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STUDY THE EFFECT OF *Citrus aurantium* LEAVES WATER EXTRACT COPER NANO PARTICLES ON THE 3th, 4th LARVAE AND PUPA OF *Culex pipiens*

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Abstract
Our study is conducted to evaluate the effect of *Citrus aurantium* leaves coper nanoparticles. Characterization of coper nanoparticles was performed using UV, FTIR. The diameter of coper nanoparticles was distributed in the range of 2 to 12 nm. Using different concentration ́s of *Citrus aurantium* leaves extract, coper sulfate and nanoparticles (100, 200, 300ppm). The result of our study showed that the higher concentration 300ppm of nanoparticles gave a higher mortality percentage on 4th larvae 100% followed with 3th 89.3% and the pupa gave 83.3% respectively, thus introduced to use *Citrus aurantium* leaves coper nano particles as a *Culex pipiens*, management agent.

Key Words: *Citrus aurantium*, UV, FTIR, *Culexpipiens*.

INTRODUCTION

Manage mosquito by eco-friendly strategies. The green nanoparticle is advantageous over chemical and physical methods, since it is cheap, single-step, and does not require high pressure, energy, temperature, instead of using highly toxic chemicals botanical products used as sources of reducing and capping metabolites (Li and Jiang 2009). Novel eco-friendly strategies to manage mosquito vectors are urgently needed (Shobha, et al., 2014). The plant-mediated fabrication of nanoparticles is advantageous over chemical and physical methods, since it is cheap, single step, and does not require high pressure, energy, temperature, or the use of highly toxic chemicals (Muhammad et al, 2013). In the latest years, a growing number of plant-borne compounds have been proposed for efficient and rapid extracellular synthesis of metal nanoparticles effective against mosquitoes at very low doses (Han, et al., 2005). *Citrus aurantium* has received little attention, like many other members of this genus, most of studies on the insecticidal properties of Citrus species were focused on peel and seed extracts and not focused on other part just like leaves, shoots (Ponce et al., 2005). *Culex pipiens* it is House Mosquito. The House mosquito is now targeting humans and mammals on a regular basis. The mosquito bites an infected bird and then goes to another blood meal host, whether a human or another bird, and bites that new victim, injecting it with the virus from the original bird. Life cycle, Egg**,** Larva**,** Pupa**,** Adult **(**Huang, et al., 2008)**.** The aim of our study was to preparing green nanoparticle by using *Citrus aurantium* leaves water extract as insecticide by evaluation its effect on 3th, 4th larvae and pupa of *Culex pipiens*.

MATERIAL AND METHODS

Water extract : Five grams of the *Citrus aurantium* leave powder were add to 200 ml of distilled water; stirred well on hot plate till boiling for fifteen minutes, the solution was filtered and kept for 48 hours at a temperature of 4°C the methods was described previously by Rafiq et al., (2013).

Total Phenols: The total phenolic compounds were detected according (Abdulalsalam et al., 2017). Taking 200µL of plant leaves crude extract 3mg/ml D.W. was added Folin Ciocalteu reagent and mixed well then 2 ml of 20% sodium carbonate were added, incubate the mixture in dark for 60 minutes. Absorbent was measured at 650 nm.

Detection of Flavonoids: Measured flavanoid content according to Wadood et al., (2013). 0.1g of leaves extract were added to 5ml distilled water, then 5ml ammonia soluteion was added, mixed well with 1.0 ml sulfuric acid. Appearing yellow color refers to flavonoids component.

Detection of Alkaloids: 0.5 g of leaves extract was added to 3 ml of hexane mixed well then 5 ml of 1% HCl, was added heating the mixture till boiling, 1-3 drops of picric acid

were added. Yellow colored precipitate appeared indicated to alkaloids component.

Detection of Terpenoids: Terpenoids content was determined as described by Lundberg (2002). Leaves extract powder 0.5g was mixed with 10ml 90% methanol, then 2ml of chloroform and 3 ml of sulphuric acid were added and mixed well. Presence of terpenoids compound, reddish brown color appeared.

