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# Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physiology

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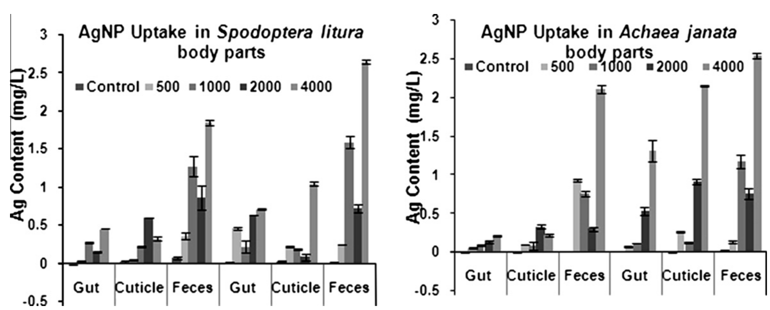
## HIGHLIGHTS

- Silver nanoparticle treatments decreased body weights of lepidopteran larvae.
- Major amounts of silver were eliminated through the feces.
- Localization of silver nanoparticles can be observed in the larval gut organelles.
- Treatments had also affected the activity of detoxifying enzymes.
- Oxidative stress induced in larval guts was countered by enhanced antioxidant enzymes.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Increased use of nanomaterials in various fields of science has lead for the need to study the impact of nanomaterial on the environment in general and on insect and plant life in particular. We studied the impact of silver nanoparticles (AgNPs) on growth and feeding responses of two lepidopteran pests of castor plant (*Ricinus communis* L.) namely Asian armyworm, *Spodoptera litura* F. and castor semilooper, *Achaea janata* L. Larvae were fed with PVP coated-AgNPs treated castor leaf at different concentrations and their activity was compared to that of silver nitrate ($AgNO_3$) treated leaf diets. Larval and pupal body weights decreased along with the decrease in the concentrations of AgNPs and $AgNO_3$ in both the test insects. Low amounts of silver were accumulated in the larval guts, but major portion of it was eliminated through the feces. Ultrastructural studies of insect gut cell using Transmission Electron Microscopy (TEM) showed accumulation of silver nanoparticles in cell organelles. Changes in the antioxidative and detoxifying enzymes of the treated larva were estimated. The effect of treatments showed differences in the activities of detoxifying enzymes, carboxylesterases (CarE), glucosidases (Glu) and glutathione S-transferases (GST) in the larval gut. Activities of superoxide dismutase, catalase, and peroxidase were also altered in the larval bodies due to the AgNPs treatments, suggesting that exposure of larvae to nanoparticles induces oxidative stress, which is countered by antioxidant enzymes. Induction of these enzymes may be an effective detoxification mechanism by which the herbivorous insect defends itself against nanoparticle treatment.



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## 1. Introduction

Nanomaterial usage has increased over years in various fields namely medicine, cosmetics, toys, other consumer products, etc. They have versatile properties such as self-assembly, specificity, encapsulation, stability, and biocompatibility. Among the available nanomaterials, silver nanoparticles (AgNPs) form a major part of the medical and agricultural industry due to their possession of antimicrobial activity. In recent studies, it has been reported that one third of the silver products in present use may pose risk to the environment (Luoma, 2008). Despite their increasing use, there is a lack of information on the possible effects of silver nanoparticles on the environment and ecosystem. Limited studies are reported on toxic effects of silver treatments where genetic damage in human cell lines (Ahamed et al., 2010a), onion plant (Kumari et al., 2009) apart from measuring their effects on several plant species (Lin and Xing, 2007; Yin et al., 2011) and marine organisms (Fabrega et al., 2011). Our previous study on effect of AgNPs treatment on castor seed germination revealed that there was no adverse effect of treatment on seed germination. However, enzymatic changes due to the accumulation of silver were found in the treated seedlings (Jyothsna and Usha Rani, 2013). Studies on ecotoxicological effects of biosynthesized AgNPs on *Daphnia magna* were found to be safer when compared to chemically synthesized AgNPs (Usha Rani and Rajasekharreddy, 2011). It has also been reported that there might be biomagnification of nanoparticles (NPs) in plants and further through plants to other organisms such as insects and humans (Judy et al., 2011, 2012; Ahamed et al., 2010a).

