International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 04-01-2024, Accepted: 21-01-2024, Published: 05-02-2024
Volume 9, Issue 2, 2024, Page No. 20-28

Characterization of *Euphorbia hirta* synthesized zinc nanoparticles and its genotoxicity against tobacco cutworm, *Spodoptera litura* Feb. (Lepidoptera; Noctuidae)

V S Kavitha, M Vimala Devi, V Uthayakumar, R Chandirasekar
[1] Department of Zoology, Vellalar College for Women, Erode, Tamil Nadu, India
[2] Department of Zoology, Sri Vasavi College, Erode, Tamil Nadu, India
[3] Department of Zoology, Human Molecular Genetics Laboratory, Sri Vasavi College, Erode, Tamil Nadu, India

Abstract
This study examined the efficacy of green nanoparticles derived from *E. hirta* methanol extract as biopesticides against the tobacco cutworm *Spodoptera litura* Feb. (Lepidoptera). The utilization of *Euphorbia hirta* leaf extract facilitated the synthesis of zinc nanoparticles, which were characterized by their rod-shaped morphology by the analysis of UV spectra, X-ray diffraction patterns, and scanning electron microscopy (SEM). The study conducted a probit analysis on the first, second, third, fourth, fifth, and sixth instars of S. litura to determine the lethal concentrations (LC_{50}) of the nanoparticles and plant extract. The results showed a positive correlation between the LC50 values and the older instars. The LC_{50} values for the plant extract varied between 286.515 ppm and 468.526 ppm, while the LC_{50} values for ZnONP ranged from 8.984 ppm to 26.074 ppm (larva I). The extract of *E. hirta* significantly reduced fertility at a concentration of 50ppm and lowered longevity at a concentration of 500ppm. The ZnONP that was produced through biological synthesis exhibited notable DNA fragmentation in the treated worm, suggesting its potential to induce genotoxicity. Eh-ZnONP was found to be the most potent biogenic compound for decreasing the population of S. litura, while also being environmentally benign.

Keywords: Zinc nanoparticles, *Spodoptera litura*, *Euphorbia hirta*, longevity, fecundity, TEM

Introduction

Environmental and human health concerns over the excessive use of synthetic chemical insecticides in numerous nations are motivating the creation and advocacy of alternative and safer pest management strategies. Repeated exposure to chemical insecticides has prompted concerns over the health of farmers. The emergence of insecticide resistance has sparked interest in developing integrated pest management strategies that minimize the use of pesticides and promote a more sustainable agricultural system. This is driven by the growing awareness of the potential negative impacts associated with excessive insecticide application [1, 2].

Nanotechnology has significantly transformed agricultural research by achieving cost-effective and environmentally sustainable objectives in terms of crop productivity and food security. The study by Benelli and Lukehart [3] presents the latest techniques for creating novel nanoscale particles (ranging from 1 to 100 nm) using various nanomaterials, including silver, zinc, copper, iron, boron, and others. These particles are commonly used in a range of IPM (Integrated Pest Management) programs. These nanoparticles, referred to as nano-pesticides, surpass current pest control techniques like heat destruction, resistance development, costly formulations, and the eco-toxicity of insecticides [3]. Zinc oxide nanoparticles (ZnONP) have garnered significant attention within the field of inorganic nanoparticles due to their remarkable optical properties and widespread application in biomedical science. ZnONP remains a subject of active research due to its notable antibacterial, antifungal, wound-healing, and UV-filtering properties. Zinc oxide nanoparticles (ZnO NP) have the ability to be compatible with human cells. As a result, they have been used as carriers for medical purposes [4]. The application of nanotechnology in insect pest management has garnered significant interest in recent years [5]. The efficacy of silver nanoparticles fabricated from *Hypnea musciformis* and *Euphorbia prostrata* was tested against moths [6]. Additionally, silver nanoparticles fabricated from *Euphorbia prostrata* were tested against beetles, while silver nanoparticles encapsulated with *Azadirachta indica* were tested against economically significant arthropods. Prior research conducted by Gonzalez *et al.* [7] investigated the efficacy of *Ocimum basilicum* extract in eradicating *Acanthoscelides obtectus*.

The Lepidoptera family members exert influence on agricultural output at both global and Pakistani levels. The tobacco cutworm, scientifically known as *Spodoptera litura* Fab. (Lepidoptera; Noctuidae), is a widespread pest that feeds on a variety of crops and is capable of causing damage to over 180 different plant species globally. This pest is particularly problematic in Asian countries, posing the greatest threat to agricultural and horticultural industries in the region [8, 9]. This pest has the ability to utilize a diverse array of host plants, including cotton, maize, rice, peanuts, caster, and various other grains, legumes, and vegetable crops. This is due to its development of resistance to pesticides and its increased capacity for mobility and spread. It originated in Asia and subsequently spread to Africa, Australia, the Middle East, Southern Europe, and the Pacific Islands. Furthermore, it disseminated to both tropical and temperate regions of Asia. The host plant has significant yield loss (ranging from 25.8% to 100%) due to hypognathous chewing, depending on the stage of the crop and the severity of the epidemic. Higher emergence is observed due to the short duration of its life cycle, which lasts approximately five weeks.

The noxious arthropod is presently being combated utilizing artificial insecticides. In their study, Sabri *et al.* [10] found that methylfenozide had a significant detrimental effect on

cutworms, while spinosad, indoxacarb, and emamectin benzoate showed only minimal resistance. Nevertheless, due of the adverse consequences of these pesticides, such as the development of insect resistance and cross-resistance, environmental toxicity, and potential health hazards for humans, scientists are currently prioritizing the development of alternative environmentally friendly means of control. Therefore, the utilization of organic insecticides has resulted in the depletion of beneficial insects, the reappearance of pests, and the emergence of pest resistance [11]. Nanoparticles can be utilized as a means of crop protection, ensuring both food and environmental safety, in order to overcome the limitations in managing tobacco cutworms. There is a lack of research on the beneficial effects of nanoparticles on lepidopterous pests in terms of toxicity and cost-benefit assessments. The objective of this study was to produce zinc nanoparticles using an extract obtained from *E. hirta* and evaluate their effectiveness in killing larvae, their ability to deter feeding, their lifespan, and their impact on reproduction, in comparison to plant extracts, against *Spodoptera litura* in different stages of development.