Detection of Tannins :Tannins was measured according to Prema, (2011) by adding 0.5g of leaves extract added to 10ml distilled water then added 2% of $FeCl_3$. A blue-green color was appeared indicated to tannins (Prema, 2011).

Green Copric-Oxide Nanoparticles: Synthesis of green nanoparticles was done by method as reported by Dhas, (1998) 0.03M cupric sulfate was added to 500 ml of *Citrus aurantium* leaves water extract. The solution heated at 80^{o}C for ten minute, the color of the mixture changed to black, and thenfurther mixing for 1 hr at room temperature kept at 4^{o}C.

UV-VIS Spectra Analysis: UV- VIS spectrum between 300-500nm**,** used for detection nano -particle hydrosol by using shemadzu equipment.

FTIR spectroscopy: The absorption and intensity of different active functional group indicate geometry features of functional groups, by using spectra of Fourier transform infrared generated by the radiation of electromagnetic absorption in the frequency range 500 to 4000cm^{-1} using Shimadzu model.

Bio assay: Collect insect eggs from stagnant water at the workplace. The eggs were grown in plastic containers with dimensions of 4 liters of water and incubated at a temperature of 27 ± 2 m, 10 ± 5% relative humidity and 12 hours illumination. The larvae were fed with a mixture consisting of a mixture of 1: 5 yeast, fish food and macaroni with at 120±1° C and 15 minutes (after 15 minutes), and in the pots 400 ml of water were placed at a rate of 50 virgins per vessel, made of organic glass 40 × 90 × 40 and door 10 × 10 cm in the same laboratory conditions above. Adult males were fed a cotton saturated with a 10% glycerin solution in Petri dishes and fed the female pigeon blood. After 2-3 days the eggs were collected by the females and were transferred to the above mentioned larval breeding ground. In our study we have taken the 3th4th larvae phase and pupa.

Preparation of pesticides: The material under study *Citrus aurantium* leave water extract, cupric sulfate salt and cupric nano particles) were used in concentrations 100, 200 and 300ppm, in addition to the control solution treatment. The test was carried out in 250 mL plastic containers usingper concentration. We read the results after 24 hours of implementation.

Statistical analysis: The percentages of mortality corrected according to the Abbott equation. Formulaand subjected to statistical analysis by the method of least significant difference, below a potential level p≤0.01.

RESULT AND DISCUSSION

Citrus aurantium leaves water extract consist of different groups of active components such as saponin, terpenoid, poly phenols, flavanoids, carbohydratesand tannins (Table1). Flavonoids are secondary metabolites acts as signaling molecules Bala (2010).

Table 1: Showed qualitative analysis of Active components in *Citrus aurantium*

Test	Results
Total phenol	+++
Saponin	++++
Terpenoids	+++ +
Carbohydrates	+++
Tannin	+++
Alkaloids	+++

Absorption spectroscopy in the UV-Visible spectral region, to detect the absorbances of nanoparticle peak at the range 300- 420 nm. The UV Visible spectrum of copric nanoparticles as shown in Figure 1. The absorption peaks at wavelengths of absorption peaks at 300-420 nm indicate the formation of cupric nanoparticles. The absorption of coper nanoparticles is responsible for this peak. The size distribution of nanoparticle measuring by using (DLS) Dynamic light scattering was shown in Figure 2. It showed that coper nanoparticle distributed in the range of 2-12 nm.