It is interesting to know how these NPs show impact on insect communities through their food chain. The studies on the effects of nanomaterials on insects may provide some basis on bioaccumulation/biomagnifications. These studies also may reflect the effects or risks associated with nanosilver, which may further be useful to foresee possible future effects on humans. Electron microscopical studies on the nanoparticle accumulation in insect gut cells would aid in better insights on their accumulation and localization in the cell organelles. In Drosophila, AuNPs accumulation and changes occurring due to treatments have been shown (Pompa et al., 2011). Recent study with silver nanoparticles of three different sizes on *Drosophila melanogaster* showed that the larval progression was impeded at the tested concentrations. F1 generation of the flies had reduced body pigmentation along with the shortened lifespan and abnormal climbing and cuticular demelanization (Key et al., 2011; Panacek et al., 2011). Earlier reports with regard to the specific toxic effects of AgNPs in *D. melanogaster*, have stated that there was induction of mutations during development with an upregulation of oxidative stress, biomarkers like heat shock proteins, ER stress markers, lipid peroxidation, heme oxygenase, metallothionein, cell death activators like caspases, etc. (Asharani et al., 2009; Ahamed et al., 2010a,b; Roh et al., 2009). The present work was aimed to study the effects of silver nanoparticles on growth and development as well as their localization in certain agricultural pests.

Insects possess capacity to adapt to various forms of stress (biotic and abiotic) and evolve resistance through undergoing changes at physiological, cellular and organismal levels (van Ooik et al., 2007; Janssens et al., 2009). Of these, enzymes involved in neutralizing xenobiotics are carboxylesterases (CarE), glucosidases (Glu) and glutathione S-transferases (GST) (Singh et al., 2001). GSTs are involved in detoxification of xenobiotics (Grant et al., 1991; Vontas et al., 2001), while glucoproteins, CarEs are involved in the metabolism of lipids and fats as reported by Campbell et al. (2003) in case of *Drosophila* larvae. Furthermore, CarEs can also degrade juvenile hormone and its analogs in haemolymph, thus regulating reproduction and diapause of insects (Vermunt et al., 1997). The primarily work on the effects of silver nanoparticles has been carried out mostly on *Drosophila* and reports on other agricultural insects are scanty.

Metal ions in insects are implicated for change in activities of detoxifying enzymes (Kafel et al., 2012) including the antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). But during the process of detoxification, potentially toxic hydrogen peroxide ($H_2O_2$) is generated, where CAT and POD act to remove these peroxides, while SOD dismutates superoxide anions directly (McCord and Fridovich, 1969). Reactive oxygen species (ROS) are highly reactive molecules due to the presence of unpaired valence shell electrons that indiscriminately interact with essential macromolecules, such as DNA, proteins and lipids specially those in cell membrane leading to the disturbances of physiological processes (Cnubben et al., 2001). No published data is available on the effects of AgNPs or silver nitrate which is a bulk form of silver (or other ionic forms) on defense system of lepidopteran insects.

The current study is therefore, aimed at investigating the direct influence of nanosilver feeding on the growth and development of two important lepidopteran insects, castor semilooper, *Achaea janata* L. and asian armyworm, *Spodoptera litura* F. We assume that antioxidative and detoxifying enzymes are key biomarkers in assessing tissue injury due to silver nanoparticles and silver nitrate treatments in insects. Studies were also carried out to investigate whether the nanoparticle accumulation and localization of the AgNPs occurs in the larval gut cells of the lepidopteran insects. We investigated the hypothesis that supplementing of silver suspensions to lepidopteran diet produces stress in the larval midgut tissue. This stress leads to crippled antioxidative defense systems and metabolic dysfunction. Hence to know the possible effects and risks associated with silver nanoparticles exposure, we have evaluated its effects on detoxification system in larval gut and also altered growth and metabolism in these two insects after feeding.

## 2. Material and methods

### 2.1. Nanomaterial

Silver nanoparticles (AgNPs) powder stabilized with polyvinyl pyrrolidone (PVP) (size < 100 nm) were obtained from Sigma Aldrich (Sigma Aldrich Inc., USA). Silver nitrate ($AgNO_3$) was obtained from Sisco Research Laboratory, Hyderabad. Suspensions of AgNPs and $AgNO_3$ were prepared using Millipore water and sonicated for 30 min to avoid aggregation. Silver nitrate being a precursor for preparation of silver nanoparticles, the same level of concentrations was used in the study for comparison. Different techniques were used to characterize various properties of the silver nanoparticles. Preliminary feeding experiments were done using 100 and 200 mg $L^{-1}$ concentrations against both the test insect species. Since no effects were observed at these levels further higher concentrations of AgNPs and $AgNO_3$, viz. 500, 1000, 2000 and 4000 mg $L^{-1}$ were used in the main experiments to ascertain the maximum effective concentration.

### 2.2. Nanoparticles characterization

Physico-chemical properties of silver NPs tested were characterized in Millipore water. Particle size and zeta potential were determined with the aid of a Malvern Instruments Zetasizer Nano Series, in order to confirm the manufacturers' specifications of the silver NPs. Approximately 1 mL of the sample suspension of NPs in Milli-Q water was taken in polystyrol/polystyrene cuvettes and