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RESEARCH ARTICLE

Killing Insecticides Silver Nanoparticles (KISN) from Fruit Peels Waste for Larvicidal and Antihelmenthic Activity

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ABSTRACT:
Larvicidal and antihelmenthic properties of silver nanoparticles have been demonstrated against *Culexquinquefasciatus* and *Eduriluseugeniae*. The insecticidal effect was observed in silver nanoparticles synthesised from POBAfruit peel extractsand were characterized through UV Visible spectroscopy, x-ray diffraction (XRD)and transmission electron microscopy (TEM). The TEM results suggested that the size of nanomaterials produced was approximately 20nm. Mortality was observed 100% in 100 µL/mL and 25% in 20 µL/mL. TheLC_{50}=60.89 µL/100 mL; LC90=100.78 µL/100 mL C. quinquefasciatus. This is a perfect ecological and inexpensive approach for the control of mosquito larvae. Further, KISN exhibited antihelminthic properties with short period of time as compared to standards.

KEYWORDS: Fruit peel extract; silver nanoparticles; Green Synthesis; POBA.

1 INTRODUCTION:
According to World Health Organization, diseases spread by mosquitoes are the best known and biggest killer among all the vector borne diseases[1]. Dengue, Malaria, Yellow Fever and Chikungunya are different type of vector borne diseases which bring serious threat to the mankind in terms of high rate of morality[2]. Larvicidial is a doing well way of sinking mosquito densities in their propagation places earlier than they appear into adults[3,4]. Lymphatic filariasis is caused by Filarioidea nematodes (i.e., Wuchereriabancrofti, Brugiamalayi, and Brugiatimori). Microfilariae are transmitted to humans by different mosquitoes. Culex species, with a special reference to *Culexquinquefasciatus*, are the most familiar vectors across urban and semi-urban areas of Asia.

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Culexquinquefasciatus, is a widely distributed tropical disease with around 120 million people infected worldwide and 44million people having common chronic manifestation[5]. Mosquito is frequently found due to poor drainage system especially during rainy seasons, fish pond, and irrigation ditches and rice fields. This provides a better breeding place for mosquitoes[6]. There is an urgent need to check the proliferation of the population of vector mosquitoes in order to reduce vector borne diseases by appropriate control methods[7,8].

Likewise, Helminthiasis is one among the most widespreadmacro parasitic infection with parasitic worms such as roundworms (*Ascarislumbricoides*), whipworms (*Trichuristrichuris*) or hookworms (*Nectatoramericanus*) in humans and animals and represent a significant socio-economic concern[9]. The disease affects the health of large fraction of the human population including animals. Anthelmintic drugs have been used either prophylactically or curatively to control these parasites. Albendazole, is a benzimidazole carbamate highly effective in ascariasis, intestinal capillariasis, entrobiasis, trichuriasis, and hookworm infections,[10,11] thus inhibiting its polymerization or assembly into microtubules.

Insecticide applications, although highly efficacious against the target vector species control, are facing a threat due to the enlargement of resistance to chemical insecticides resulting in rebounding vectorial capability[12]. At the moment nanoparticles have drawn the attention of scientists, because of their extensive application in the development of new technologies in the areas of electronics, material sciences, catalyst and drug at the nanoscale[13,14]. It offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use deadly chemicals in the synthesis protocols[15,16]. In our previous investigation,[17] we have used multiple fruits peel wastes of POBA for the synthesis of silver nanoparticles which has been reported to have antimicrobial, antioxidants and anticancer cell line studies[18]. The aim of this study was to investigate the larvicidal activity of C. quinquefasciatus and vermifugal activity of E.eugeniaeusing the novel killing insecticides silver nanoparticles (KISN) obtained from POBA extract.

2. MATERIALS AND METHOD:

2.1 Preparation of SNP:

We have synthesized SNP using POBA extract with our previous work [17,18]. The attained SNPs were analyzed by using UV-Visible spectroscopy, XRD, FT-IR and TEM.