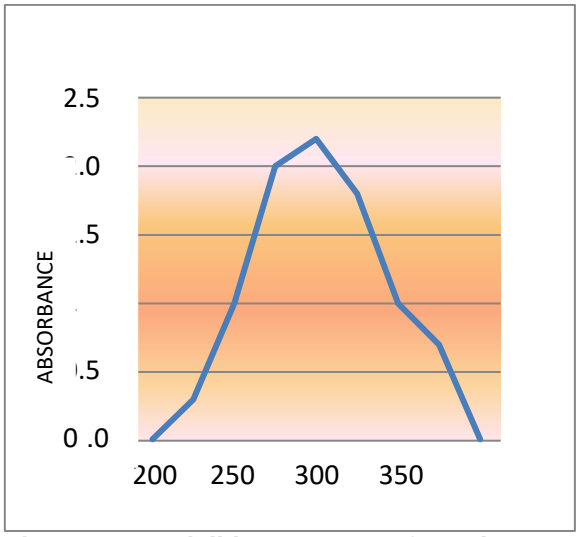

Figure 1: UV Visible spectrum of copric nanoparticles

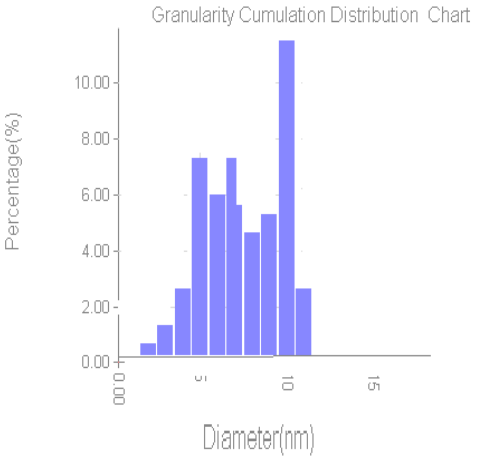

Figure 2: Size distribution by using DLS

Citrus aurantium leaves functional groups determined by FTIR analysis and the band intensities in different regions of the spectrum for coper nano particle. The broad and intense absorption peak at around 3,394cm 21 corresponds to the O–H stretching vibrations of phenols and carboxylic acids. The peak 2,355cm 21 located at around was attributed to the N–H stretching or the C=O stretching vibrations (Lahib et al., 2017).The shift from 3,394-3,388cm^{-1} may indicate the involvement of O–H functional group in the synthesis of nanoparticles. The peaks in the range 650-1000 cm^{-1} of copper nanoparticles are shifted slightly to higher wave numbers, the soluble elements present in *Citrus aurantium*extract could have acted as capping agents preventing the aggregation of nanoparticles in solution, and thus playing a relevant in their extracellular synthesis and shaping (Figure 3).

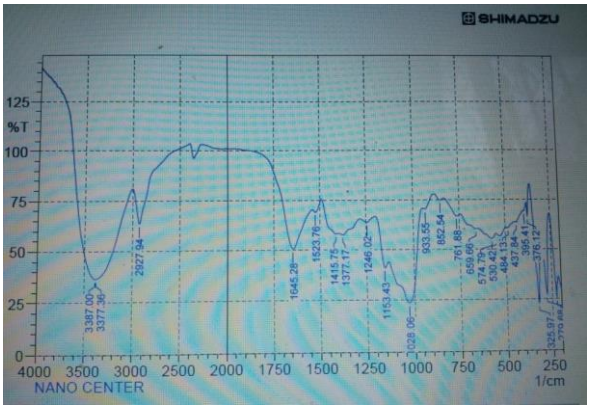

Figure 3: FTIR spectrum of synthesized copper nanoparticles

Figure 4 showed the effect of different concentration of the water extract in destruction *Culex pipeins*. The percentage of mortality for the 3th larvae was 6.6, 9.9 and 11.3. We found that the treatment with cupric sulfate as shown in Figure 5 (43.2, 53.2, 36.6%). Figure 6 presented the, respectively (Sarmad et al., 2017), in compare with control effect of nanoparticle on the 3th larvae mortality 65.3, 77.6, 89.3%, respectively.