Materials and Methods

Preparation of Euphorbia hirta extract

We gathered mature and healthy *E. hirta* leaves from the grounds of Bharathiar University. Following the removal of surface dust and filth using double distilled water (ddH2O), the leaves underwent a 12-hour drying procedure in a hot air oven (Robus Technologies, UK). The dried leaves were pulverized into a fine powder using a mill and pestle. The standardized liquid was placed in a sterilized glass container. The LabTech EV311H rotary evaporator was utilized to dissolve 15.0 g of E. hirta fine powder in 1000 mL of ddH2O, without the need for a vacuum pump. The solution was thereafter heated to a temperature of 45°C and agitated at a speed of 40 revolutions per minute for a duration of 40 minutes. Once the extract reached room temperature, it was centrifuged at a force of 10,000 times the acceleration due to gravity for 10 minutes. Afterwards, the liquid layer above the sediment, referred to as the supernatant, was meticulously transferred to a fresh glass container equipped with a screw-top cover. The experiment concentrations were created utilizing the aforementioned stock solutions.

Spodoptera litura rearing

The *S. litura* larvae were obtained from the Central Institute of Cotton Research, situated in Coimbatore, India, and associated with the Indian Council of Agricultural Research. The participants were raised in a controlled laboratory environment and given *Ricinius communis* leaves as needed. The temperature was kept at a constant 27 ± 2˚C, the relative humidity was maintained between 75−85%, and there was a light-dark cycle of 14:10 (L:D). The pre-pupae of S. litura were isolated and given vermiculite clay, which acts as an excellent substrate for pupation. The pupae of *S. litura* were placed in a specialized enclosure intended for the emergence of adult insects. The cage was positioned on a layer of cotton within Petri dishes. To promote egg-laying, freshly hatched moths were fed a diet containing a 10% sucrose solution, along with a small quantity of a vitamin cocktail known as MULTI DEC Vitamin drops. Moths were introduced into the oviposition cages with the previously specified adult diet, maintaining a 1:1 ratio of males to females. In order to promote egg laying, a layer of white muslin fabric was positioned on top of the cage housing *S. litura* eggs. To inhibit the transmission of viruses, the egg's protective coverings were daily removed and cleansed with a solution containing 10% formaldehyde. To expedite the hatching process, the egg casings were moistened and placed in a plastic container. This strategy enabled a reliable supply of experimental insects [12].

E. hirta-mediated synthesis and characterization of ZnO nanoparticles

The aqueous extract of E. hirta was made by mixing 10g of well prepared and finely chopped leaves with 300 mL of sterilized double distilled water. Afterward, the mixture was boiled for 5 minutes and then let to settle for one hour. Ultimately, the mixture was passed through Whatman No. 1 filter paper to separate its components. Afterwards, it was kept at a temperature of 15 °C and tested within a 5-day timeframe. A solution of zinc nitrate with a concentration of 0.05 millimolar (mM) was added to the filtrate and continuously stirred using a magnetic stirrer. The solution mixture was held at a temperature of 90 °C for a period of four to five hours, while being stirred vigorously without interruption. By employing this procedure, a precipitate exhibiting a profound yellow coloration was produced. The mixture was subjected to a 15-minute centrifugation at 7000 revolutions per minute, resulting in the removal of the dark yellow precipitate. After further stirring, the liquid was heated to a temperature of 150 °C for 1 hour, leading to the production of a solid precipitate with a slight yellow color. After air-drying, the resulting solid was extensively purified using methanol. The product was subjected to a heat treatment at a temperature of 400 degrees Celsius for a period of one hour. The result produced a powder that was white in color. The subsequent investigation utilized a powdered substance known as ZnO NP. The zinc nitrate was acquired from Precision Scientific Co., which is situated in Coimbatore, India.

The ZnO nanoparticles suspended in water were examined using UV-Vis spectrophotometry. The optical properties of ZnONP were examined using Shimadzu's UV-3600 ultraviolet and visible absorption spectroscopy, covering a wavelength range of 200 to 800 nm. The ZnONP-containing powder sample underwent Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR) Spectroscopy, and X-ray Diffraction (XRD) analyses (Dinesh *et al*., 2015; Suresh *et al*., 2015) [13, 14]. The FTIR spectrum was obtained and examined using a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer operating in the diffuse reflectance mode, with a resolution of 4 cm-1. The structure and content of freeze-dried, pure ZnONP were analyzed using an FEI QUANTA-200 TEM and a 10 kV ultra-high resolution scanning electron microscope. A 25 mL amount of the sample was thinly layered onto a copper stub using sputter coating. The TEM analysis was performed utilizing a JEOL-MODEL 6390 instrument. The XRD analysis was performed using a diffractometer operating at 40 kilovolts and 30 milliamperes. The 2θ scanning range spanned from 35˚ to 85˚, with an incremental step size of 0.02˚. The XRD pattern was matched with phase using match software version 1.10c Inc. The International Centre for Diffraction Data (ICDD) gave the standard values. The Hkl indices of the observed peaks, obtained by Bragg's reflection for the

face-centered cubic structure, were 111, 200, and 311. The dimensions of ZnONP were evaluated utilizing the Malvern Zetasizer-Nanosizer, a device specifically designed for analyzing particle sizes. The scattering laser was employed to examine the minor alteration on ZnONP, allowing for the measurement of supplementary ZnONP dimensions.

Toxicity against the tobacco cutworm *Spodoptera litura*

The toxicity against S. litura larvae and pupae was assessed using a no-choice technique in the leaf disc experiment. Zinc oxide nanoparticles (ZnONP) were synthesized via the dipping technique. Subsequently, these nanoparticles were given to larvae of the F2 generation on cotton leaf discs that had previously been treated with varying quantities of E. hirta extract. Following a 24-hour duration, the individuals were relocated to unprocessed, freshly harvested cotton leaves. The leaves were substituted everyday. After a 96-hour therapy session, deaths were observed. Fifty larvae (n = 50) were divided into five replicates, with each replicate consisting of 10 larvae, for each treatment. Goswami *et al*. [15] employed the following formula to compute the mortality rate:

Corrected mortality = (Mortality in treatment − mortality in control) / (100−mortality in control) ×100.

Larvae that remained alive until they entered the pupal stage were provided with untreated cotton leaves as their food source. Pupal mortality was assessed by subtracting the number of emerging adults from the total number of pupae.

Antifeedant activity of *S. litura*

The leaf disc no choice bioassay method was employed to evaluate the antifeedant activity of plant extracts and ZnONP. Various plant extracts and different concentrations of ZnONP were applied to freshly cut castor leaf discs with a surface area of 1350 square millimeters. After the solvent had fully evaporated, the leaf disc was transferred to separate petri plates of 9 cm in diameter and kept at room temperature. Each petriplate contained a single third-instar Spodoptera litura larva that had been subjected to pre-starvation. The larva were given the incubated discs for periods of 24, 48, and 72 hours. The leaf discs that were moistened with water were employed as the negative control. There were a total of twenty-five replicates, with five replicates conducted for each of the five studies. The unused section of the leaf disc was measured using a leaf area meter at the conclusion of the experiment. The percentage of antifeedant activity was calculated using the methodology established by Singh and Pant [16]. Later, the data was subjected to analysis of variance.

