Indian Journal of Experimental Biology
Vol. 61, March 2023, pp. 224-229
DOI: 10.56042/ijeb.v61i03.68093

Note

Effect of *Calotropis procera* (Aiton) Dryand. based zinc oxide nanoparticles on the cotton pest *Spodoptera litura* Fab.

M Chitra Devi[1], M Sithi Jameela[1], A Asharaja[2], G Raaja Rajan[3], M Iyyadurai[4] & A Daniel Reegan[5]*

[1]Department of Zoology and Research Centre, Sadakathullah Appa College (Autonomous), Affiliated to Manonmaniam Sundaranar University, Rahmath nagar, Tirunelveli - 627 011, Tamil Nadu, India

[2]PG and Research Department of Zoology, Pasumpon Muthuramalinga Thevar College, Affiliated to Manonmaniam Sundaranar University, Melaneelithanallur, Sankarankovil, Tenkasi - 627 953, Tamil Nadu, India

[3]JFA – FSSAI, Food analysis laboratory, TNFSD, Tirunelveli 627 002, Tamil Nadu, India

[4]Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli - 627 012, Tamil Nadu, India

[5]National Centre for Disease Control, Bengaluru Branch, 2nd Floor, Hosakerehalli BMTC Building, Banashankari 3rd Stage, Bengaluru – 560 085, Karnataka, India

Received 31 October 2022; revised 02 February 2023

Major loss in agricultural crops is caused by insect pests. In India, various synthetic insecticides are used against pests. These are much expensive and cause environmental hazards. The nanoparticles, as an alternative approach is gaining considerable interest in this field. In the present study, we explored the biological synthesis of zinc oxide nanoparticles using Giant milkweed, *Calotropis procera* (Aiton) Dryand. and its effects on the tobacco cutworm, *Spodoptera litura*. The reduction of zinc ions (Zn2+) to zinc nanoparticles (ZnO NPs) was prepared by mixing 50 g of *C. procera* leaves with 100 mL of single distilled water in a 250 mL glass beaker. To synthesize nanoparticles, 50 mL of *C. procera* leaf extract was taken using a stirrer-heater and 5 g of zinc oxide was added at 60ºC, boiled, then kept in a hot air oven at 70ºC for 24 h. Finally, the obtained light yellow coloured powder was carefully collected and characterized using X-ray diffraction (XRD) analysis. The results revealed that the biologically synthesized zinc oxide nanoparticles pesticide was highly effective against the pest. The weight of the pest decreased from low concentration to high concentration. It is concluded that the *Calotropis Procera* based zinc oxide nanoparticles could be used for the control of *Spodoptera litura*.

Keywords: Apple of sodom, Biocontrol, Calotrope, Giant milkweed, Nano formulation, Pest control, Sodom apple, Tobacco cutworm

Spodoptera litura Fab., commonly called Tobacco cutworm, is a polyphagous lepidopteran pest that feeds on 87 plant species of 40 botanical families including many economically important crops. It is found in Asia, Africa, Australia, Europe, Pacific islands, and the Middle East. It is an extremely serious pest of several field crops in India. Arthropods are destroying an estimated 18–20% of the annual crop production worldwide and loss of more than US$470 billion[1]. *S. litura* alone caused a loss of 1.39 billion rupees during an epidemic in India[2]. Currently, pesticides are used against *S. litura* and the residues affect human health and the environment[3,4]. Hence, biosynthesized nanoparticles would be alternative to pesticides and their utilization in agriculture has wide scope[4-6].

Nanotechnology is a field, where synthesis, identification, investigation, and application of nanosized (1-100 nm) materials for the advancement of science. Though chemical methods are effectively investigated to shape the synthesis of metal nanoparticles in various shapes and sizes, it is harmful to the environment and other beneficial insect species. Recent research shows that the utilization of botanicals is a novel method for the synthesis of metal nanoparticles, which has many advantages over the existing chemical methods. Zinc oxide (ZnO) is considered to be a remarkable material having a wide spectrum of applications, such as that of a semiconductor (Eg = 3.37eV), electroluminescent material, magnetic material, piezoelectric sensor, UV-absorber, actuator, nanostructure varistor, field emission displaying material, thermoelectric material, gas sensor, and as a constituent of cosmetics, etc.[7,8].