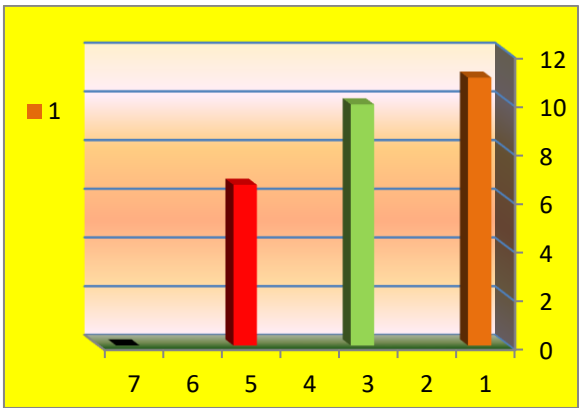

Figure 4: The effect of different concentration of the water extract in destruction third larvae phase of *Culex pipeins*. R.L.S.D. for the effect of concentration (p< 0.01) 3.1

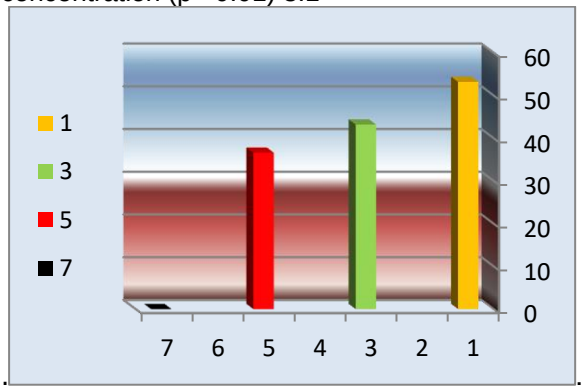

Figure 5: The effect of different concentration of the coper sulfate in destruction third larvae phase of *Culex pipeins*. *Culex pipeins*. R.L.S.D. for the effect of concentration (p< 0.01) 3.1

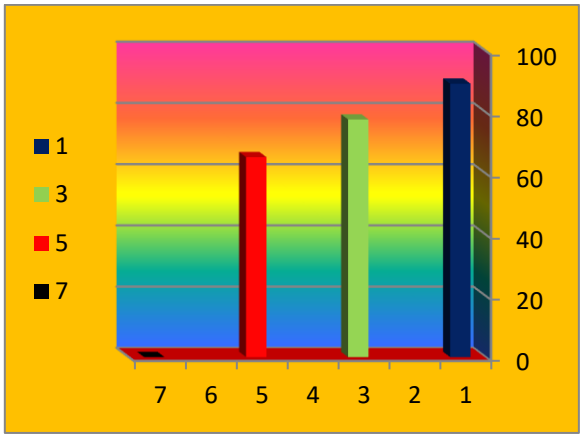

Figure 6: The effect of different concentration of the nanoparticle in destruction third larvae phase of *Culex pipeins*. R.L.S.D. for the effect of concentration (p< 0.01) 3.1

Figure 7, 8, 9 Showed the result of the effect of water extract, copric sulfate and nano particle on 4th larvae 16.6,19.9 and 26.6, 49.9, 56.6 and 69.8 and 100, 100 and 100, respectively.

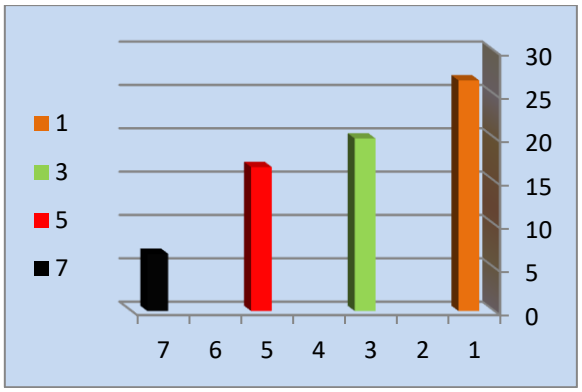

Figure 7: The effect of different concentration of the in water extract destruction fourth larvae *Culexpipeins*. R.L.S.D. for the effect of concentration (p< 0.01) 3.1

