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Green synthesis of silver nanoparticles using *Euphorbia hirta* (Euphorbiaceae) leaf extract against crop pest of cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT

Biosynthesis of insecticides from plant extracts is currently under exploitation. Plant extracts are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of synthetic and other chemical insecticides. So the present study was carried out to establish the larvicidal effect of synthesized silver nanoparticles (AgNPs) using leaf extract of *Euphorbia hirta* (Euphorbiaceae) against the first to fourth instar larvae and pupae of the crop pest of cotton boll worm , *Helicoverpa armigera*. The production of the AgNPs synthesized using leaf extract of *Euphorbia hirta* was evaluated through a UV–Vis spectrophotometer in a wavelength range of 200 to 700 nm. An SEM micrograph showed 30-60-nm-size aggregates of spherical- and cubic-shaped nanoparticles. EDX showed the complete chemical composition of the synthesized nanoparticles of silver. The results showed that the considerable larval mortality was found in the synthesized AgNPs against the I instar to IV instar larvae and pupae of *Helicoverpa armigera*. The leaf extract exhibited larval toxicity against the I instar to IV instar larvae and pupae of *Helicoverpa armigera*. The chi-square value was significant at $p<0.05$ level. Nanoparticle treatment showed toxicity against larval instars of *Helicoverpa armigera* and had impact on the biological parameters of *Helicoverpa armigera*. Treated larva and pupa showed extended their durations. Similarly, longevity of male and female and fecundity also reduced. Treated insects had less consumption index at growth. Decreased food utilization efficiency measures (ECI and ECD) and concomitant decreased in the level of digestive enzyme profiles in the midgut after the treatment indicate that nanoparticle administration affected gut physiology of insects.

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Key words: *Helicoverpa armigera*, Silver nano particles, biology, feeding, food utilization, *Euphorbia hirta* and cotton boll worm.

INTRODUCTION

Indian economy is largely determined by agricultural productivity. India is basically an agro-based country; more than 80% of Indian population depends on agriculture. Insect-pests are known to cause significant damage to crops and affect agricultural productivity. The environmental hazards posed by synthetic pesticides provide an impetus for investigations into some ecofriendly and biorational alternatives (Subashini *et al.,* 2004).

Nanoparticles play an important role in pharmaceutical, industrial, and biotechnological applications. In particular, silver nanoparticles are proved to have potential antibacterial, antifungal, and antiplasmodial and larvicidal properties (Saxena *et al*., 2010; Elumalai *et al*., 2010). But the synthesis of nanoparticles using chemical and physical methods requires high pressure, energy, temperature, and toxic chemicals. In this regard, plants and plant part extract-based biosynthesis has been found to be cost-effective and environment-friendly (Casida and Quistad, 2005). With a decrease in the size, nanoparticles have a higher surface area-to-volume ratio, and specific surface area is relevant for catalytic activity and other related bioactive properties such as antimicrobial (Fayaz *et al*., 2010; Wen-Ru *et al.*, 2010), antifungal (Ales Pana *et al.,* 2009), antiviral (James *et al.*, 2008), anti HIV (Lara *et al.,* 2010), and very recently, mosquito larvicidal (Sap-Iam *et al.,* 2010) activity of AgNPs. Silver nanoparticles are nanoparticles of silver, i.e. silver particles of

between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms (Lu *et al.*, 2011).

Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods (Mohanpuria *et al*., 2008). Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis (Shankar *et al.*, 2004). Synthesis of nanoparticles using microorganisms or plants can potentially eliminate this problem by making the nanoparticles more biocompatible.

Plants and microbes are currently used for nanoparticle synthesis. The use of plants for synthesize of nanoparticles are rapid, low cost, eco-friendly, and a single-step method for biosynthesis process (Huang *et al*., 2007). Silver nanoparticles (AgNPs) may be released into the environment from discharges at the point of production, from erosion of engineered materials in household products (antibacterial coatings and silver-impregnated water filters), and from washing or disposal of silver-containing products (Benn and Westerhoff, 2008).

Euphorbia hirta (Euphorbiaceae) a small annual herb common to tropical countries (Soforowa, 1982). It can grow to a height of 40 cm. The stem is slender and often reddish in colour, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lanceolate, and are usually greenish or reddish underneath, measuring about 5 cm long. In the axils appear very small dense round clusters of flowers (Fig.1). The small green flowers constitute the inflorescence characteristic of the euphobias. The stem and leaves produce white or milky juice when cut (Lind and Tallantire, 1971). The aerial parts of the plant are qualitatively well investigated for presence of diterpenoids, triterpenoids, flavonoids, phenolics, tannins, carbohydrates, hydrocarbons (Chen 1991; Mallavadhani and Narasimhany, 2009), and of scopoletin, scoparone, isoscopoletin, quercetin, isorhamnetin, pinocembrin, kaempferol, luteolin and gallic acid (Yi *et al*., 2012) etc. from this species.