Calotropis procera (Aiton) Dryand., commonly known as Apple of Sodom or Giant milkweed, is widely distributed in tropical and subtropical regions of Asia and Africa including India, Pakistan, Afghanistan, Nepal, Iran, Iraq, Algeria, Israel, Kuwait, Niger, Kenya, Nigeria, Oman, Saudi Arabia, Vietnam, Yemen, the United Arab Emirates, Zimbabwe and China[9]. It is a desert plant and referred as “Madar” in Greeco-Arab medicine., and also used in Indian traditional medicine to treat fever, muscular spasms, allergy, dysentery, rheumatism, asthma, and as an expectorant, analgesic, anti-inflammatory and antitumor agent[9-11]. *C. procera* secretes a milky liquid called latex. During mechanical damage, the tissues of this plant break and secrete the milky latex, which

*Correspondence:
E-Mail: danielreegan85@gmail.com

possesses many active compounds, alkaloids, resins, and tannins. Principally, milky latex contains numerous alkaloidal compounds like calotropin, calcilin, catotoxin, gigatin[11,12]. Milky latex of *C. procera* is used as a reducing material as well as a surface stabilizing agent for the synthesis of spherical-shaped ZnO-NPs[13]. The structure and morphology of the synthesized NPs are characterized by X-ray diffraction (XRD) analysis.

Inorganic NPs have special properties due to their quantum size effects. Many unsaturated atoms and polymeric materials containing inorganic NPs display magnetic, catalytic and optical properties. Such properties of nanoparticles could be exploited for production of new insecticides[14,15]. Nanoparticles, as carrier, ensure gradual release of the chemical, and thereby ensures effective protection against a variety of insect pests[16]. In the present study, we have examined the potential effects of *Calotropis procera* based ZnONP's on the tobacco cutworm *Spodoptera litura* under laboratory conditions.

Materials and Methods

Insect culture

A colony of the test insect *Spodoptera litura*, was established in the insectary (Department of Microbiology, Sadakathullah Appa College, Tirunelveli, India) from field collected, disease free under a constant temperature of 25±1°C, 70% RH with a photoperiod of 14:10. *S. litura* was reared in sterile petri plates on fresh leaves of castor (*Ricinus communis*). The second stage larvae of *S. litura* were used in the present experiment.

Preparation of leaf extract

Fresh leaves of *C. procera* plant were collected in the Sadakathullah Appa college campus. The leaves were washed three times with tap water to remove the dust particles and then shade-dried for a week as per the method of Naseer et al. (2020)[17] with slight modifications. The reduction of zinc ions (Zn2+) to zinc nanoparticles (ZnO NPs) was prepared by mixing 50 g of *C. procera* leaves with 100 mL of single distilled water in a 250 mL glass beaker. This mixture was boiled till the colour of the mixture changed from watery to light yellow. The extract was then cooled to room temperature of 33 ± 1°C and filtered using filter paper. The extract was subjected to a rotary vacuum evaporator and the dried extract was stored at 4°C for further evaluation.

Green synthesis of zinc oxide nanoparticles and characterization

To synthesize nanoparticles, 50 mL of *C. procera* leaves extract was taken using a stirrer-heater and 5 g of zinc oxide was added at 60°C. The mixture was then boiled well till it condensed to a deep yellow-coloured paste. The resulting paste was collected in a ceramic container and heated well using the hot air oven at 70ºC for 24 h as per the method of Naseer *et al*.[17] with few modifications. Finally, the obtained light yellow coloured powder was carefully collected and characterized using X-ray diffraction (XRD) analysis. The X-ray diffraction (XRD) pattern of the prepared sample of zinc oxide nanoparticles was recorded by XPERT-PRO diffractometer, using CuKα1 radiation (λ = 1.54060 Å), CuKα2 radiation (λ = 1.54443 Å), CuKβ radiation (λ = 1.39225 Å), 40 kV- 30mA, 2θ/Ω scanning mode. Data was taken for the 2θ range of 25°C with a step of 0.0170 degree.

