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Biogenic silver nanoparticles mediated stress on developmental period and gut physiology of major lepidopteran pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)—An eco-friendly approach of insect pest control

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ABSTRACT

In the present work, laboratory trial was carried out to determine the cytotoxicity of nanoparticles on *Spodoptera frugiperda*-21cell lines (SF-21) by MTT assay, developmental influence and gut physiology like nutritional index parameters approximate digestibility, efficiency of conversion ingested and digested food, gut enzymes, gut microbiota against major lepidopteran polyphagous pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). Silver nanoparticles synthesized from Pomegranate (*Punica granatum*) peel extract and the various analytical techniques used to characterize the synthesized nanoparticles revealed the uniform, monodispersive, highly stable particles with the size range of 14–28 nm. Nanoparticles at different concentration tested on SF-21cell lines and life stages of *Spodoptera litura* revealed that the nanoparticles were highly effective against all the tested parameters as dose dependent manner by showing effective inhibition of cell viability on SF-21 cell line, larval and pupal mortality, least lethal concentration 50 (LC_{50}), lethal time 50 (LT_{50}), reduced larval, pupal period, reduced adult emergence and adult longevity. Gut physiology study on third instar of *Spodoptera litura* showed distinct reduction effect on all the tested parameters.

1. Introduction

Spodoptera litura (Lepidoptera: Noctuidae) is a chief polyphagous pest, invading more than 180 plant species [1–4]. Nowadays, manmade pesticides have been extensively used for controlling this pest on diverse crops, but adverse side effects of manmade pesticides, comprising the frequency of resistance, have imposed a shift to more eco-friendly attitudes for controlling this pest. In recent years, the use of manmade organic pesticides in crop insect pest management programs around the globe has occasioned in mutilation to the environment, pest resurrection, pest resistance to pesticides and fatal effects on non-target organisms. Due to these sways of chemical pesticides provoked a quest for alternate practices for insect pest management [5–8]. One possible way to lessen the high ingesting of manmade pesticides is through the appliance of biopesticides, commonly contemplated to be environmental and medical safe. Biopesticides cater an alternative to manmade pesticides because of their subdued environmental pollution, low toxicity to humans and other benefits [9]. Plant extracts, secondary metabolites of bacteria, fungi and essential oils and their constituents have been reported to be an operative fount of biopesticides [10,11]. The mounting cognizance of the perils of excessive use of pesticides universally has led researchers to explore for safer and added environment friendly methods for pest control. Research on natural products could be replacements to manmade pesticides [12–14]. The neonate larvae primarily affecting the foliage of the plants and the later stage feeds on evolving seeds in the pod. The selected pest are contemplated as the staid pests of several economically important crops such as tobacco, castor, cabbage, cotton, groundnut, chilly, mustard and tomato as well as some legumes etc., and also they have established resistance in almost all commercially existing chemical pesticides. Furthermore, literatures relating to the control of this pest using biopesticides are sparse. These pests have developed resistance to a variety of pesticides due to the haphazard use of chemical pesticides. Pesticides affect the non-target organisms and

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human beings, indirectly or directly. Biopesticides are operational against an array of agricultural insect pests; they are clearly degradable. This is favourable for both the environment and agriculture product regulars [15].

The practice of nanotechnology in agriculture has shaped a prodigious interest, propounding the potential for notably enhanced agricultural production and efficacy with less cost and litter [16,17]. Prominently, the advent of these applications in agriculture and areas has also risen up safety concerns over environmental and humanoid health; the ensuing field of nanotoxicology has expounded in an effort to riposte critical queries of exposure, hazard and risk [18]. In agriculture, nanotechnology can promote improved pest management and crop fortification through excellent efficiency of pesticides and other agrochemicals such as fertilizers and growth agents [19–21]. Targeted nanoparticles often exhibit novel characteristics such as extraordinary strength, more chemical reactivity, and possessing a high electrical conductivity. Thus, nanotechnology has become one of the most promising new approaches for pest control in the recent years [22]. Nanoparticles represent a new generation of environmental remediation technologies that could provide cost-effective solution to some of the most challenging environmental clean-up problems [23]. Silver has been used in many applications in pure free metal or in compound form because it possesses antimicrobial activity against pathogens, yet it is nontoxic to humans [24,25]. Although there have been numerous studies on the toxicity effects of nanoparticles on bacteria, fungi, and animal pathogens [26–29] little research has carried out to investigate the toxicity effect of nanoparticles on insects. The present investigation is aimed to evaluate pesticidal effect of silver nanoparticles synthesized from peel extract of pomegranate (*Punica granatum*) against major lepidopteran pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) under laboratory conditions by determination of developmental period, cumulative mortality and gut physiology stress. The present study results suggest that the biogenic silver nanoparticles may have potential for use as eco-friendly biopesticide against insect pest associated with economic important crops.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used were of analytical reagent grade. Silver Nitrate ($AgNO_3$) and Grace's TNM-FH insect media were obtained from Sigma-Aldrich. Chemicals used for cytotoxicity and gut enzyme studies such as the substrates used in enzyme studies, Fetal calf serum, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), penicillin, streptomycin, nutrient agar, potato dextrose agar, chloramphenicol were purchased from Himedia, Mumbai. Dimethyl Sulphoxide (DMSO) was obtained (Merck). All solutions were made with Millipore water.