E. hirta has been studied by various workers and a number of active constituents have been isolated. Afzelin (I), quercitrin (II), and myricitrin (III) have been isolated from the methanolic extract of *E. hirta*. (Liu, 2007). The chemical investigation of *E. hirta* has led to the isolation of rutin (IV), quercitin (V), euphorbin-A (VI), euphorbin-B (VII), euphorbin-C (VIII), euphorbin-D (IX), 2,4,6-tri-*O*-galloyl-β-D-glucose, 1,3,4,6-tetra-*O*-galloyl-β-D-glucose, kaempferol, gallic acid, and protocatechuic acid.(Rastogi RP, 2002a,b) *E. hirta* also contains β-amyrin, 24-methylenecycloartenol, β-sitosterol, heptacosane, *n*nonacosane,(Williamson, 2002) shikmic acid, tinyatoxin, choline, camphol, and quercitol derivatives containing rhamnose and chtolphenolic acid (Ogbulie *et al*., 2007).

In central and north India, it is the major pest affecting cotton. *H. armigera* has a long history of resistance to conventional insecticides. Variety of chemical insecticides and pesticides are used to control *H. armigera*. However, harmful effects and persistent nature of the chemical pesticides demand for eco-friendly alternatives. Economic loss due to this pest in India accounts for 5,000 cores (Manjunath *et al.*, 1985). The cotton bollworm, or corn earworm, *Helicoverpa armigera*, is a moth, the larvae of which feed on a wide range of plants, including many important cultivated crops. It is a major pest in cotton and one of the most polyphagous and cosmopolitan pest species. The cotton bollworm is very variable in both size and colour. The body length varies between twelve and twenty milimetres with a wingspan of thirty to forty milimetres. The forewings are yellowish to orange in females and greenish-gray in males, with a slightly darker transversal band in the distal third. The external transversal and submarginal lines and

the reniform spot are diffused. The hind wings are a pale yellow with a narrow brown band at the external edge and a dark round spot in the middle (Waring, 2003). Hence, in the present study, we report that the synthesis of AgNPs, reducing the silver ions present in the solution of silver nitrate by the cell-free aqueous leaf extract of *Euphorbia hirta* and tested against toxicity against the biology and feeding of crop pest of cotton boll worm, *Helicoverpa armigera*.

MATERIALS AND METHODS

Collection of plant and preparation of extract

The leaves of *Euphorbia hirta* were collected from in and around Coimbatore, Tamil Nadu, India and leaves were washed with tap water and shade dried at room temperature (28±2 °C) for 5 to 7 days. The air-dried plant materials (leaves) were powdered by an electrical blender. From the leaf, 1 kg powdered was macerated with 3.0 L of methanol sequentially for a period of 72 h and filtered. The yield of the *Euphorbia hirta* leaves crude extract was produced by methanol (21.5 g). The extracts were concentrated at reduced temperature on a rotary vacuum evaporator and stored at a temperature of 4 °C. One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1 % stock solution. From this stock solution, concentrations were prepared ranging from 100, 150, 200, 250, and 300 ppm, respectively. AgNO3 was purchased from the Precision Scientific Company, Pvt Ltd., Coimbatore, Tamil Nadu.

Culture of *Helicoverpa armigera*

Helicoverpa armigera larvae were collected from the Central Institute of Cotton Research, Indian Council of Agricultural Research, Govt. of India, Coimbatore, India and were cultured in the laboratory and fed with *Gossypium hirsutum* leaves *ad libitum* at 27±1°C; 10: 14 L.D: 75% Rh. Pre-pupa of *Helicoverpa armigera* were separated and provided with vermiculate clay which is a good medium for pupation. Pupae of *Helicoverpa armigera* were kept on cotton in Petri dishes inside an adult emergence cage. The emerging moths were fed with 10% sucrose solution fortified with a few drops of vitamin mixture (MULTDEC drops) to enhance oviposition. Moths in the ratio of one male to two females were allowed inside oviposition cages containing the adult food mentioned above. The egg cage of *Helicoverpa armigera* was covered with white muslin cloth for egg laying. The egg clothes were removed daily and surface sterilized using 10% formaldehyde solution to prevent virus infection. The egg clothes were moistened and kept in a plastic container for the eggs to hatch. This process facilitated un-interrupted supply of test insects.