Body mass assay

Ten second stage larvae of *S. litura* were released on the prepared castor leaf discs (4×4 cm) and treated with various concentrations of the nanoparticle solutions as per the leaf-dip technique bioassay method[12]. The effects of the ZnO NPs on second stage larvae were determined by dipping infested leaves in various concentrations viz., 10, 20, 30 and 40 ppm following previous reports[18].

Insecticidal assay

Insecticidal activity was assessed as per the method of Paul & Jayaraj[19] and Pittarate *et al*.[20] with slight modifications. Ten larvae of *S. litura* were released for each replication on the castor leaf discs and were incubated at 25±1°C with 70% RH in a BOD incubator. Concentrations were 100, 200, 300 and 400 ppm. Each treatment was replicated five times and leaf discs were treated with only water assisted as control. The number of larvae that died was recorded after 24 h interval and the percent mortality was computed.

Statistical analysis

The data sets of *S. litura* insect weight were subjected to Analysis of Variance (ANOVA - SPSS, Inv., 1999 version 10.00) and means were separated by the Tukey's test at P =0.05. The data were also analyzed by Probit Analysis[21] to determine the LC_{50} values of *C. procera* based zinc-oxide nanoparticles.

Results

The XRD analysis confirmed that the prepared particles are zinc-oxide nanoparticles having face-

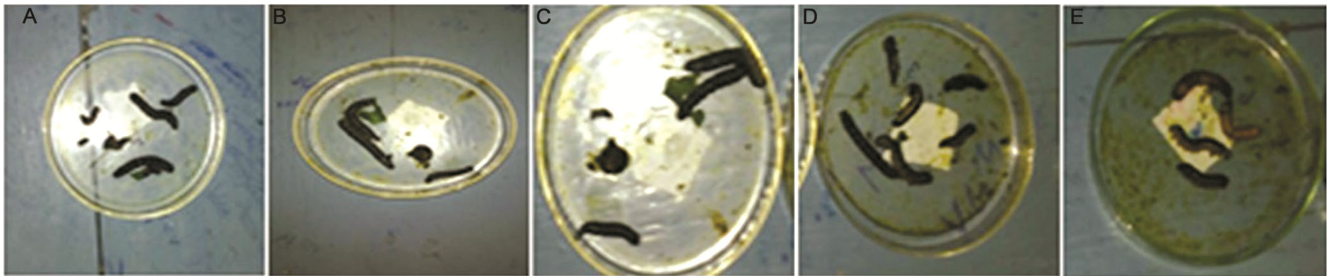

Fig. 1 — Effect of *Calotropis procera* based ZnO NPs on treated *Spodoptera litura larvae* compared with control

Table 1 — Peak list and their relative intensity of ZnO NPs obtained in XRD analysis

Pos. (°2Th.)	Height (cts)	FWHM Left (°2Th.)	d-spacing (Å)	Rel. Int. (%)
18.6720	75.73	0.4015	4.75231	32.37
26.3243	233.96	0.2007	3.38565	100.00
29.3077	125.43	0.2342	3.04743	53.61
35.8443	130.48	0.2676	2.50529	55.77
37.7632	19.80	1.6059	2.38228	8.46
41.2201	67.49	0.3011	2.19013	28.85
44.2191	38.73	0.4015	2.04830	16.55
46.1553	44.37	0.3346	1.96678	18.97
50.5242	24.90	0.8029	1.80649	10.64
54.9583	65.74	0.2676	1.67077	28.10
55.9287	30.05	0.4015	1.64406	12.84
57.0909	33.75	0.4015	1.61333	14.43
61.9945	27.93	0.4015	1.49697	11.94
74.1654	26.34	0.4015	1.27857	11.26

Table 2 — Lethal concentration (in ppm) of *Calotropis procera* based ZnO NPs against the larvae of *Spodoptera litura*