Impact on longevity and fecundity of *S. litura*

Twenty individuals of both male and female *S. litura* were housed in wooden cages, keeping an equal ratio of males to females. After ten hours since they emerged, the subjects were given an artificial meal consisting of 20 mg of sucrose, 1 mL of honey mixed with 1 mL of sterile distilled water, and 1 mL of an aqueous solution containing either 10, 20, 30, 40, or 50 parts per million (ppm) of plant-synthesized zinc oxide nanoparticles (ZnO NP), or 100, 200, 300, or 400 ppm of *E. hirta* extract. The control diet was devoid of ZnO nanoparticles or plant extract. The population of female individuals suitable for reproduction (n=20) was divided by the number of eggs deposited over a span of four days on five recently emerging *G. hirsutum* leaves. The average lifespan of each adult was computed, and the daily rate of death was observed.

DNA fragmentation analysis

The *S. litura* cells were immersed individually in a 10 mL solution of buffer, including 10mM Tris HCl and 10mM EDTA (pH 8.0). The cells were then treated with a 10 mL solution containing 10 millimolar Tris HCl, 10 millimolar EDTA (pH 8.0), 2% SDS, and 20 milligrams per milliliter proteinase K. The solution was incubated at a temperature of 37 degrees Celsius for a period of 3 hours. Afterwards, DNA extraction was conducted using a solution composed of phenol, chloroform, and isoamyl alcohol at a ratio of 25:24:1. The DNA was treated with DNase-free RNase at a concentration of 20 mg/mL, at a temperature of 4◦C for 45 minutes. Afterwards, it was isolated by adding 100 mL of 2.5 M sodium acetate and 3 times the volume of ethanol. A DNA fragmentation analysis was conducted using 10µg of DNA isolated from both control cells and treated cells at different time points. The procedure entailed electrophoresis on a 2% agarose gel supplemented with ethidium bromide, performed for a duration of 1 hour at a voltage of 20V.

Data analysis

A two-variable ANOVA was used to analyze acute toxicity data for multiple insect species. The analysis focused on the specific instar being targeted and the dose being evaluated. The Tukey's HSD test was utilized to differentiate across means, using a significance level of $P < 0.05$. The mortality data was subjected to probit analysis. The LC50 and LC90 values were determined utilizing the Finney method, as described in reference [17]. The study utilized a two-way analysis of variance (ANOVA) with two factors: the treatment including the ZnONP plant extract, and the dosage. The aim was to examine the impact of these factors on lifespan and reproductive capacity. The Tukey's honestly significant difference (HSD) test was used to distinguish between the means, with a significance level of $P<0.05$. The data were analyzed using the SPSS Statistical Software Program, especially version 17.0. Values were considered significant if their probability threshold was $P<0.05$.

Results and Discussion

Characterization of ZnO nanoparticles

UV-Vis spectroscopy is a crucial instrument for understanding the production, structure, and longevity of nanoparticles. Zinc oxide nanoparticles (ZnONP) were produced by combining an aqueous extract of E. hirta with a 0.05 mM solution of zinc nitrate. The inclusion of ZnONP surface Plasmon absorption led to a distinct peak at 360 nm in the UV-Vis spectra (Fig. 1a). Ali *et al*. [18] detected a peak in ZnONP absorption at 375 nm as a result of surface Plasmon resonance, which is consistent with our own research. Patil and Taranath [19] reported that the production

of ZnONP utilizing *Limonia acidissima* resulted in a peak with the highest absorbance at 374 nm. Figure 1b illustrates the X-ray diffraction (XRD) pattern, which confirms the presence of crystalline ZnONP formed by E. hirta. The XRD pattern displayed distinct peaks that were attributed to the crystalline structure of ZnONP and matched the crystal planes of (111), (200), (220), and (311). The X-ray diffraction (XRD) pattern produced from the production of ZnO nanoparticles using *Polygala tenuifolia* was indistinguishable. Djuricic and Pickering [20] discovered that the wide peaks indicate that the particles have a crystalline size inside the nanoscale range, suggesting that the biosynthesized particles are exceptionally minute and delicate. The use of nanosized ZnO allows for the formation and breakdown of ZnONP by applying an extract obtained from *E. hirta* leaves [21]. Further corroboration of the results of this investigation is provided by XRD data acquired from ZnO nanoparticles produced by plants [22, 23, 24].

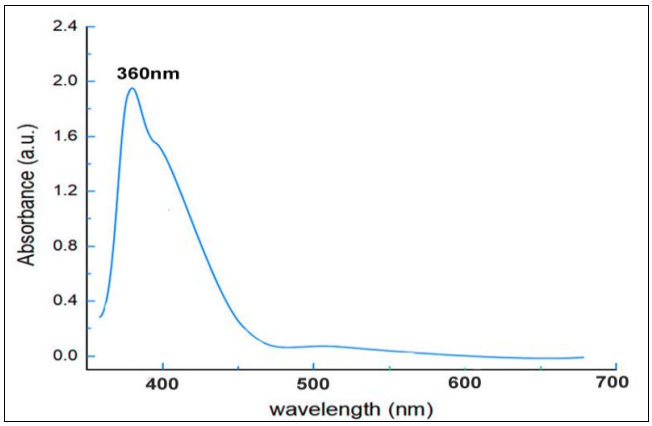
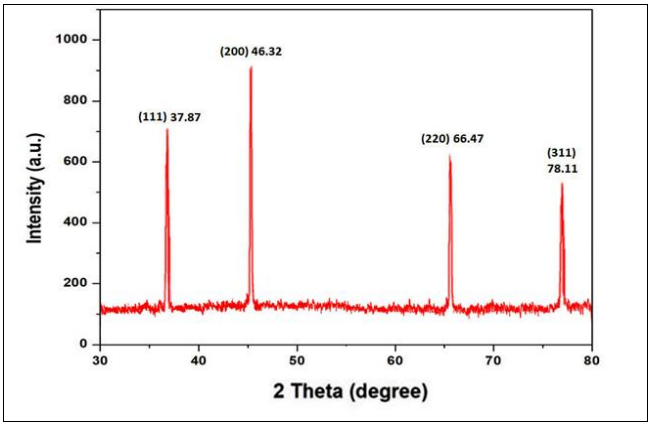