Treatments

The fresh *Gossypium hirsutum* leaves were coated with different concentrations of silver nanoparticle solution and air dried. The control leaves were treated with water alone. The 3 hr starved different larval instars were individually fed with different concentrations of silver nanoparticle treated and untreated leaves. Every 24 hr, the uneaten leaves were removed and placed with fresh treated and untreated leaves. A minimum of 20 larvae/ concentration were used for all the experiments and replicated for 5 times.

Quantitative Food Utilization Measures

A gravimetric technique was used to determine weight gain, food consumption and faces produced. All weights were measured using a monopam balance accurately to 0.1 mg. The newly molted I, II, III, IV, V and VI instar larvae were starved for 3 hr. After measuring the initial weight of the larvae, they were individually introduced into separate containers. The larvae (20 larvae / concentration, five replicates) were allowed to feed on weighed quantities of treated and untreated *Gossypium hirsutum* leaves, for a period of 24hr. Larvae were again weighed. The difference in weight of the larvae gives the fresh weight gained during the period of study. Sample caterpillars were weighed, oven dried (48 hrs at 60^0C) reweighed to establish a percentage dry weight of the experimental caterpillars. The leaves remained all the end of each day were oven dried and re-weighed to establish a percentage dry weight of the diet (dry weight) remaining at the end of each experiment from the total dry weight of diet provided. Faces were collected daily and weighed, oven dried and reweighed to estimate the dry weight of excreta.

The experiment was continued for four days and observations were recorded for every 24hr. Consumption, growth rates and post digestive food utilization efficiencies (all based on dry weight) were calculated in the traditional manner (Waldbauer, 1968; Slansky, 1985; Slansky and Scriber, 1985; Murugan and Ancy George, 1992)

Consumption index (CI) = E/TA

Relative growth rate (RGR) = P/TA

Approximate digestibility (AD) = 100 (E-F)

Efficiency of conversion of ingested food (ECI)

= 100 P/E

Efficiency of conversion of digested food (ECD)

= 100 P/ (E-F)

Where,

A = mean dry weight of animal during

E = dry weight of food eaten

F = Dry weight f food eaten

P = dry weight gain of in insect

T= duration of experimental period

Larval Growth and Mortality Bioassay

The *Gossypium hirsutum* leaves were treated with different concentration of synthesized AgNPs using *Euphorbia hirta.* Control leaves were treated with water alone. The leaves were allowed to dry at room temperature for 10 min and then placed on a wet filter paper disc in 10 cm diameter plastic Petri dishes. The experiments were carried out with newly molted, 3 hr starved I,II, III, IV, V and VI instar larvae and pupae using one larva Petri dish in five replicates (20 larvae / concentration). After 4 days, the larvae were transferred to fresh untreated *Gossypium hirsutum* leaves and maintained until the molted into adults or died. Total numbers of normal adult that survive were noted. The larvae were observed for mortality and morphological changes associated with growth disrupting effects. The per cent mortality data after corrections were subjected to profit analysis for calculating mean lethal concentration (LC_{50}) and (LC_{90}) (Finney, 1971). Results were corrected for control mortality by using Abbott's (1925) formula.

$$Pt = Po - Pc/ 100 – Pc \times 100$$

Where,

Pt = Corrected per cent mortality

Po = Observed mortality

Pc = Observed mortality in control

From the mean lethal concentration (LC_{50}) the physiological doses were selected for biological, nutritional and biochemical studies. In another experiment, mortality of IV instar larva was recorded in hours after the treatment of *Euphorbia hirta* methanolic leaf extract and synthesized AgNPs using *Euphorbia hirta* at different concentrations.

Mortality Rate Measurement

Mortality rates (MR) were measured (according to Eslin and Pre´ vost, 1998) on groups of 5–20 larvae of *Helicoverpa armigera*. Series of 20 controls, *Helicoverpa armigera* larvae were allowed to complete their development under the conditions of light and temperature MR were estimated as follows:

$$MR\ (\%) = \frac{\text{Number of dead larvae}}{\text{Initial number of larvae}} \times 100$$

Food consumption and faecal pellet weight measures

The newly emerged third, fourth and fifth instars were allowed to feed on *Euphorbia hirta* methanolic leaf extract and synthesized AgNPs using *Euphorbia hirta* treated and untreated *Gossypium hirsutum* leaves for three days. The amount of food consumed and weight of fecal pellet produced were recorded and was converted into percentage.