Insect species	Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ^2
			LL	UL		LL	UL			
Spodoptera litura	*Calotropis procera* based ZnO NP's	232.55	203.90	264.32	530.81	432.49	740.20	3.5±0.4	3.4±1.1	5.3*

[*P ≤0.05, level of significance of chi-square values. ZnO NP's – zinc oxide nanoparticles]

Table 3 — Weight of the *Spodoptera litura* insect pest in different days treated with *Calotropis procera* based ZnO NPs

Insect	Concentration	24 h	48 h	72 h	96 h	120 h	144 h
Spodoptera litura	10 ppm	0.2933±0.004^{d}	0.2892±0.007^{d}	0.2839±0.008^{d}	0.2789±0.01^{d}	0.2716±0.01^{d}	0.2676±0.02^{d}
	20 ppm	0.3881±0.008^{c}	0.3679±0.006^{c}	0.3507±0.01^{c}	0.3427±0.007^{c}	0.3212±0.004^{c}	0.3125±0.01^{c}
	30 ppm	0.4325±0.01^{b}	0.4117±0.01^{b}	0.3937±0.008^{b}	0.3937±0.008^{b}	0.3877±0.008^{b}	0.3719±0.005^{b}
	40 ppm	0.6604±0.01^{a}	0.6286±0.008^{a}	0.6182±0.009^{a}	0.5920±0.004^{a}	0.5672±0.01^{a}	0.5512±0.008^{a}
	Control (without any NPs)	0.2521±0.005^{e}	0.2591±0.003^{e}	0.2658±0.005^{d}	0.2689±0.005^{d}	0.2725±0.005^{d}	0.2772±0.005^{d}

[Each value represents mean of five replicates ± SD; Values carrying different letters in a column are statistically different by ANOVA Tukey's test at P = 0.05]

centered cubic crystal structure. The XRD peak image is given in Fig. 1. The details of the XRD peaks are given in Table 1. The insecticidal activity results of the *Calotropis procera* based ZnONPs against *Spodoptera litura* pest are given in Table 2. In 24 h, *C. procera* based ZnO NP's caused increasing mortality in different concentrations in 100, 200, 300 and 400 ppm. *C. procera* based ZnO NPs caused more mortality in 400 ppm concentration when compared to the different concentrations like 300, 200 and 100 ppm, and there was no mortality observed in control. The lethal concentration LC_{50} value was found to be 232.55 ppm, and the LC_{90} value was 530.81 ppm, respectively (Table 2). Further, there was no mortality in ZnO NPs alone treated larvae (Table 2).

In this study, we also observed the weight of the pest was decreasing across the treatment (Table 3). The weight of the pest decreased from low concentration to high concentration. In 144 hours, 0.5512 ± 0.008 was the weight of the pest in the highest concentration of 40 ppm. The weight was 0.6604 ± 0.01 at the same concentration in 24 h (Table 2). This shows that the weight of the pest *S. litura* decreased steadily and considerably.