2.2 Collection of mosquito larvae:

The mosquito larvae were composed from stagnant water samples from drainage canals, temporary water pools and ponds around Ambattur Industrial Estate, Chennai district, Tamil Nadu, India, using sterile wide mouth container. The identified Culexsp. mosquito larvae were kept in plastic and enamel trays containing tap water, maintained and reared in laboratory as per the previous method[19]. The larvicidal activity was assessed following WHO (1996)[20]with minor modification[21].

2.2.1 Statistical Analysis:

The percentage of mortality was calculated from the results of anti-larval study by which the percentage variances of mortality. The significance was noted by using linear regression analysis. The EPA Propit analysis program (version 1.5) was used for calculating LC50 and LC90 values.

2.2.2 Test for larvicidal activity:

Testing of the KISN for larvicidal activity was carried out at different concentrations (20, 35, 50, 65, 80 and 100µl) using total of 20 reared mosquito larvae which was placed in 150 ml of double distilled sterile water. Sterile distilled water was used as control. The larval mortality in both treated and control was recorded after 24 hours and the percentage of mortality was calculated using Abbott's formula[22].

2.3 Collection of E. eugeniae

Preceding to testing, E. eugeniae were composed from damp soil and reserved for numerous days in darkness in cow dung at a constant temperature of 25°C. The earthworms of 4-6 cm in measurement lengthwise and 0.2-0.3 cm in breadth were utilized in the investigational protocol.

2.3.1 Test for antihelminthicmotion:

Mature motility in-vitro studies were performed on grown-upexistmature worms*E.eugeniae* according to the standard method. The KISNsolution was checked for its antihelminthic property.Standard antihelmenthic drug (albendazole, 100mg/ml) was equipped in purified water and normal saline were utilized as a control. Six collections of the same size of worms *E.eugeniae*were liberated into the petridish. Time period taken for paralysis and death of individual worms was observed and was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C.

3. Characterization of the synthesized nanoparticles

3.1 UV-Visible Spectrum Analysis

To identify the spectrum of the synthesized KISN using POBA extract for this purpose an aliquot 1 mL solution was scanned in the range of 400-800 nm.

3.2 XRD Pattern:

To know crystalline nature and size of the formed KISN were predicted by XRD studies.X-ray diffraction (XRD) patterns observed from Bruker, Germany, D8 Advance model, equipped with Ni filter, a graphite mono-chromatized Cu-Kα radiation (λ=0.154nm) operated at 40-kV accelerating voltage and 30 mA. The samples were scanned at step mode with 2° min-1 scan rate. The particle size of the synthesised SNps was determined from Debye-Scherrer equation[23].

$$D=K\lambda/\beta \cos\theta \qquad (1)$$

Where K is a shape factor (0.9), β is the full width at the half maximum height of the diffraction peak of (200) reflection plane at angle θ, D is the crystallite size and λ the wavelength.

3.3 TEM Analysis:

Transmission Electron Microscope (TEM) images were recorded on a TecnaiT20 G2 200kV, FEI Brand (Netherlands) microscope and operating at an accelerating voltage of 200 kV.

4. RESULTS AND DISCUSSION:

4.1 UV-Vis spectrum of KISN:

KISN formation using POBA were illustrated in the UV–Vis spectra (Fig.1). The band seen at 427 nm which obviouslyassigned the development of KISN and exposed that the size of 20 nm[17,24].Further it confirmsthe broadening of the peak illustrate that the oxidation of silver to silver oxide, this may be due to the fruit extracts contain various organic compounds especially flavonoids. It play a vital role which have various oxygen containing functional groups easily coordinate with silver ions to form coordination bonds due to their ability to donate electrons[25].