Rate of development of *Helicoverpa armigera*

The rate of development from I instar larvae, fed with different concentration of *Euphorbia hirta* methanolic leaf extract and synthesized AgNPs using *Euphorbia hirta* treated and untreated leaves were studied based on the total body weight and duration of post embryonic development. For assessing the duration of post – embryonic development, freshly laid eggs were separated and observations were made based on their incubation period and duration of each larval stages and adult longevity of *S. litura* after treatment.

Synthesis of silver nanoparticles

Leaves were washed with distilled water and dried for 2 days at room temperature. A plant leaf broth was prepared by placing 10 g of the leaves (finely

cut) in a 300-ml flask with 100 ml of sterile-distilled water. This mixture was boiled for 5 min, decanted, stored at−4 °C, and used in our tests within 1 week. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature. As a result, a brown–yellow solution indicating the formation of AgNPs was found and that aqueous silver ions can be reduced by aqueous extract of plant parts to generate extremely stable silver nanoparticles in water (Fig. 1).

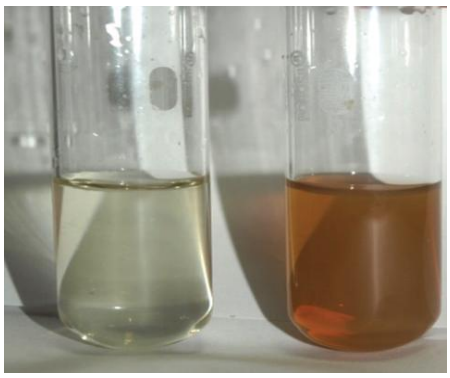

Figure 1. Colour change in reaction mixture (silver nitrate + *Euphorbia hirta* leaves extract) (a) at 0 hours; (b) after 2 hours.

Characterization of silver nanoparticles

Synthesized silver nanoparticles were confirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV–Vis spectra, at the wavelength of 200–700 nm in UV-3600 Shimadzu spectrophotometer at 1-nm resolution. Further, the reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min, and the resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45µm). An aliquot of this filtrate containing silver nanoparticles was used for scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), and Fourier transform infrared (FTIR) studies. The structure and composition of freeze-dried purified silver particles were analyzed by using a 10-kV ultra-high resolution scanning electron microscope with 25 µl of sample sputter coated on copper stub and the images of nanoparticles were studied using FEI QUANTA-200 SEM. The surface groups of the nanoparticles were qualitatively confirmed by using FTIR spectroscopy (Stuart, 2002), with spectra recorded by a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. In addition, the presence of metals in the sample was analyzed by EDS.

Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's multiple range tests by Alder and Rossler (1977). The average larval and pupal mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95 % fiducial limits of upper fiducial limit and lower fiducial limit, and chi-square values were calculated using the SPSS Statistical software package 16.0 version. Results with $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The results of larvicidal and pupicidal activity of *E. hirta* leaf extract at various concentrations against *H. armigera* were noted and presented in Table 1. The graded concentrations at various 20ppm, 40ppm, 60 ppm, 80 ppm and 100 ppm of *Euphorbia hirta* leaf extract were treated against first to six instars larvae and pupae of *H.armigera.* Considerable mortality was evident after the treatment of *E. hirta* for all larval instars and pupae. *Helicoverpa armigera* mortality was increased as the concentration increased; for example, in first instar stage at 20ppm concentration, the larval mortality was 45%, whereas at 100ppm concentration, it was increased to 94%. In pupal mortality at 20 ppm concentration, it was increased to 38%, and it increased to 90% at 100ppm. A similar trend has been noted in *Helicoverpa armigera* mortality which was increased as the concentration increased; The LC_{50} values of instar larvae and pupae of 33.383, 34.056, 37.843, 42.475, 55.473, 70.805 and 44.890 ppm and LC_{90} values of 97.146, 86.572, 96.278, 103.166, 123.487, 145.734 and 115.781 ppm respectively (Table 1). *Helicoverpa armigera* had LC_{50} values of and LC_{90} values of and ppm. The 95 % confidence limits LC_{50} (LCL–UCL) and LC_{90} (LCL–UCL) were also calculated.