Fig 1a: UV-vis spectroscopy analysis of *E. hirta*



1b) XRD pattern of *E. hirta* synthesized ZnO NP

Figure 2 displays transmission electron microscopy (TEM) images of produced green zinc oxide nanoparticles (ZnONP). The photograph demonstrates that ZnO nanoparticles had a rectangular shape [25]. Most zinc nanoparticles derived from plants have a rectangular shape [26, 27]. The ZnONPs produced by L. acidissima were mainly evenly distributed, having a round form, with an average size ranging from 12 to 53 nm [28]. Furthermore, Banumathi *et al.* [29] identified the presence of both spherical and hexagonal ZnONPs in *Lobelia leschenaultiana*. The nanoparticles exhibited a size range of 20 to 65 nm. According to Ali *et al.* [18], ZnONPs synthesized with Aloe vera exhibited diverse morphologies such as spherical, oval, and hexagonal, with an average diameter ranging from 8 to 18 nm. The particle size distribution analysis of our Zn nanoparticles, synthesized by *E. hirta*, was conducted using dynamic light scattering. The results obtained were in agreement with the analysis performed using transmission electron microscopy (Fig. 3a). The particles had a size distribution spanning from 10 to 110 nm, with an average particle size of 80 nm (Fig. 3b). The work done by Dwivedi and Gopal [30] demonstrated that silver and gold nanoparticles produced from *Chenopodium album* maintained stability over a wide range of pH levels, thanks to their high zeta potential. This finding aligns with prior study undertaken by Ahmad *et al.* [31], Rajaganesh *et al.* [32], and Rajapandian and Kadarkarai [7]. The FTIR analysis was used to determine the functional groups that influenced the synthesis and stability of ZnONPs. The graph in Figure 4 shows a distinct and broad band that may be found at the following coordinates: 1423, 1510, 1605, 2396, 2934, and 3380 cm-1. The intense peak at around 440 cm-1 is a result of the stretching motion of the zinc-oxygen bond [33]. The absorption peaks at 1510 and 1390 cm-1 are indicative of the C=O stretching vibrations in diisobutyl phthalate, hexahydrofarnesyl acetone, tannins, and flavonoids. The presence of phenols is indicated by the stretching vibration at 3380 cm-1, while the existence of a carboxylate group is shown by the band at 1605 cm-1 [28]. The band at 1605 cm-1 [29] corresponds to the C=O stretching vibrations of the amide group. Considering the detected peaks, it is likely that ZnONP has been coated with diverse functional groups originating from biomolecules. The purpose of this coating is to inhibit the agglomeration of particles, hence ensuring the stability of the medium.

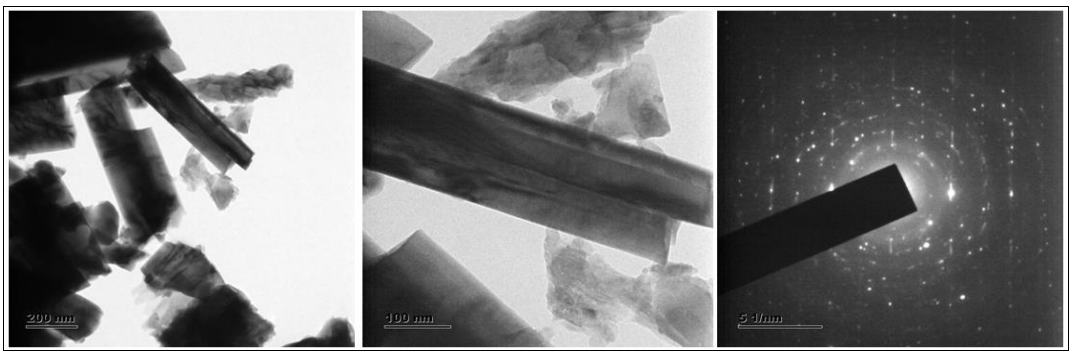


Fig 2: TEM image of *E. hirta* synthesized ZnO NP

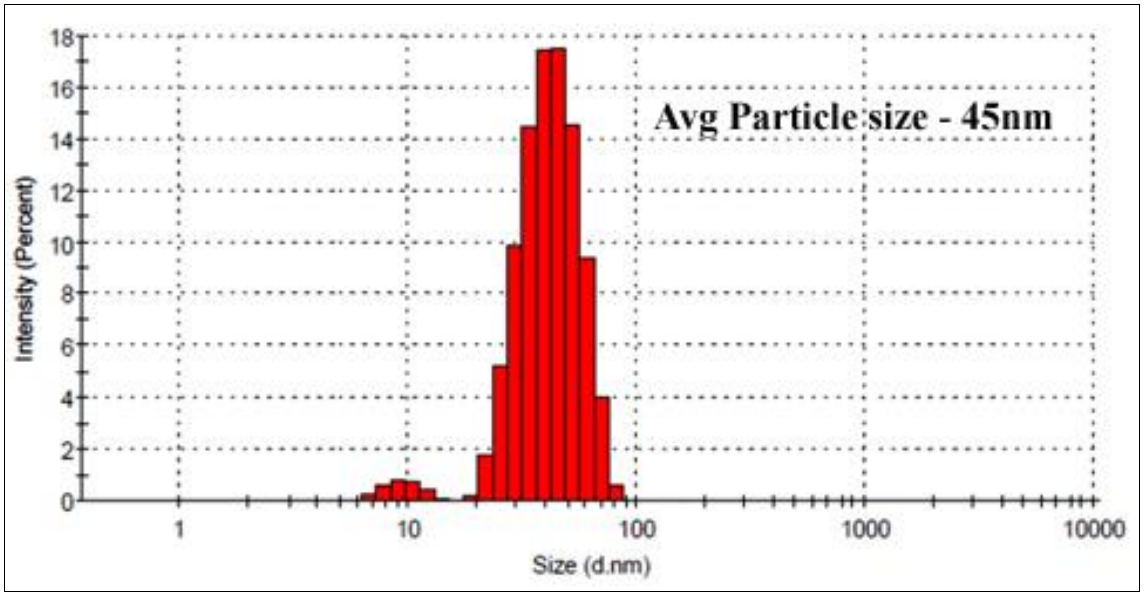
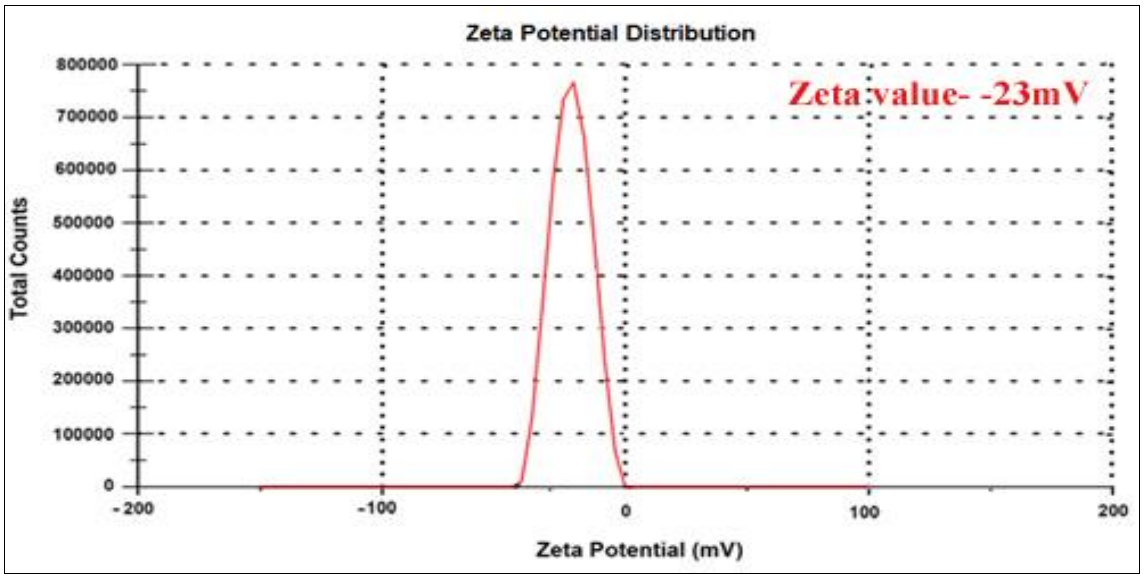