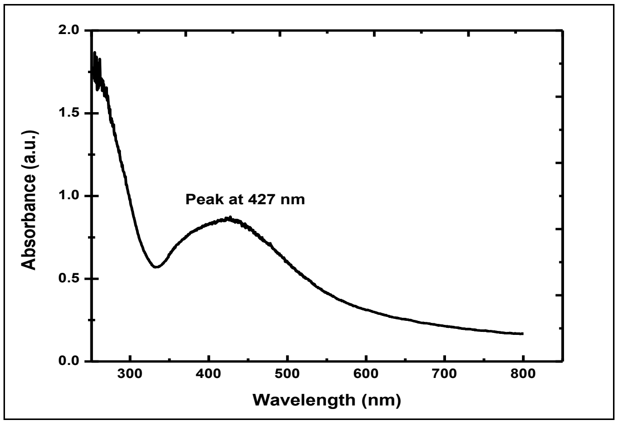


Fig. 1 UV-Visible Spectra of KISN at 427 nm

4.2 XRD pattern of KISN:

Fig. 2 shows the representative the XRD pattern of KISN. The result of the diffraction peaks at 2θ=44.51°, 63.76° and 76.85° corresponding to the (111), (131) and (022) planes of Ag, could be attributed to FCC lattice of metallic silver and the obtained outcomes were coincide with earlier report[26,27].

4.3 TEM Analysis of KISN:

TEM analysiswas determined to know the size, shape of nanoparticles and particle morphology of the KISN. Fig.3 clearly shows the morphology of the obtained nanoparticles was spherical and with an average size 20 nm[28]. The results also confirmed that the uniform dispersed appearance of the nanoparticles indicates the stabilization of particles is well defined without addition of capping agent.

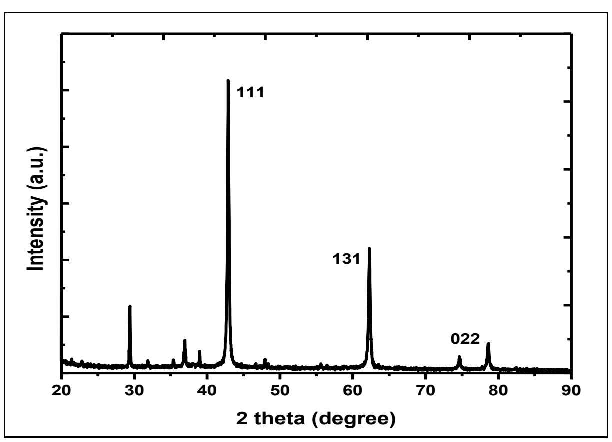


Fig. 2 XRD pattern of KISN

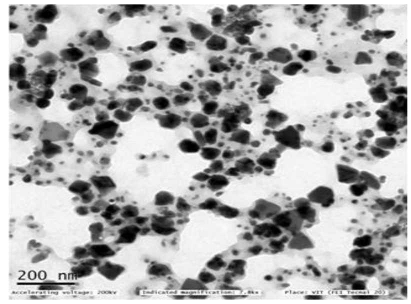


Fig. 3 TEM Image of KISN

4.4 Anti-larvicidal activity of KISN:

In this present work, the larvicidalactivity of different concentration of KISN with POBA extract was testedagainst larvae of *C. quinquefasciatus*. The larval mortality of of KISN against*C. quinquefasciatus*was shown in Fig. 4, the result predicts the highest and lowest mortality was observed100% in 100 µL/mL and 25% in20 µL/mL.The LC50=60.89 µL/100 mL; LC90=100.78 µL/100 mL*C. quinquefasciatus* (Table 1). The control (distilled water) showed nil mortality in the concurrent assay. The complete mortality was observed for KISN for *C. quinquefasciatus* at concentration 10 mg/L (Table 1).The 100% of lower confidential level (LCL) and upper confidential level (UCL) of LC50 and LC90 were 60.97-61.85 µL/100 mL and 100.86 -101.57 µL/100 mL, respectively. The regression values R2 are 0.959 and 0.990 where shown in Table 1. Mosquitoes are the most deadly vector for several diseases. Many plant extracts and essential oils manifest repellency activity against different mosquito species.[29] To the best of our knowledge there is no report in the literature for the control of mosquito population by using synthesized KISN using POBA extract. This is an ideal eco-friendly approach for the control of dengue and filariasis vectors.