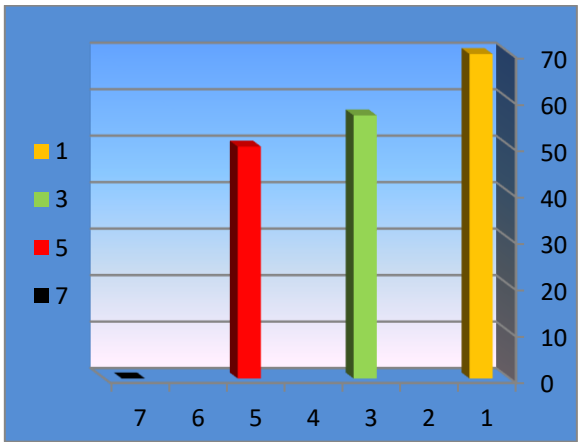

Figure 8: Effect of different concentration of the coper sulfate in destruction fourth larvae phase of *Culex pipeins*.R.L.S.D. for the effect of concentration (p< 0.01) 3.1

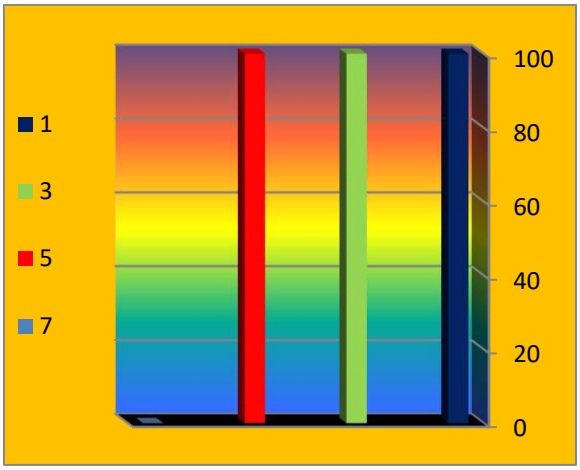

Figure 9: Effect of different concentration of the nano particles in destruction 4th larvae phase of *Culex pipeins*.R.L.S.D. for the effect of concentration (p< 0.01) 3.1

Figures 10, 11 and 12, presented the effect of material under study on *Culex pipeins* pupae phase 3.3, 3.3, 6.6, 6.6, 16.6, 20.3 and 69.9, 76, 83.3, respectively (Sabar, 2017).

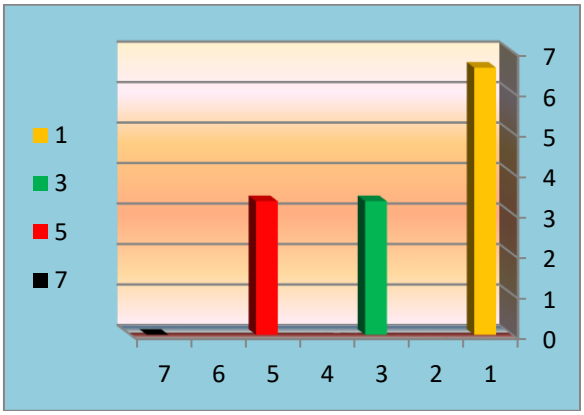

Figure 10: Effect of different concentration of the in water extract destruction Pupae phase *Culex pipeins*. R.L.S.D. for the effect of concentration (p< 0.01) 3.1

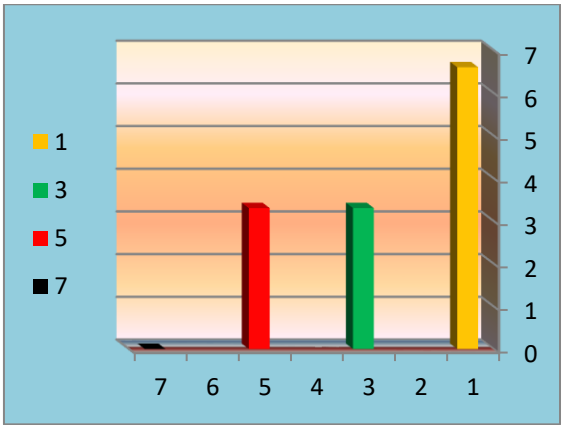

Figure 11: Effect of different concentration of the coper sulfate in destruction Pupae phase of *Culex pipeins*. R.L.S.D. for the effect of concentration (p< 0.01) 3.1