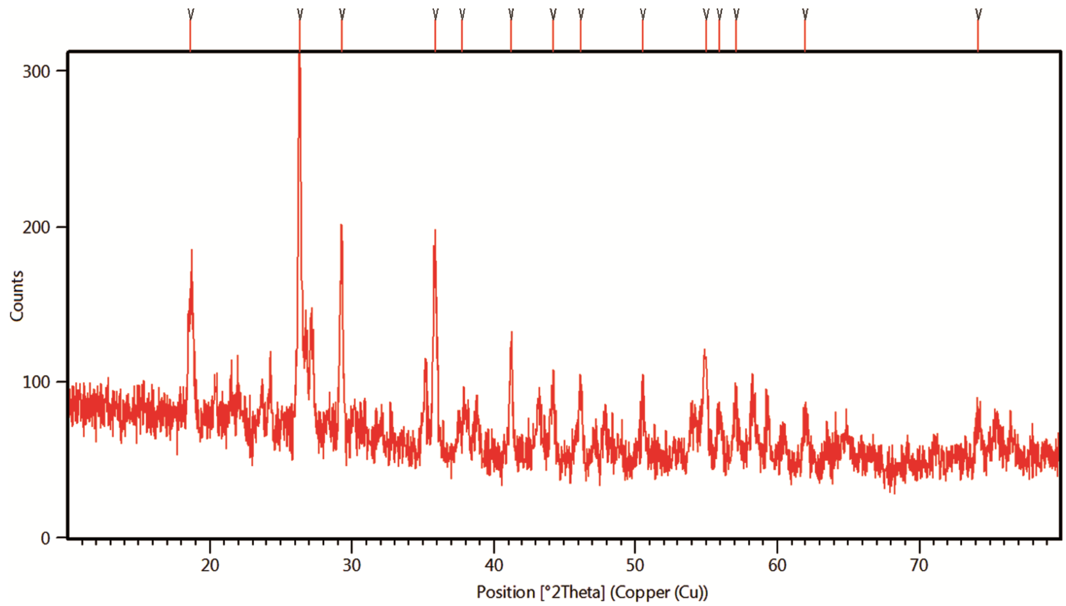

Fig. 2 — XRD image of *Calotropis procera* based zinc oxide nanoparticles (ZnO NPs)

Similarly, in all the other concentrations, the weight of the pest *S. litura* was found to be decreased (Table 2). However, in the control, the weight of the pest *S. litura* was found to be increased (Table 2). Further, *C. procera* based ZnO NP's treated *S. litura larvae* were blackened and showed less movement (Fig. 2).

Discussion

Spodoptera litura Fab. (Lepidoptera: Noctuidae) is a major pest of several agricultural crops[22]. It is extensively distributed throughout tropical regions of Asia, Australasia, and the Pacific Islands[23]. *S. litura* attack many host plants and mainly feed on tobacco, groundnut, cabbage, jute, rice, lucerne, cotton, tea, maize, and soybeans, potato, cauliflower, capsicum and castor[24]. Several outbreaks of this pest on cotton, tobacco, and chilies have been reported in India[25] and the economic damage range is calculated to be 26-100% depending upon the stage and species of crop and its level of infestation[26]. It has developed resistance against almost all the insecticide groups[27] including the new insecticides like lufenuron[28]. The adverse effects due to synthetic pesticides on beneficial insects and their subsequent impacts on ecological imbalance demand suitable alternatives[26].

Nanoparticle-based biopesticides are one such new alternative. Nanoparticles possess insecticidal properties due to novel characteristics like extraordinary strength, chemical reactivity, and electrical conductivity. They have distinct physical, biological, and chemical properties associated with their atomic strength[27]. Nanoparticles are usually clustered atom via atom; their size and shape can be maintained exactly according to the need[29], and nanoparticles can be arranged into a sequenced layer. This self-linking is due to the strong forces viz. hydrogen bonding forces, surface tension, gravity, dipolar forces, hydrophilic and hydrophobic interactions, etc.

Among all the metal oxide nanoparticles (NPs) zinc-oxide is interesting because it has wide applications, in the field of biotechnology, medical diagnostic, catalyzes, high-performance engineering material, diagnostic, magnetic recording media optical devices, catalyzes, DNA labeling, and drug delivery, etc[30]. However, the green synthesis of zinc oxide nanoparticles is an interesting topic in nanoscience, and has a wide application including creams, paints etc[31]. In several earlier reports, the botanical mediated nanoparticles revealed that green synthesized (ZnO) nanoparticles exhibited strong antimicrobial activities on pathogenic microbes and insecticidal activities on pest insects[32,33]. The use of the microbial extract, plant extract, or plant biomass could be alternative to the chemical and physical method for the production of an ecofriendly manner of nanoparticles[34]. In this study, the *C. procera* based zinc-oxide nanoparticles are active against *S. litura*. However, its mode of action needs to be explored.

Conclusion

The present study results have demonstrated that the green synthesis of *Calotropis procera* based zinc oxide nanoparticles are effective against the tobacco cutworm *Spodoptra litura*. In particular, the weight of the pest was observed to be decreasing across the treatment and the treated larvae showed less movement. Thus, it could be used in the integrated pest management program to effectively control the tobacco cutworm.