Table 1: Larvicidal activity of KISN

LC50	LC90	r^2
UCL-LCL 60.97-61.85	UCL-LCL 100.86-101.57	0.959
60.89	100.78	0.905

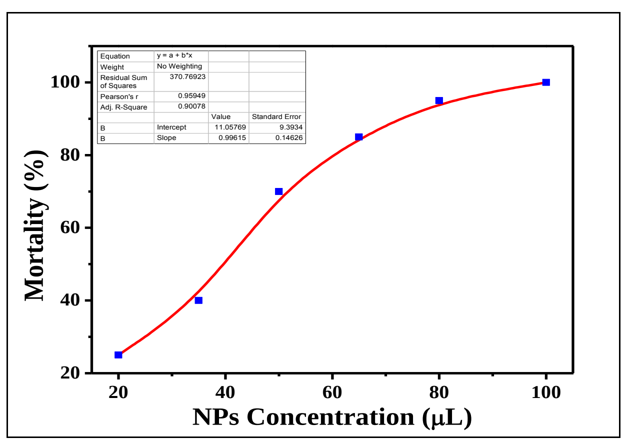


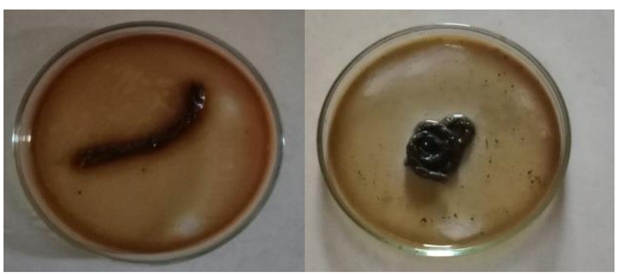
Fig. 4.The larval mortality of KISN against *C. quinquefasciatus*

4.5 Antihelminthic activity of KISN:

This study was conducted by exposing the worms to three different concentrations of KISN (15, 35 and 50 µL/ml in distilled water). The in-vitro studies were performed according to the standard method. The time taken for paralysis and death of E. eugeniaewas studied at different concentrations of silver nanoparticles and standard Albendazole drug was shown in the Table 2. From the results, it can be confirmed that within short period of time 7 min at a concentrationof 50 µL/ml of KISN exhibited significant antihelminthicactivity. Moreover the time taken for the paralytic and death stage of KISN as less as compared to standard drug (Fig. 5). It can be concluded that potent activity of KISN may be due to the presence of flavonoid and other phenolic phytochemicals[30]in the POBA extract. Because of easy availability, earthworms have been used extensively for the preliminary in vitro evaluation of anthelmintic compounds in vitro.[31]

Table 2: Lethal time vs concentration of KISN and standard drug:

Concentration (µL)	Albendazole	SNP
15	24	21
35	17	11
50	11	07



Paralytic Stage **Death Stage**

Fig. 5.Antihelmenthic activity of KISN

4.6 Mechanism of KISN on Insecticidal Activity:

The KISN can bind sulphur containing proteins or phosphorous containing compound to occupy within a cell, most important to destroy the characteristic properties of a protein or other biological macromolecules[32]. Thereafter, the reducing the selectivity permeability of a membrane and interruption in proton motive force required for ATP construction are persuaded which reason themisplaced of cellular biological function and lastly cell death will occur[33].

5. CONCLUSION:

The result of this study is evident of potent larvicidaland anthelmintic activities of KISN obtained from POBA extract. SNP with their unusualpossessions, have varied into in vitro and in vivo biological uses. Presence of various phytoconstituents e.g. phenolic, flavonoids, etc. in the crude materials could be the reason for this pharmacological activities. In the search of safer insecticide, SNP has more selective modes of actions and reduce the risk of non-target organisms as well the environment, the development of plant derived compounds are capable of target insects on inexpensive way. Further attention has to be carried out for isolation and characterization of the active components to establish an effective drug resource scientifically. More targeted approaches and ongoing research into the capabilities and possibilities for silver colloids could yield novel marketable products of use to man-kind.

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