Fig 3a: Average particle size analysis of E. hirta synthesized ZnO NP



3b) Zeta potential analysis of *E. hirta* synthesized ZnO NP

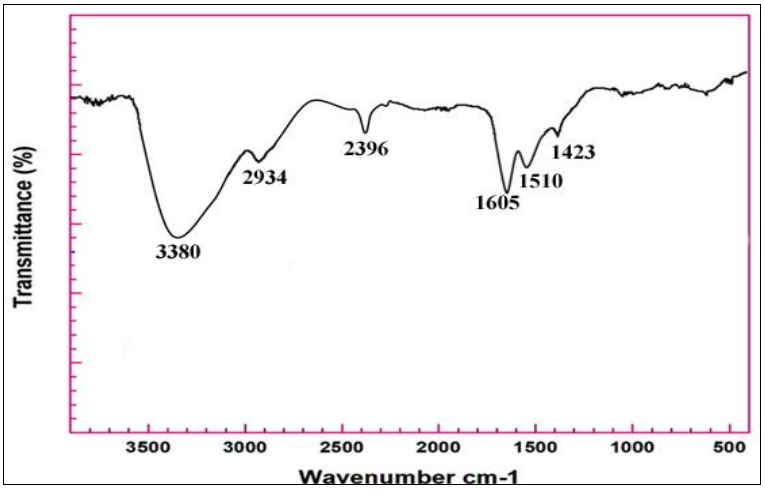


Fig 4: FTIR spectrum of *E. hirta* synthesized ZnO NP

Toxicity of zinc nanoparticles on *Spodoptera litura*

The larvae of S. litura were subjected to the toxic properties of the *E. hirta* leaf extract. The LC_{50} values for compounds I, II, III, IV, V, and VI were 286.515 ppm, 311.751 ppm, 345.815 ppm, 389.458 ppm, 426.436 ppm, and 468.526 ppm, respectively, as indicated in Table 1. In the study

conducted by Suresh *et al*. [34], they found that the LC_{50} values of the aqueous extract from the mangrove plant *Suaeda maritima* had varying effects on the larvae and pupae of Spodoptera litura. The concentration needed to cause mortality ranged from 20.937 ppm for larva I to 46.896 ppm for pupa. Deshmukhe *et al*. [35] discovered that the aqueous crude extract derived from the leaves of *Lantana camara*, when administered at a concentration of 40%, resulted in the death of fourth instar *S. litura* larvae. In addition, the ZnONP produced by E. hirta shown significant toxicity against *S. litura*. The LC_{50} values for compounds I, II, III, IV, V, and VI were 8.984 ppm, 10.978 ppm, 15.855 ppm, 19.034 ppm, 22.547 ppm, and 26.074 ppm correspondingly, as shown in Table 1. Metal nanoparticles are regarded as a promising class of possible biopesticides for the control of many plant pests, among other options. In their investigation, Khooshe-Bast *et al*. [36] discovered that both ZnONPs and *Beauveria bassiana* TS11 had a substantial lethal impact on the greenhouse whitefly, *Trialeurodes vaporariorum*. The LC_{50} value of ZnONPs was 7.35 ppm, whereas *B. bassiana* obtained an LC_{50} value of 3.28×10^5 spore ml-1.

Table 1: Larvicidal activity of E. hirta extract and ZnONP against tobacco cut worm *Spodoptera litura*

Treatment	Life stages	LC_{50} (LC_{90}) (ppm)	95% confidence Limit		Regression equation	χ^2 (*df*=4)
			LC_{50} (LC_{90})			
			LCL	UCL		
E. hirta extract	I Instar	286.515 (577.159)	258.866 (524.241)	313.444 (653.456)	y = -1.263+ 0.004x	2.380 *n.s*
	II Instar	311.751 (615.145)	283.790 (555.984)	340.554 (702.043)	y = -1.317 + 0.004x	1.288 *n.s*
	III Instar	345.815 (677.221)	315.689 (604.904)	379.743 (787.846)	y = -1.337+ 0.004x	1.767 *n.s*
	IV Instar	389.458 (743.935)	355.768 (656.717)	431.935 (882.461)	y = -1.408+ 0.004x	0.022 *n.s*
	V Instar	426.436 (801.998)	387.855 (699.678)	479.637 (970.683)	y = -1.455+ 0.003x	0.302 *n.s*
	VI Instar	468.526 (884.422)	421.003 (756.277)	541.241 (1109.391)	y = -1.444+ 0.003x	0.709 *n.s*
E. hirta- ZnONP	I Instar	8.984 (43.994)	1.748 (39.123)	13.706 (51.523)	y = -0.037+ 0.037x	2.471 *n.s*
	II Instar	10.978 (49.507)	3.645 (43.674)	15.749 (58.940)	y = -0.365 + 0.033x	0.902 *n.s*
	III Instar	15.855 (53.251)	10.192 (47.172)	19.860 (62.920)	y = -0.543+ 0.034x	1.025 *n.s*
	IV Instar	19.034 (58.759)	13.748 (51.649)	22.920 (70.407)	y = -0.614+ 0.032x	0.877 *n.s*
	V Instar	22.547 (63.008)	17.889 (55.180)	26.243 (75.955)	y = -0.714+ 0.032x	0.828 *n.s*
	VI Instar	26.074 (68.588)	21.743 (59.535)	29.849 (83.986)	y = -0.786+ 0.030x	2.010 *n.s*

