to find out that how many molecules are used for reduction of copper ion and balancing of copper particle by capping action. With the study of FTIR it was observed that leaf extract shows distinct peak with copper nitrate solution at 500 – 4000cm. The FTIR spectrum of copper nanoparticles has shown the band at 464.86, 661.61, 1022.31, 1388.79, 1548.89, 1641.48, 2879.82, 3431.48, and 3738.17 which confirms O-H, C=O and C-C, groups (Fig. 3).This represents the presence of biomolecule inside the *Thujaorientalis* plant extract.

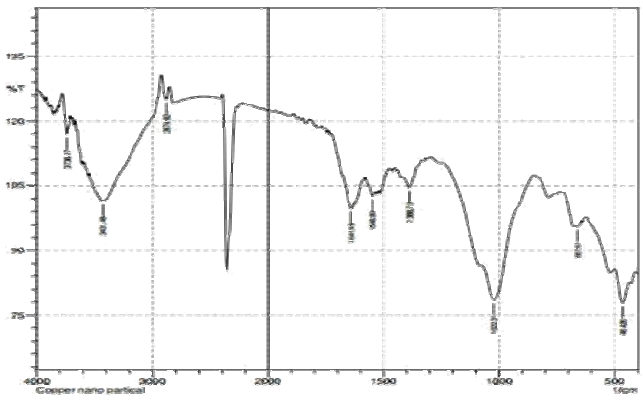

Fig. 3: FTIR Spectra Synthesis of Copper nanoparticle produced by *Thujaorientalis*

Study of mosquito larvicidal activity

The impact of copper nanoparticleson *Anopheles* larvae was observed to be highest at the highest dose of copper nanoparticles after 72 hours (Table.1).To check theeffect of nanoparticles triplicate set were run of each dose (i.e.150, 200, 250 and 300 ppm). The highest mortality rate was found at 300 ppm dose later followed by 250, 200 and 150 ppm. The mortality rate of each dose was observed after 3, 6, 12, 24, 48 and 72 hours, respectively. The mortality rate increases with the increase of copper nanoparticles. (Prasher *et al*., 2018) At 150 ppm, the mortality rate of larvae was 23.43%. But when the dose was increase at 200ppm the mortality ratewas 40%. In the last at higher dose of nanoparticle 250 ppm mortality rate of larvae was 26.67%. At 300ppm the mortality rate of larvae was and 33.34 %. This indicates the mortality rate was increased with the increase of dose.

Table 1: Effect of different doses of *Thuja* nanoparticles on mortality of 4th instar of *Anopheles stephensi*larvae

Dose (ppm)	Percent Mortality (In Hours)					
	3	6	12	24	48	72
150	23.34± 1.41	40.00± 0.83	53.34± 0.70	60.00± 0.00	70.00± 1.41	100±0 .00
200	26.67± 0.70	43.34± 0.83	63.34± 1.41	73.34± 0.83	86.67± 0.70	100±0 .00
250	33.34± 0.70	50.00± 1.41	68.67± 0.00	76.67± 0.83	90.00± 0.70	100±0 .00
300	41.67± 0.83	53.34± 1.41	71.67± 0.70	80.00± 0.70	93.34± 0.83	100±0 .00
Control	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00±0 .00	0.00± 0.00

% Percent; ± SD

Mortality of *Anopheles* larvae was found due to the presence of toxic substance present in *Thujaorientalis* leaves. When the color changed after the mixing of both the compounds, it showed that there was many type of compound present in Thuja leaves which are toxic for the larvae. Plant commonly used as medicinal plant because it has many compounds which helps in the killing of microorganism present in the human body. In other report, Mondal and Hajra, (2016) it was found that the highest mortality rate was found at 72 hours with leaf extract of sun flower. In another report, Muthumaran *et al*., 2016 noticed the activity of nanoparticles against the adult of *Anopheles stephensi*, *Aedesaegypti*, *Culexquinquefasciatus* after the treatment of *C.asiatica* for mosquito vector. The activity of larvae was found restless at higher dose of nanoparticles with abnormality and after some time larvae was found dead. Rate of mortality directly proportional to dose. The highest mortality was observed *An. stephensi* (LD50= 26.60µg/ml;LD90=48.34 µg/mL) and at *Ae. Aegypti* (LD50=29.16 µg/mL; LD; 90=52.84 µg/mL and *Cu. quinquefasciant* (LD50=32.23 µg/mL; LD90= 58.24 µg/mL), respectively. In control sets, no mortality was observed.

Plants has many effective ingredients which target the mosquitoes and they are safe to environment, an author confirmed the effect of another plant has mosquito larvicidal properties of *Eucalyptus globulus* and *Aloe vera* against *Anopheles stephensi* at 90pmm and 550ppm concentration of extracts, respectively (Riat and Kocher, 2017). Suman *et al.,* 2015 also reported the larvicidal activity of both synthesized titanium dioxide nanoparticle by using *Morindacitrifolia*and aqueous root extract of *M. citrifolia*against the three species of mosquitoes' larvae (*Anopheles stephensi, Aedesaegypti*, *Culexquinquefasciatus*).The present study reveals the formation of copper nanoparticles with the help of Thuja plant by very simple technique as many procedures are available which requires expensive chemicals for the formation of nanoparticles. The nanoparticles formed by the present technique is eco-friendly and effective against mosquitoe control too. The FTIR indicated the formation of nanoparticles in a study. Similar result formation by Ethylene groups detected by FTIR have been reported by Li *et al*. (2014) to can act as reducing or capping agent.

Conclusion

Anopheles larvae was commonly found in pond water, salty water and in rice fields, larvae cause malaria disease in case of infant. The malarial disease was controlled by various methods, one of the commonest method is with the help of synthetic insecticides. But due to its side- effects, natural larvicides like the formation made in this report i.e. *Thuja* nanoparticles will be very much helpful to compete with synthetic insecticides. During the study of nanoparticles, it was found that with increase in the dose of nanoparticles and with increase in time the highest rate of mortality was found these nanoparticles can be used for the control of malaria spreading mosquito *Anophelesstephensi*.

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