Table 2 provides the effect of AgNPs at different concentrations (2ppm, 4ppm, 6ppm, 8ppm and

Table 1. Larvicidal and Pupicidal activity of *Euphorbia hirta* at various concentrations against *Helicoverpa armigera.*

Larval and Pupal stage	% Larval and Pupal mortality Concentration of AgNPs (ppm)					LC_{50} and LC_{90}	Regression equation	95% confidence Limit		Chi-square value
	20	40	60	80	100			LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	
I instar	45.0 ±1.6[a]	52.0±2.9[a]	64.0±4.9[a]	83.0±4.9[a]	94.0±4.9[a]	33.383 (97.146)	Y=-4.787 +8.587=X	24.365 (87.380)	40.164 (111.685)	4.827
II instar	40.0±2.9	55.0±3.0	67.0±1.6	89.0±3.4	96.0±1.6	34.056 (86.572)	Y=-5.742 +9.700=X	26.883 (79.003)	39.737 (97.122)	3.584
III instar	35.0 ±2.2	50.0±1.6	70.0±2.7	84.0±1.6	90.0± 2.9	37.843 (96.278)	Y=-5.877 +9.387=X	30.381 (87.416)	43.805 (108.967)	0.675
IV instar	30.0±1.6	44.0±1.6	73.0±2.7	80.0±3.0	85.0±3.5	42.475 (103.166)	Y=- 6.383 +9.349=X	22.682 (84.940)	54.927 (146.852)	5.508
V instar	25.0±2.7	37.0±1.6	55.0±1.6	70.0±2.9	78.0±1.6	55.473 (123.487)	Y=-7.414 +8.712=X	48.913 (110.429)	61.636 (143.399)	0.657
VI instar	20.0±2.2	30.0±2.7	40.0±2.2	58.0±1.6	67.0± 3.0	70.805 (145.734)	Y=-7.145 +5.705=X	62.475 (120.920)	84.141 (195.612)	0.453
Pupa	38.0±3.0	44.0±2.2	56.0±3.0	69.0±1.6	90.0±2.0	44.890 (115.781)	Y=-5.873 +8.237=X	18.702 (91.269)	60.149 (194.133)	6.301

Within a column means followed by the same letter(s) are not significantly different at 5 % level by DMRT. Each value is the mean ± SD of five replicates LFL lower fiducidal limit, UFL upper fiducidal limit, x 2 chi-square value, df degrees of freedom *p<0.0(significant at this level).

Table 2. Larvicidal and pupicidal activity of synthesized AgNPs using *Euphorbia hirta* at various concentrations against *Helicoverpa armigera.*

Larva and Pupal stage	% larval and pupal mortality Concentration of AgNPs (ppm)					LC_{50} and LC_{90}	Regression equation	95% confidence Limit		Chi-square value
	2	4	6	8	10			LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	
I instar	49.0 ±2.9[a]	55.0±1.6[a]	66.0±1.6[a]	85.0±1.6[a]	95.0±3.0[a]	2.905 (9.402)	Y=- 4.073 +8.295=X	3.473 (7.556)	4.387 (14.496)	5.779
II instar	47.0± 2.9	53.0±1.6	63.0±4.6	81.0±4.6	93.0±4.9	3.191 (10.171)	Y=-4.220 +8.013=X	2.156 (9.068)	3.940 (11.875)	5.130
III instar	45.0±1.6	50.0±2.9	60.0±3.0	77.0±1.6	90.0±2.0	3.558 (11.096)	Y=-4.407 +7.672=X	2.515 (9.811)	4.320 (13.152)	4.416
IV instar	42.0±1.6	49.0±2.2	57.0±3.0	73.0±3.5	89.0±1.6	3.948 (11.621)	Y=-4.812 +7.638=X	2.969 (10.250)	4.687 (13.835)	4.650
V instar	40.0±1.6	44.0±1.6	54.0±1.6	70.0±1.6	84.0±4.9	4.485 (12.748)	Y=-5.109 +7.276=X	3.531 (11.126)	5.238 (15.462)	3.476
VI instar	37.0±1.6	40.0±1.6	50.0±3.5	69.0±1.6	80.0±3.0	5.054 (13.407)	Y=-5.684 +7.273=X	4.186 (11.671)	5.797 (16.326)	3.476
Pupa	48.0±2.7	53.0±1.6	62.0±4.6	82.0±4.9	90.0±2.0	3.086 (10.658)	Y=-3.795 +7.541=X	1.919 (9.421)	3.902 (12.642)	4.507

Within a column means followed by the same letter(s) are not significantly different at 5 % level by DMRT. Each value is the mean ± SD of five replicates LFL lower fiducidal limit, UFL upper fiducidal limit ,x 2 chi-square value ,df degrees of freedom *p<0.05 (significant at this level) 4.830 (14.456)

Table 3. Food Utilization measures of VI instar larvae of *Helicoverpa armigera* after the treatment of *Euphorbia hirta*