Antifeedants activity of *E. hirta* synthesized zinc nanoparticles on *Spodoptera litura*

Researchers determined the antifeedant activity of *E. hirta* extracts and generated ZnONP by analyzing the average leaf consumption in treated and control leaves. A study was done to evaluate the ability of plant extracts and synthetic ZnONP to inhibit the eating behavior of fourth instar larvae of *Spodoptera litura*. *Spodoptera litura* ingested a smaller amount of leaf discs treated with extract and produced ZnONP, as compared to the control group. Quinones, phenols, alkaloids, lactones, diterpenoids, and tripinoids are the most efficient insecticides [37]. Thorough investigation of the plant has resulted in the discovery of multiple active constituents extracted from *E. hirta*. Afzelin, quercitrin, and myricitrin were derived from the methanolic extract of *E. hirta* [38]. The chemical analysis of E. hirta has led to the discovery and 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. *E. hirta* contains several compounds including amyrin, 24-methylenecycloartenol, -sitosterol, heptacosane, nnonacosane, shikmic acid, tinyatoxin, choline, camphol, and derivatives of quercitol with rhamnose and chtolphenolic acid [39]. After being treated with E. hirta extract for 72 hours, the antifeedant effect on 4th instar S. litura larvae was measured to be 91.2% at a concentration of 100ppm, 74.2% at 200ppm, 57.2% at 300ppm, 25.0% at 400ppm, and 13.4% at 500ppm. The antifeedant action was evaluated using the leaf dip method, as described in Table 2. The inhibitory effect of ZnO nanoparticles produced by *E. hirta* was assessed after a period of 72 hours. The antifeedant activity was measured at different concentrations: 89.6% at 10ppm, 70.6% at 20ppm, 48.2% at 30ppm, 20.2% at 40ppm, and 7.2% at 50ppm. Typically, a greater antifeedant proportion signifies a decrease in the rate at which food is consumed. The study found comparable outcomes in the unprocessed ethanolic extracts of five medicinal plants (*Berberis lyceum* L, *Hedera nepalensis* L, *Acorus calamus* L, *Zanthoxylum armatum* L, and *Valeriana jatamansi* L). These plants have a distinctive mechanism of influencing insects by utilizing a blend of many components [40].

Table 2: Antifeedants activity of *E. hirta* extract and ZnONP against tobacco cut worm *Spodoptera litura* (IV Instar)

Treatment	Post treatment hours	Leaf Feeding Area (%) ± Mean Standard Error					
		Control	100ppm	200ppm	300ppm	400ppm	500ppm
E. hirta extract	24 hours	52.8±3.27^a	43.6±2.30^b	36.8±2.16^c	32.4±2.70^d	22.4±1.81^e	6.8±1.64^f
	48 hours	76.4±1.94^a	74.4±2.40^b	61.2±2.38^c	42.8±2.58^d	23.2±2.77^e	8.8±1.64^f
	72 hours	97.6±2.50^a	91.2±1.30^b	74.2±2.68^c	57.2±1.92^d	25.0±1.87^e	13.4±1.51^f
E. hirta- ZnONP		Control	10ppm	20ppm	30ppm	40ppm	50ppm
	24 hours	53.6±4.03^a	38.8±1.30^b	34.6±2.07^c	27.6±1.81^d	19.8±2.38^e	5.2±1.64^f
	48 hours	77.4±2.30^a	70.8±1.48^b	58.8±2.94^c	36.4±2.88^d	19.2±1.92^e	7.0±1.22^f
	72 hours	98.6±1.34^a	89.6±1.14^b	70.6±1.94^c	48.2±2.68^d	20.2±2.48^e	7.2±1.92^f

Values are means±SD of five replicates,

Within each row, means followed by the same letter(s) are not significantly different (P<0.05),

Impact of zinc nanoparticles on *Spodoptera litura* longevity and fecundity

The application of *E. hirta* extract and plant-derived ZnONP treatments resulted in reduced adult longevity and fecundity in *S. litura*, as shown in Table 3. The treatment with 50 ppm E. hirta generated ZnONP resulted in a decrease in longevity to 19.6 days, compared to 28.4 days in the control group. Following the administration of ZnONP generated by *E. hirta*, there was a significant reduction in fecundity. The control group had 157 eggs, whereas the treatment groups at 10, 20, 30, 40, and 50 ppm had 145.8, 135.6, 124.8, 105.8, and 83.2 eggs, respectively (Table 3). There is a scarcity of field research that examine the impact of plant-derived nanoparticles on the reproductive capacity and lifespan of agricultural pests. A study conducted by Sahayaraj *et al*. [41] found that nanoparticles generated from pungam oil, specifically Ag and Au nanoparticles, reduced the lifespan of *Pericallia ricini*. Chitra *et al*. [42] demonstrated that using a methanolic extract of *Senna alata* effectively reduced the ability of *S. litura* to reproduce and live for a long time, thereby acting against crop pests.

Table 3: Longevity and fecundity of *Spodoptera litura* after the treatment of *E. hirta* extract and ZnONP

Treatment	Concentration (ppm)	Adult longevity (Days)		Fecundity (no. eggs)
		Male	Female	
E. hirta extract	Control	28.2 ± 1.30^{a}	33.6 ± 1.14^{a}	156.2 ± 3.27^{a}
	100	27.6 ± 2.30^{b}	33.2 ± 1.92^{b}	154.4 ± 3.78^{b}
	200	25.2 ± 1.92^{c}	31.2 ± 1.30^{c}	150.6 ± 2.96^{c}
	300	22.8 ± 2.28^{d}	28.0 ± 1.22^{d}	145.6± 3.36^{d}
	400	18.6 ± 1.34^{e}	23.4 ±1.14^{e}	133.2 ± 2.58^{e}
	500	15.4 ± 1.14^{f}	20.4 ± 2.30^{f}	124.2 ± 2.86^{f}
E. hirta- ZnONP	Control	28.4 ± 1.34^{a}	34.2 ± 1.92^{a}	157.2 ± 3.56^{a}
	10	24.8 ± 1.48^{b}	31.6 ± 1.51^{b}	145.8 ± 3.49^{b}
	20	22.4 ± 2.07^{c}	26.6 ± 1.14^{c}	135.6 ± 3.36^{c}
	30	19.8 ± 1.48^{d}	23.0 ± 1.41^{d}	124.8 ± 2.94^{d}
	40	14.8 ± 1.48^{e}	19.2 ± 2.16^{e}	105.8 ± 2.77^{e}
	50	9.6 ± 1.14^{f}	14.2 ± 1.48^{f}	83.2 ± 1.30^{f}