2.2. Collection and processing of plant material

Healthy and matured fruits of Pomegranate (*Punica granatum*) were collected in the fruit supermarket in Chennai, Tamilnadu. The collected fruit material was gently washed with Millipore water to remove the surface dirt and debris. The arils and peel were separated; the separated peel was dried at 50 °C in hot air oven overnight and homogenized in a domestic mixer, transferred to the sterile plastic container for further studies.

2.3. Preparation of peel extract

Extract prepared from dried peel material used as source of nanoparticle synthesis was carried out by dissolving 1.5 g of finely ground dried peel powder in 100 mL of Millipore water and heated for 30 min at 50 °C. The extract was cooled and centrifuged at 10,000*g* for 10 min. The supernatant was collected into a clean screw cap vial.

2.4. Synthesis of silver nanoparticles

30 mL of the collected supernatant was added to 70 mL of 1 mM of silver Nitrate ($AgNO_3$) solution. The solution was allowed to react in room temperature under magnetic stirrer at dark condition. The initial pale yellow colour of reaction eventually turned to brown colour indicating silver nanoparticles synthesis. Stirring was continued for one hour to complete the reaction. The biosynthesized silver nanoparticles solution was centrifuged at 10,000*g* for 10 min and pellet was redispersed in Millipore water, lyophilized. Lyophilized powder was stored in a sterile screw cap vial for further studies.

2.5. Characterization studies

2.5.1. UV–visible spectroscopy

Primary confirmation of synthesized silver nanoparticles was carried by UV–vis spectroscopy to determine surface Plasmon absorbance maxima. UV–vis spectrum analysis was recorded in Shimadzu-1800 spectrophotometer (800–200 nm range). The sample was sonicated prior to measurement for uniform dispersion.

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

Further characterization of silver nanoparticles was done by Fourier Transform Infrared Spectroscopy (FTIR) to detect the changes in functional groups. FTIR spectra were measured using Bruker Optic GmbH Tensor 27. The silver nanoparticles were mixed uniformly with potassium bromide at 1:5 (sample: KBr) ratio, respectively. The KBr discs were prepared by compressing the powders (mixture of sample and KBr) at pressure of 5 ton for 5 min in a hydraulic press. The discs were scanned in the range of 400–4000 cm^{-1}.

2.5.3. Field emission scanning electron microscopy (FESEM) and Energy Dispersive Atomic Spectroscopy (EDAS)

Particle Shape and size morphology was studied by field emission scanning electron microscopy. In a SEM setup, the silver nanoparticles coated to be conductive is scanned in a high vacuum chamber with a focused electron beam using Supra 55- Carl Zeiss (Germany) magnification range of 35–10,000, resolution 200 Å, acceleration voltage 19 kV.

2.5.4. High Resolution Transmission Electron Microscopy (HRTEM)

High transmission electron microscopy (HRTEM) images were obtained by a JEOL 3010 microscope with UHR pole piece and a lattice resolution of 0.14 nm at an accelerating voltage of 120.0 kV. The samples for HRTEM studies were prepared by drying a drop of the aqueous suspension of films on carbon coated copper grid under ambient conditions. Prior to the HRTEM measurements, the sample was ground into small pieces at liquid nitrogen temperature to improve the depth of resolution.

2.5.5. X-ray diffraction (XRD)

X-ray diffraction (XRD) measurement of the silver nanoparticles was carried out using Rigaku Smart lab instrument operated at a voltage of 30 kV and a current of 100 mA with Cu K beta radiations with a scan speed/duration time of 4 °/min.

2.5.6. Atomic force microscopy (AFM)

Surface Topology of silver nanoparticles was visualized with an atomic force microscope (AFM). A thin film of the sample was