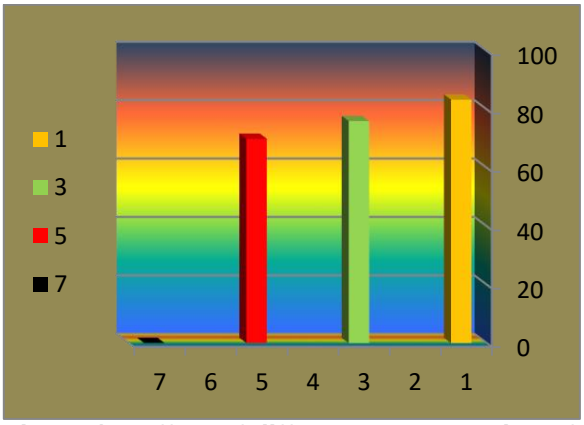

Figure 12: Effect of different concentration of the nanoparticles in destruction Pupae phase of *Culex pipeins*. R.L.S.D. for the effect of concentration (p< 0.01) 3.1

Our study had shown that the nano particles presented the highest mortality effect on 4th larvae phase it gave 100% mortality in all concentrations, followed by 3rd larvae 300ppm concentration gave the highest potential in compare with the other concentrations 89.3%, while pupae 83.3% (figure 13).

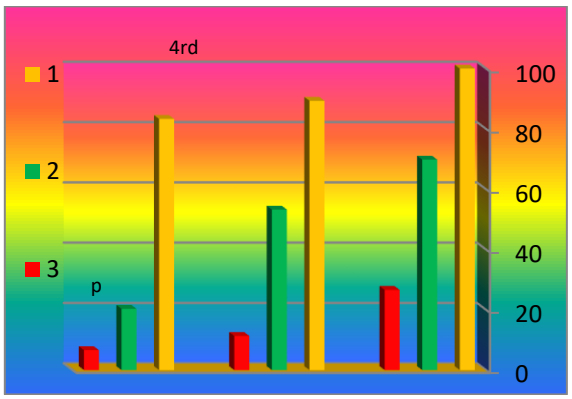

Figure 13: Effect of different concentration of the nanoparticles in destruction 3 th.4th larvae and Pupae phase of *Culex pipeins*. R.L.S.D. for the effect of concentration (p < 0.01) 3.1

The responses and mortality of *Culex* larvae and pupa observed in the present investigation could be attributed to phytochemicals present in the leaf extracts of *Citrus aurantium* (Gusmao et al., 2002). The other studies observed that phytochemicals have a major role in mosquito mannegment (Mancebo, et al., 2001). Saponin from the bark of *Quillaja saponaria* was a natural larvicidal against Culex pipens.

Conclusion

In the present study the leaf extract of *Citrus aurantium*copric nanoparticles was found to have larvicidal activity again, thus introduced to use *Citrus aurantium* leaves as a mosquito managment agent. Also, the results of this investigation indicate that the *Citrus aurantium* nano-particle was eco-friendly pesticide.

REFERENCES

Totsuka, A. and C. Fukazawa, Affinity purification of α-amylase originating from plant using cyclo-maltohexose-immobilized sepharose 6B in the presence of ammonium sulphate. Prot. Expr. Purif. 4: 333-336 (1993).

Barns, S.M., C.R. Delwiche, J.D. Palmer and N.R. Pace, Perspective on archaeal diversity, thermophily and monophyly from environmental rRNA sequence. Proc. Natl. Acad. Sci. USA 93: 9188-9193 (1996)

Abdulalsalam, H., N.M. Ardalan and S.H. Ahmed, Evaluation of anti-inflammatory effect by using iron nanoparticles prepared by Juglansregia water extract. Current Research in Microbiology and Biotechnology 5(4): 1151-1156 (2017).