Within each tested product, values followed by the same letter(s) are not significantly different (ANOVA, Tukey's HSD, α =

Genotoxicity analysis of ZnONP

Figure 5 shows the pest groups treated with biosynthesized ZnONP and analyzed using DNSA fragmentation analysis. The figure shows that M represents a DNA Marker with a range of 1000bp. The streak pattern seen in the various concentration treated groups reveals that the DNA of the treated group larvae was severely damaged. The animal in the control group was not subjected to any particles. The results demonstrate that the treated larvae exhibited an increase in DNA fragmentation as the concentration of biosynthesized particles rose. The produced particles are capable of internalizing the gut of the larvae, where they intercalate with the genomic DNA, resulting in fragmentation.

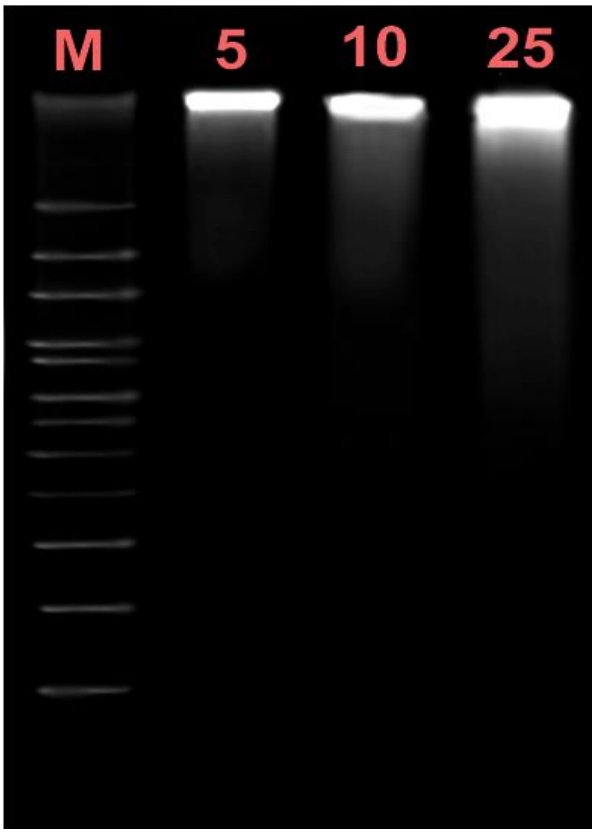


Fig 5: DNA fragmentation analysis of tobacco cut worm *Spodoptera litura*: lane M (Marker), lanes 5, 10 and 25µg treated group.

Conclusion

Therefore, with the utilization of a low-cost aqueous extract of *E. hirta* as both a reducing and stabilizing agent, we achieved successful biosynthesis of ZnONP. E. hirta synthesized rectangular ZnO nanoparticles with a crystalline structure in a face-centered cubic arrangement. Our research indicates that extracts from *E. hirta* and ZnONP produced using *E. hirta* have significant promise as eco-friendly agents for pest control in agricultural crops. In summary, the current approach employed in our laboratory effectively assists in the management and regulation of *S. litura* field population resistance to several pesticides now available. The genotoxicity of the substance was demonstrated using DNA ladder analysis, indicating a substantial level of damage. Considering its environmentally friendly nature and its non-hazardous impact on human health, it may be concluded that Eh-ZnONP is a suitable option for the safe management of *S. litura*. This study has also laid the groundwork for future research on the practical use and commercial development of environmentally friendly nanoparticles for managing important moth pests.

Conflict of Interest

The authors declare that they no competing interest

References

1. Carter HO. Agricultural sustainability: an overview and research assessment. Calif, Agric,1989:43:13–17.
2. Nathan SS, Kalaivani K. Combined effects of azadirachtin and nucleopolyhedrovirus (SpltNPV) on Spodoptera litura Fabricius (Lepidoptera: Noctuidae) larvae. Biological control,2006:39(1):96-104.
3. Benelli G, Lukehart CM. Applications of green-synthesized nanoparticles in pharmacology, parasitology and entomology. J. Clust. Sci,2017:28:1-2.
4. Murugan K, Roni M, Panneerselvam C, Suresh U, Rajaganesh R, Aruliah R, *et al. Sargassum wightii*-synthesized ZnO nanoparticles reduce the fitness and reproduction of the malaria vector *Anopheles stephensi* and cotton bollworm *Helicoverpa armigera*. Physiol. Mol. Plant Pathol,2018:101:202-213.
5. Rajapandian R, Kadarkarai M. Encapsulation of silver nano crystals using Salvinia molesta against the Anopheles stephensi and oxidative stress enzyme activity of larvivorous fish. Journal of Natural Pesticide Research,2023:3:100022.
6. Roni M, Murugan K, Panneerselvam C, Subramaniam J, Nicoletti M, Madhiyazhagan P, *et al.* Characterization and biotoxicity of *Hypnea musciformis*-synthesized silver nanoparticles as potential eco-friendly control tool against *Aedes aegypti* and *Plutella xylostella*. Ecotoxicol. Environ. Saf,2015:121:31-38.
7. Rodríguez-González Á, Álvarez-García S, González-López Ó, Da Silva F, Casquero PA. Insecticidal properties of *Ocimum basilicum* and *Cymbopogon winterianus* against *Acanthoscelides obtectus*, insect pest of the common bean (*Phaseolus vulgaris*, L.). Insects,2019:10(5):151.
8. Edwin ES, Vasantha-Srinivasan P, Ponsankar A, Thanigaivel A, Selin-Rani S, *et al.* Effects of temperature and nonionizing ultraviolet radiation treatments of eggs of five host insects on production of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) for biological control applications. J. Asia-Pac. Entomol,2016:19(4):1139-1144.
9. Ahmad JN, Mushtaq R, Ahmad SJN, Malik MA, Manzoor M, Tahir M, Aslam Z, Maqsood S, Ahuja I, Bones AM 6 Sub-lethal dose reponses of native polyhydroviruses and spinosad for economical and sustainable management of Spodoptera litura in Pakistan. Pak. J. Zool,2020:52(3):989-999.
10. Sabri MA, Islam MS, Hussain D, Saleem M. Evaluation of lethal response of biorational insecticides against *Spodoptera litura* (Lepidoptera: Noctuidae). J. Entomol. Zool. Stud,2016:4:270-274.
11. Krishnappa S, Kalikeri S, Garampalli RK, Kachintaya CK. A brief review of the impact of silver nanoparticles on agriculture and certain biological properties: A case study. International Journal of Health and Allied Sciences,2022:11(1):9.
12. Murugan K, Senthil Kumar N, Jeyabalan D, Senthil Nathan S, Sivaramakrishnan S, Swamiappan M. Influence of *Helicoverpa armigera* (Hübner) diet on its parasitoid *Campoletis chlorideae* Uchida. Inter. J. Trop. Ins. Sci,2000:20:23-31.
13. Dinesh D, Murugan K, Madhiyazhagan P, Panneerselvam C, Mahesh Kumar P, Nicoletti M, Jiang W, Benelli G, Chandramohan B, Suresh U. Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from Aloe vera extracts: towards an effective tool against the malaria vector Anopheles stephensi ? Parasitology research,2015:114:1519-29.
14. Suresh U, Murugan K, Benelli G, Nicoletti M, Barnard DR, Panneerselvam C, *et al*. Tackling the growing threat of dengue: Phyllanthus niruri-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector Aedes aegypti (Diptera: Culicidae). Parasitology research,2015:114:1551-62.
15. Goswami A, Roy I, Sengupta S, Debnath N. Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. Thin solid films,2010:519(3):1252-1257.
16. Singh G, Pant PC, Bhan VM. Studies on the Critical Period of Weed Control in Spring Planted Sugarcane1. Indian Journal of Weed Science,1980:12(2):120-4.
17. Finney DJ. Probit Analysis, Cambridge University, London, 1971, 68e78
18. Ali K, Dwivedi S, Azam A, Saquib Q, Al-Said MS, Alkhedhairy AA, *et al*. *Aloe vera* extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. J. Coll. Inter. Sci,2016:472:145-156.
19. Patil BN, Taranath TC, Limonia acidissima L. leaf mediated synthesis of zinc oxide nanoparticles: A potent tool against Mycobacterium tuberculosis. International journal of mycobacteriology,2016:5(2):197-204.
20. Djuričić B, Pickering S. Nanostructured cerium oxide: preparation and properties of weakly-agglomerated powders. Journal of the European Ceramic Society,1999:19(11):1925-34.
21. Ambika S, Sundrarajan M. Antibacterial behaviour of Vitex negundo extract assisted ZnO nanoparticles