Conflict of interest

Authors declare no competing interests.

References

1 Sharma S, Kooner R & Arora R, Insect Pests and Crop Losses. In: *Breeding Insect Resistant Crops for Sustainable Agriculture*, (Ed. R. Arora, S. Sandhu; Springer Nature, Singapore), 2017, 45.

2 Sharma K, Sridhar J & Sharma RK, Effect of dietary antioxidants on growth and development of Taro caterpillar, *Spodoptera litura* (Fab.). *Indian J Exp Biol*, 56 (2018) 129.

3 Kong F, Song Y, Zhang Q, Wang Z & Liu Y, Sublethal Effects of Chlorantraniliprole on *Spodoptera litura* (Lepidoptera: Noctuidae) Moth: Implication for Attract-And-Kill Strategy. *Toxics*, 9 (2021) 20.

4 Sudha B, Athinath KS, Swabna V & Sumathi S, Keratinase mediated fabrication and partial characterization of gold nanoparticles and its antibacterial potential. *Indian J Exp Biol*, 61 (2023) 59.

5 Yooboon T, Pengsook A, Ratwatthananon A, Pluempanupat W & Bullangpoti V, A plant-based extract mixture for controlling *Spodoptera litura* (Lepidoptera: Noctuidae). *Chem Biol Technol Agric*, 6 (2019) 1.

6 Bakavathiappan Ga, Baskaran S, Pavaraj M & Jeyaparvathi S, Effect of *Calotropis procera* leaf extract on *Spodoptera litura* (Fab.). *J Biopest*, 5 (Suppl.) (2012) 135.

7 Park S, Lee JH, Kim HS, Park HJ & Lee JC, Effects of ZnO nanopowder dispersion on photocatalytic reactions for the removal of Ag ions from aqueous solution. *J Electroceramics*, 22 (2009) 105.

8 Wang ZL, Energy harvesting for self-powered nanosystems. *Nano Res*, 1 (2008) 1.

9 Tripathi M, Shukla PK, Sikarwar RLS, Tiwari AK, Dwivedi N & Tripathi S, Pharmacognostic study of *Calotropis procera* (Aiton) W.T. Aiton root and stem. *Indian J Nat Prod Resour*, 13 (2022) 374.

10 Mathur R, Gupta SK, Mathur SR & Velpandian T, Anti-tumor studies with extracts of Calotropis procera (Ait.) R.Br. root employing Hep2 cells and their possible mechanism of action. *Indian J Exp Biol*, 47 (2009) 343.

11 Silva MCC, Silva ABD, Teixeira FM, Sousa PCPD, Rondon RMM, Junior JERH, Sampaio LRL, Oliveira SL, Holonda ANM & Vasconcelos SMMD, Therapeutic and biological activities of *Calotropis procera* (Ait.) R. Br. *Asian Pac J Trop Med*, 3 (2010) 332.

12 Alam P & Ali M, Phytochemical investigation of *Calotropis procera* Ait roots. *Indian J Chem*, 48 (2009) 443.

13 Mohamed NH, Ismail MA, Abdel-Mageed WM & Shoreit AAM, Antimicrobial activity of latex silver nanoparticles using *Calotropis procera*. *Asian Pac J Trop Biomed*, 4 (2014) 876.

14 Owolade OF, Ogunleti DO & Adenekan MO, Titanium Dioxide affects disease development and yield of edible cowpea. *Elect J Environ Agri Food Chem*, 7 (2008) 2942.

15 Namasivayam SKR & Bharani RSA, Silver nanoparticles loaded pyrrole based pesticidal metabolites (AgNps-PFM) nanoconjugate induced impact on the gut microbion and immune response against lepidopteron pest *Spodoptera litura* (Fab.). *Indian J Biochem Biophy*, 58 (2021) 478.

16 Thabet AF, Boraei HA, Galal OA, El-Samahy MFM, Mousa KM, Zhang YA, Tuda M, Helmy EA, Wen J & Nozaki T, Silica nanoparticles as pesticide against insects of different feeding types and their non-target attraction of predators. *Sci Rep*, 11 (2021) 14484.