Almukhtar, S.A., Effect of growth regulators and sucrose on the induction and production od\f flavonoids in callus of *vitisvinifera(L) in vitro*. Pak. J. Biotechnol, 14 (4): 803-809 (2017).

Al-Shawi, S.G., A.K. Zena and A.A.F. Nada, Study of cumin antibacterial and antioxidant activity of alcoholic and aqueous extracts. Pak. J. Biotechnol. 14 (2): 227 -231 (2017).

Bala, B., P.K. Kar, U.K.Haldar and S.B Mazumder, Evaluation of anticancer activity of *Cleomegynandra*on Ehrlich's ascites carcinoma treated mice. Journal of Ethnopharmacol.129(1): 131–134. (2010).

Dhas, N.A., C.P. Raj and A. Gedanken, Synthesis, characterization, and properties of metallic copper nanoparticles. Chem Mater. 10:1446–1452 (1998).

Gusmao , D.S.,V. Pascoa, L. Mathias, I. Vieira,J.C. Braz, R. Filho, and F.J.A. Lemos, *Derris*(*Lonchocarpus*) *urucu* (Leguminosae) extract modifies the peritrophic matrix structure of *Aedesaegypti* (Diptera: Culicidae). Mem. Inst. Oswaldo Cruz. 97 (3):371-375 (2002).

Han W.K., J.W. Choi, G.H. Hwang, S.J. Hong, J.S. Lee and S.G. Kang, Fabrication of Cu nano particles by direct electrochemical reduction from CuO nano particles. App Surf Sci, 252: 2832–2838 (2005).

Huang Z., F. Cui, H. Kang, J. Chen, X. Zhang, and C. Xia, Highly dispersed silica-supported copper nanoparticles prepared by precipitation-gel method: A simple but

efficient and stable catalyst for glycerol hydrogenolysis. Chem. Mater. 20:5090–5099 (2008).

Lundberg, K. An environmental impact assessment model for malaria control in developing countries.M.Sc. Thesis in Biology-Department of Landscape Planning-Ultuna, EIA center-Sveriges Lantbruks Universities Pp. 46 pp (2002).

Mancebo, F., L. Hilji, G.A. Mora, V.H. Castro, and R. Salazar, Biological activity of *Rutagraveolens* (Rutaceae) and *Sechiumpittieri* (Cucurbitaceae) extracts on *Hypsipyla grandella* (Lepidoptera: Pyralidae) larvae. Revistade Biologica Tropical 49(2): 501-508 (2001).

Mohammed, L.A. and T.A. Kareem, Characterization of some bioactive compounds from citrus limon L. and evaluating the biological effects on thyroid hormone and cellular toxicity Pak. J. Biotechnol. 14 (3): 551-558 (2017).

Ponce, A.A. and K.J. Klabunde, Chemical and catalytic activity of copper nanoparticles prepared via metal vapor synthesis. J. Mol. Catal. 225: 1–6 (2005).

Prema, P., Chemical mediated synthesis of silver nanoparticles and its potential antibacterial application. Analysis and Modeling to Technol. Applications 1(8): 151-166 (2011).

Rafiq, M., U.M. Dahot, S.H.A. Naqvi and A. Nadir, Insecticidal activity of Azadirachtin related limonoids from callus and cells suspension biomass extracts of neem against Jassids, whitefly and thrips. Pak. J. Biotechnol, 10(1): 11- 19 (2013).

Shobha, G., M. Vinutha and S. Ananda, Biological synthesis of copper nanoparticles and its impact - a review. Int. J. Pharmaceut. Sci. Invent. 3: 28–38 (2014).

Wadood, A., G. Mehreen, B.J. Syed, N. Muhammad, K. Ajmal, G. Rukhsana and A. Snad, Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochem. Anal. 2: 144 (2013).

LiDC, X.K. and J.G. Jiang, Identification and quality control of Chinese medicine based on the fingerprint techniques. Curr. Med. Chem. 16: 3064–3075 (2009).