against pathogenic bacteria. Journal of Photochemistry and photobiology B: Biology,2015:146:52-7.
22. Dobrucka R, Długaszewska J. Biosynthesis and antibacterial activity of ZnO nanoparticles using *Trifolium pratense* flower extract. Saudi J. Biol. Sci.2016:23(4):517-523.
23. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol,2006:51: 45-66.
24. Supraja N, Prasad TN, Krishna TG, David E. Synthesis, characterization, and evaluation of the antimicrobial efficacy of *Boswellia ovalifoliolata* stem bark-extract-mediated zinc oxide nanoparticles. App. Nanosci,2016:6:581-590.
25. Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T, Bagavan A, *et al.* Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi. Spectrochimica Acta Part A: Molecul. Biomol. Spectros.,2012:90:78-84.
26. Benelli G. Green synthesized nanoparticles in the fight against mosquitoborne diseases and cancer e a brief review, Enzym. Microb. Technol,2016a:95:58-68.
27. Benelli G. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review, Parasitol. Res,2016b:115:23-34.
28. Patil BN Taranath TC. *Limonia acidissima* L. leaf mediated synthesis of zinc oxide nanoparticles: A potent tool against *Mycobacterium tuberculosis*. Inter. J. Mycobacterial,2016:5(2):197-204.
29. Banumathi B, Malaikozhundan B, Vaseeharan B. *In vitro* acaricidal activity of ethnoveterinary plants and green synthesis of zinc oxide nanoparticles against *Rhipicephalus (Boophilus) microplus*. Veter. Parasitol,2016:216:93-100.
30. Dwivedi AD, Gopal K. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. Coll. Surf. A: Physicochem. Engin. Asp,2010:369(1-3):27-33.
31. Ahmad A, Senapati S, Khan MI, Kumar R, Ramani R, Srinivas V, *et al*. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. Nanotechnology,2003:14(7):824.
32. Rajaganesh R, Murugan K, Panneerselvam C, Jayashanthini S, Roni M, Suresh U, *et al*. Fern-synthesized silver nanocrystals: towards a new class of mosquito oviposition deterrents? Research in Veterinary Science,2016:109:40-51.
33. Kwon YJ, Kim KH, Lim CS, Shim KB. Characterization of ZnO nanopowders by the polymerized complex method via an organochemical route. J. Cer. Process. Res,2002:3(3/2):146-149.
34. Suresh U, Murugan K, Panneerselvam C, Rajaganesh R, Roni M, Al-Aoh HA, *et al*. Suaeda maritima-based herbal coils and green nanoparticles as potential biopesticides against the dengue vector Aedes aegypti and the tobacco cutworm Spodoptera litura. Physiological and molecular plant pathology,2018:101:225-35.
35. Deshmukhe PV, Hooli AA, Holihosur SN. Effect of *Lantana camara* (L.) on growth, development and survival of tobacco caterpillar (*Spodoptera litura* Fabricius). Karnataka J. Agricul. Sci,2011:24(2):137-139.
36. Khooshe-bast Z, sahebzadeh N, Ghaffari-Moghaddam M, Mirshekar A. Insecticidal effects of zinc oxide nanoparticles and *Beauveria bassiana* TS11 on *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae). Acta Agricul. Sloven,2016:107(2):299-309.
37. Schoonhoven LM. Biological aspects of antifeedants. Entomologia experimentalis et applicate,1982:31(1):57-69.
38. Liu Y, Murakami N, Ji H, Abreu P, Zhang S. Antimalarial Flavonol Glycosides from Euphorbia hirta. Pharmaceutical Biology,2007:45(4):278-81.
39. Ogbulie JN, Ogueke CC, Okoli IC, Anyanwu BN. Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. Afric. J. Biotech, 2007, 6(13).
40. Tewary DK, Bhardwaj A, Shanker A. Pesticidal activities in five medicinal plants collected from mid hills of western Himalayas. Industrial crops and Products,2005:22(3):241-7.
41. Sahayaraj K, Madasamy M, Radhika SA. Insecticidal activity of bio-silver and gold nanoparticles against *Pericallia ricini* Fab. (Lepidaptera: Archidae). J. Biopest,2016:9(1):63.