Treatment (ppm)		CI (mg mg day)	RGR (mg mg̅ day̅1)	ECI (%)	ECD (%)	AD (%)
Control		21.00±1.18	4.98±0.23	22.02±2.18	35.01±3.40	62.70±5.85
Euphorbia hirta	20	19.45±1.16	4.86±0.19	27.68±1.00	30.76±2.78	58.50±5.80
	40	17.65 ±1.12	4.35±0.13	25.87±0.95	24.45±2.55	52.45±5.30
	60	15.00±1.00	3.15±0.11	20.46±0.77	18.66±1.65	46.40±4.15
	80	12.80±0.92	2.10±0.09	14.87±0.62	12.28±0.89	40.67±3.95
	100	10.55±0.87	2.00±0.02	09.22±0.38	10.20±0.72	35.48±3.55

10ppm) on the mortality and toxicity of different larval instars and pupae of *H.armigera*. Considerable mortality was evident after the treatment of silver nanoparticles. In *H. armigera*, the lowest mortality recorded after treating with synthesized of AgNPs was 37.0 % at 2 ppm on the VI instars and the highest mortality was 95 % at 10 ppm on first instars. Similar trend has been noted for all the instars and pupae of *H. armigera* at various concentrations of treatment. AgNPs against the crop pest of *H. armigera* first to six instar larvae and pupae against had the following LC_{50} and LC_{90} values: *H. armigera* had LC_{50} values of 2.905, 3.191, 3.558, 3.948 and 4.485ppm, and LC_{90} values of 9.402, 10.171, 11.096, 11.621, 12,748 and 13.407ppm, respectively. In our study, the toxic effect was studied on larvae instar and pupae after 24 h exposure. The results of larvicidal activity clearly indicate that the percentage of mortality being directly proportional to the concentration of the extract. This proves that concentration plays important role in larvicidal activity. Each test included a control group with five replicates for each individual concentration. All the tested components that showed lethal effect and mortality were positively dose-dependent.

Food Utilization measures of VI instar larvae of *H. armigera* after the treatment of *E. hirta* is given in Table 3. Consumption index of larvae of *H. armigera* did not reduced by *E. hirta* even in higher concentration 100ppm tested (as does not deter the insect). The initial leaf disc choice bioassay assessed show how effectively the synthesized AgNPs using *E. hirta* deterred the feeding of the insect. The Consumption index of synthesized AgNPs using *E. hirta* has reduced significantly, which was dose dependent. At 20ppm concentration, the synthesized AgNPs using *E. hirta*, it shows highly reduced Consumption Index 19.45 (mg mg day), when compared to the control 21.00 (mg mg day). More falls on the consumption index was shown by the synthesized AgNPs using *E. hirta* at 20ppm to 100 ppm. The relative growth rate (RGR) of control was 4.98 mg mg day. The relative growth rate of *H. armigera* had fallen significantly in methanolic leaf extract of *E. hirta* treatment at all concentrations 40ppm to 100 ppm tested. At 100 ppm concentration the RGR was 2.00 mg mg day. *H. armigera* treated with synthesized AgNPs using *E. hirta* also shows fall in RGR 4.86 to 2.00 mg mg day. Significantly reduced regulative growth rate ($P<0.05$).

ECI and ECD values of control were 22.02%, 35.01% respectively. In the treatment with methanolic leaf extract of *E. hirta* the ECI and ECD values decreased with the increase in dose, which shows the toxic nature of the tested *E. hirta.* At 100 ppm concentration of methanolic leaf extract of *E. hirta* the ECI and ECD values were 9.22%, 10.20%. *E. hirta* shows the increased ECI and ECD values, 27.68%, and 30.76% with the increase in dose. The ECI and ECD values had extended to 25.87%, 20.46%, 14.87% to 24.45%, 18.66%, and 12.28% at the highest concentration tested. The AD has also been affected by these treatments and was dose dependent. Approximate digestibility of control was 62.70%. In case of methanolic leaf extract of *Euphorbia hirta*, at lower concentration tested the AD was slightly affected, but reduced significantly when the concentration was increased. But in case of synthesized AgNPs using *E. hirta*, AD values were more the *E. hirta*. The AD values increases with the concentrations because of the less food intake. Biological parameters of *H. armigera.*

Table 4. Pupal duration and adult longevity of *H. armigera* after the treatment of synthesized AgNPs using *E. hirta*

Treatments (ppm)		Pupal duration (Days)	Adult longevity (Days)	
			Male	Female
Control		11.5±0.45	9.0±0.30	9.5±0.35
AgNPs	2	12.7±0.51	6.0±0.25	7.0±0.26
	4	13.5±0.58	4.7±0.18	6.5±0.22
	6	15.8±0.65	3.5±0.14	6.0±0.19
	8	17.3±0.69	3.0±0.12	4.5±0.15
	10	19.0±0.75	2.3±0.10	3.7±0.13

Significant effect was shown on larval duration, weight of the larva and pupae, as well as longevity, fertility shown by both insecticides tested, although these effects were dose dependent.