17 Naseer M, Aslam U, Khalid B & Chen B, Green route to synthesize zinc oxide nanoparticles using leaf extracts of *Cassia fistula* and *Melia azedarach* and their antibacterial potential. *Sci Rep*, 10 (2020) 9055.

18 Venkateswari G, Krishnayya PV, Arjuna Rao P & Krishna Murthy KVM, Bioefficacy of Abamectin and Emamectin Benzoate against *Spodoptera litura* (Fab.). *Pestic Res J*, 20 (2008) 229.

19 Paul A & Jayaraj R, Pesticidal properties of *Anamirta cocculus*, *Cardiospermum halicacabum*, *Cocculus laurifolius* and *Strychnos nux-vomica* against *Spodoptera litura* (Lepidoptera: Noctuidae). *Indian J Nat Prod and Resour*, 11 (2020) 295.

20 Pittarate S, Rajula J, Rahman A, Vivekanandhan P, Thungrabeab M, Mekchay S & Krutmuang P, Insecticidal Effect of Zinc Oxide Nanoparticles against *Spodoptera frugiperda* under laboratory conditions. *Insects*, 12 (2021) 1017.

21 Finney DJ, *Probit analysis*. IInd Edn. (Cambridge University Press, UK), 1952, 318.

22 Su J, Lai T & Li J, Susceptibility of field populations of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. *Crop Prot*, 42 (2012) 217.

23 Monobrullah M & Shankar S, Sublethal effects of Sl NPV infection on developmental stages of *Spodoptera litura* (Lepidoptera: Noctuidae). *Biocontrol Sci Technol*, 18 (2008) 431.

24 Sharma RK & Bisht RS, Antifeedant activity of indigenous plant extracts against *Spodoptera litura* Fabricius. *J Insect Sci*, 21 (2008) 56.

25 Rao BHK, Subbaratnam GV & Murthy KSRK, Crop losses due to insect pests Spl. *Indian J Entomol*, 1 (1983) 215.

26 Punia A, Chauhan NS, Singh D, Kesavan AK, Kaur S & Sohal SK, Effect of gallic acid on the larvae of *Spodoptera litura* and its parasitoid Braconhebetor. *Sci Rep*, 11 (2021) 531.

27 Kranthi KR, Jadhav DR, Kranthi S, Wanjari RR, Ali SS & Russell DA, Insecticide resistance in five major insect pests of cotton in India. *Crop Protect*, 21 (2002) 449.

28 Hilliou F, Chertemps T, Maïbèche M & Goff GL, Resistance in the Genus *Spodoptera*: Key Insect Detoxification Genes. *Insects*, 12 (2021) 544.

29 Sangu V, Yamuna T, Preethi GA & Sineha A, Green synthesis and characterization of silver nanoparticles using ethanolic extract of *Mimosa Pudica* linn leaves. *Indian J Chem Technol*, 28 (2021) 479.

30 Wang RH, Xin JH, Tao XM & Daoud WA, Zno Nanorods grown on cotton fabrics at low temperature. *Chem Phys Lett*, 398 (2004) 250.

31 Deekala V, Babu BK & Rudraraju R, Pharmacological studies of zinc oxide nanoparticles. *Indian J Biochem Biophys*, 56 (2019) 500.

32 Sawai J, Quantitative evaluation of antibacterial activities of metallic oxide powders (Zno, Mgo, And Cao) by conduct metric assay, *J Microbiol Methods*, 54 (2003) 177.

33 Abdo AM, Fouda A, Eid AM, Fahmy NM, Elsayed AM, Khalil AMA, Alzahrani OM, Ahmed AF & Soliman AM, Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) by *Pseudomonas aeruginosa* and their activity against pathogenic microbes and common house mosquito, *Culex pipiens*. *Materials*, 14 (2021) 6983.

34 Bhattacharya D & Rajinder G, Nano Technology and potential of microorkoulsms. *Crit Rev Biotechnol*, 25 (2005) 199.