Pupal duration and adult longevity of *H. armigera* after the treatment of synthesized AgNPs using leaf extract of *E. hirta* is given in Table 4. Pupal duration was 11.5 days for the control. The synthesized AgNPs leaf extract of *Euphorbia hirta* extended the pupal duration at the higher concentration tested. It has been extended to 19.0 days in the synthesized AgNPs leaf extract of *E. hirta* treatment at 10 ppm. When the *H. armigera* was treated with the synthesized AgNPs using *Euphorbia hirta* at 2 to 10 ppm, the pupal duration was 11.5 days. Pupal duration has highly extended to 19.0 days. When the control male and female insect lives for 9.0 days, 9.5 days respectively, the *H. armigera* treated with synthesized AgNPs using *E. hirta* (2 to 10 ppm) lives for 3.8 days, 4.8 days respectively. The longevity reduces significantly to 2.3 days (male), 3.7 days (female) in the treatment with synthesized AgNPs using *E. hirta* at 2 to 10 ppm. Adult longevity has been highly affected by the synthesized AgNPs using *E. hirta*.

Pupal weight, adult emergence, fecundity and egg hatchability of *H. armigera* after the treatment of synthesized AgNPs using *E. hirta* is given in Table - 5. When the *H. armigera* was fed with control leaf, average pupal weight was 440.6 mg, where as synthesized AgNPs using leaf extract of *E. hirta* at 10 ppm it was 247.5mg. The pupal weight reduces to 396.5mg, in the treatment with synthesized AgNPs using *E. hirta* at 10ppm. Adult emergence for control was 97.0%. More reduction in adult emergence has been reduced in the highest concentrations tested in the study. Emergence was 76.8 % and 30.5% in the treatment with synthesized AgNPs using *E. hirta* at 2 to 10ppm respectively. Adult emergence has been highly affected by the treatment of synthesized AgNPs using *E. hirta* 77.6% to 15.9% decreased. Fecundity and fertility were affected when *H. armigera* treated with synthesized AgNPs using *E. hirta.* This effect was dose – related due to both reproduction parameters decreased progressively as the concentration of each insecticide increased. Fecundity was 1685.5 eggs in the control, has reduced to 1327.3 eggs and 589.3 eggs when treated with synthesized AgNPs using *E. hirta* 1074.2 eggs to 579.2 eggs decreased fecundity respectively with respect to the fertility. There was a marked reduction in the percentages of eggs hatch from females treated with synthesized AgNPs using *E. hirta* tested. Formation of AgNPs by reduction of silver nitrate during exposure to *E. hirta* leaf extract can be easily monitored from the change in colour of the reaction mixture. Silver nanoparticles bear a characteristic yellow brown colour due to the excitation of surface Plasmon vibrations. The change in colour of the reaction mixture after 2 hrs is presented in Figure 1, which indicated the formation of AgNPs. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. *E. hirta* group of plants has been a subject of intense photochemical examination and isolated compounds which include: Flavanoids, triterpenoids, alkanes, amino acids, and alkaloids. All species of Euphorbia exudes a milky juice when broken, which is more or less poisonous and used as an ingredient in arrow poisons.

Table 5. Pupal weight, adult emergence, fecundity and egg hatchability of *Helicoverpa armigera* after the treatment of synthesized AgNPs using *Euphorbia hirta*

Treatments (ppm)		Average pupal Weight (mg)	Adult emergence (%)	Fecundity (No.)	Egg Hatchability (%)
Control		440.6±17.5	97.0±4.0	1685.5±71.5	90.4.±3.5
AgNPs	2	396.5±15.0	76.8±2.9	1327.3±59.5	78.5±2.8
	4	364.5±14.2	65.2±2.2	1025.5±45.2	67.8±2.0
	6	311.4±11.5	47.0±2.0	955.5±33.5	50.2±1.5
	8	270.1±8.5	30.5±1.7	797.8±28.8	41.7±1.0
	10	247.5±6.5	16.2±1.2	589.3±22.0	30.3±0.5

E. hirta possesses antibacterial, anti-inflammatory, Galactogenic, anthelmintic, antiasthmatic, sedative, antidiarrheal, anticancer, antioxidant, antiamoebic, antispasmodic, antifertility, antifungal, and antimalarial properties (Williamson, 2007).

Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) is a very destructive polyphagous pest occurring on cotton, tomato, bhendi, chickpea, pigeon pea, chili, maize, sorghum and many other crops, inflicting substantial crop losses every year (Reed and Pawar 1982; Manjunath *et al*.1989; Sharma 2001). *H. armigera* is also characterized by its high mobility and fecundity and it has shown great capacity to develop resistance to synthetic insecticides used in its management (Armes *et al*., 1996; Kranthi 1997; Ramasubramaniam and Regupathy, 2004). The larvicidal and pupicidal activity of synthesized silver nanoparticles using an aqueous leaf extract of *Tinospora cordifolia* showed maximum mortality against the head louse *Pediculus humanus* and fourth instar larvae of *Anopheles subpictus* and *Culex-quinque fasciatus* (Jayaseelan *et al.,* 2011).

Similarly, larvicidal and pupicidal activity of methanol extract of *E. hirta* at various concentrations against the malarial vector *A. stephensi*. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 121.51 ppm; II instar was 145.40 ppm; III instar, 169.11 ppm; and IV instar, 197.40 ppm, respectively. LC_{90} value of I instar was 236.44 ppm; II instar, 293.75 ppm; III instar, 331.42 ppm; and IV instar, 371.34 ppm, respectively; and the LC_{50} and LC_{90} values of pupae were 219.15 and 396.70 ppm, respectively (Agalya Priyadarshini *et al*., 2012).

A recent study which focused on the synthesized silver nanoparticle using *N. oleander* leaf aqueous extract was observed. The larval and pupal mortality of *Anopheles stephensi* first to fourth instars after the treatment with *N. Oleander* leaf extract at different concentrations (10 to 50 ppm) The LC_{50} value of the I instar was 20.60 ppm, II instar 24.90 ppm, III instar 28.22 ppm, and that of the IV instar was 33.99 ppm. The LC_{90} value of the I instar was 41.62 ppm, II instar 50.33 ppm, III instar 57.78 ppm, and that of the IV instar was 68.41 ppm. The LC_{50} and LC_{90} values of pupae were 39.55 and 79.10 ppm, respectively (Roni *et al*., 2013).

These results on weight gain, CI, AD, ECI and ECD f of *Helicoverpa armigera* more or less similar with the report of Ramdev and Roa (1979) and Suganthi and Nagapasupathi (2005) on *Achaea janata*, Dhandapani and Balasubramanian (1980) on *Helicoverpa armigera*, Soo and Fraenkel (1996) on *Prodenia eridania*. These findings will help to understand the food utilization and biology of the particular pest and could help in its management, particularly on castor, tomato and cotton.

A recent study which focused on the food consumption and utilization indices of tobacco caterpillar *Spodoptera litura* larvae was observed. Food consumption and conservation of digested and indigested castor leaves by *S. litura* larvae varied among the larval ages from different day of treatment. The consumption index indicates the different days of 3,4,5,6 and 15and larvae of 47.00, 14.66, 7.16 and 1.7. Approximate digestibility varied with the age of the larvae that ranged from 23.16 to 30.33. A maximum ECI of 19.1 was noticed on the 10^{th} day as against 7.4% observed on the 15^{th} day of hatching. ECD of 6.8% was noticed on the 12^{th} day as against 2.7% in the 15^{th} day of hatching. The weight gained by the larvae 0.34g in the V-instar was 0.009 significantly higher than the weight gained, in the I-instar larvae. The maximum faeces of 0.12g were recorded by 12^{th} day larva as against a

minimum of 0.001 recorded by 2nd day larva. (Krishnaveni *et al*., 2013).

In the present observation, green synthesis shows that the environmentally benign and renewable source of *E. hirta* is used as an effective reducing agent for the synthesis of AgNPs. The synthesis of AgNPs, reducing the silver ions present in the solution of silver nitrate by the cell free leaf extract of *E. hirta*. Nanotechnology in agriculture plays a very important role in the slow release effects which includes pest control with increased shelf-life to various applications in the agricultural fields. The importance of nanoparticles, with respect to the life cycle of the pest controlling it biologically. Biologically synthesized nanoparticles *Euphorbia hirta* were found to produce a high pupicidal and larvicidal activity. This biological reduction of silver nanoparticles would be boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce AgNPs, involving organisms even ranging to higher plants. The formed AgNPs are highly stable and have significant crop pest of *H. armigera*.

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