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Biosynthesis of zinc oxide nanoparticles from *Memecylon talbotianum* D. Brandis leaf extract and its larvicidal activity

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Abstract
A rapid, green, and easy strategy for the Biosynthesis of Zinc oxide Nanoparticles using the leaf of *Memecylon talbotianum* D. Brandis (Melastomataceae). it is non-hazardous compared to physical and chemical synthesis methods.it is a cost-effective and eco-friendly synthesis. The current study deliberates to focus on the green synthesis of Zinc oxide nanoparticles by zinc nitrate and utilizes the bio components of plant leaf extract of *M. talbotianum*. Zinc oxide is an inorganic nanoparticle the crystals were synthesized in an eco-friendly way. Zinc oxide has a broad spectrum of applications. The structures of synthesized nanoparticles were characterized by using UV-visible absorbance spectroscopy (UV), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Energy dispersive X-ray (EDX) and scanning electron microscope (SEM) to determine the phase and morphology of nanoparticles (NPs).In UV-visible spectroscopic analysis portrayed surface Plasmon resonance at 365nm, of synthesized ZnONps, and FTIR suggested the presence of Alcohol, Aldehyde, carbon dioxide, Isothiocyanate, Carboxylic acid, Alkene, Nitro compounds, Alkane, Alkene. Synthesized particles were a flower in shape, and In XRD 2 θ values ranging from 27.3° to 75.32° values, Elemental Detection by X-Ray Diffraction method (EDX) analysis shows the mass percentage of Zinc and Oxide are 70.43% and 21.72% respectively, and provision with larvicidal activity from Culex species shown LC50 values for Methanol, Pet ether, and Zn nanoparticles showing 205.11, 307.6, 123.82, 229.08, 98.1, 21.72 mg/L to know their potential as a promising tool in vector control strategies for combining mosquito-borne diseases.

Keywords: Culex species, green synthesis, larvicidal activity, *Memecylon*, zinc oxide nanoparticles

Introduction

Nanoparticles are essential in developing a sustainable technology for the future, for humanity and the environment. The Biosynthesis of Nanoparticles by plants is a green chemistry approach that interconnects nanotechnology and plant biotechnology. Plant extracts are used for the metal ions' bio-reduction to form nanoparticles. (Parveen *et al*., 2015). Nowadays ZnO NPs have drawn the attention of many researchers for their distinctive properties, ZnO NPs have been used in numerous applications like electrical, thermal stabilities, environmental protection, and the medicinal industry. (Raha S *et al.*,2022) [13]. Mostly tropical and sub-tropical trees, shrubs, climbers, and herbs comprise approximately 5858 species in 173 genera (Michelangeli *et al*., 2020). In this sense leaves of *M. umbellatum* have traditional medicinal properties like cooling astringent, in conjunctivitis as a lotion to cure eye pain, leucorrhea, and gonorrhea (Gagana SL *et al.*,2020) [5]. In India 116 Melastome species were recorded, in that genus *Memecylon* estimated around 54 (Hooker, 1879). The genus and species were identified with shreds of evidence to conclude the species *M. talbotianum* (Gamble, 1935; Mao & Dash, 2020). Mosquitoes are the main cause of the transmission of various life-threatening diseases all over the world and create an unhealthy society. (Dass & Mariappan, 2014) [2].

This is the largest group of the phylum Arthropoda of public health importance worldwide as they can transmit diseases such as Malaria, Chicken gunya, Dengue, Filariasis, Zika, Japanese encephalitis, and yellow fever. The Culex mosquitoes are responsible for spreading various diseases, such as Japanese encephalitis, West Nile fever, St. Louis encephalitis, Filariasis, and Avian malaria (Trari *et al*., 2017) [17]. Among these diseases Japanese encephalitis is endemic in more than 21 countries, it is the main source of viral encephalitis in Asia, with 30,000- 50000 cases annually. (Erlanger *et al*., 2009) [4] Lymphatic filariasis is widely distributed in tropical and subtropical areas of the world, nearly 20 to 30 % of the population is at risk of filarial infection (Bizhani *et al.,* 2021) [1]. Control of the mosquito vector is the key challenge to prevent the transmission. This relates to the present work, which is on utilizing leaf-based synthesis of ZnO NP's of the *M. talbotianum* to evaluate the larvicidal activity against the culex species larvae.

Materials and Methods

Collection of Plant Material

The Collection of the *Memecylon talbotianum* leaf (Figure 01) was carried out through Identification, a specimen was present in Uttar Kannada District, Karnataka, India, and the collected Leaf part was cleaned by running tap water to remove waste and other organic contents, followed by Distilled water extraction. A total of 25 gm of *M.talbotianum* was cut into fine small pieces and added to the Soxhlet apparatus the extract was cooled down and filtered using WhatmanNo.1 filter paper. The final extracts were stored in the refrigerator and gathered for further analysis.

Formulation of ZnO NPs from the extract Memocylon talbotianum

The Biosynthesis of ZnONPs utilizing the plant leaf Memecylon talbotianum extract was taken using a 0.01 M zinc nitrate hexahydrate (Zn (NO3)2·6H2O) solution. Zinc nitrate hexahydrate (Zn (NO3)2·6H2O) of a reagent grade 98% was obtained from NICE Chemicals, India. (Figure 2) In essence, 5 mL of aqueous extract of Memecylon talbotianum leaves was combined with 95 mL of zinc nitrate solution (0.01 M), and the composition was stirred with a magnetic stirrer for an hour at 75 ◦C (150 rpm). The formation of the reduced precipitates detected the bioformulation of ZnNPs. Finally, minimal precipitates of ZnNPs were collected by centrifugation for 15 min at 5000 rpm. After discarding the supernatants, the materials were mashed in a mortal pestle to get a fine nature for characterization and further analysis. (Yassin MT *et al.,* 2022) [19]

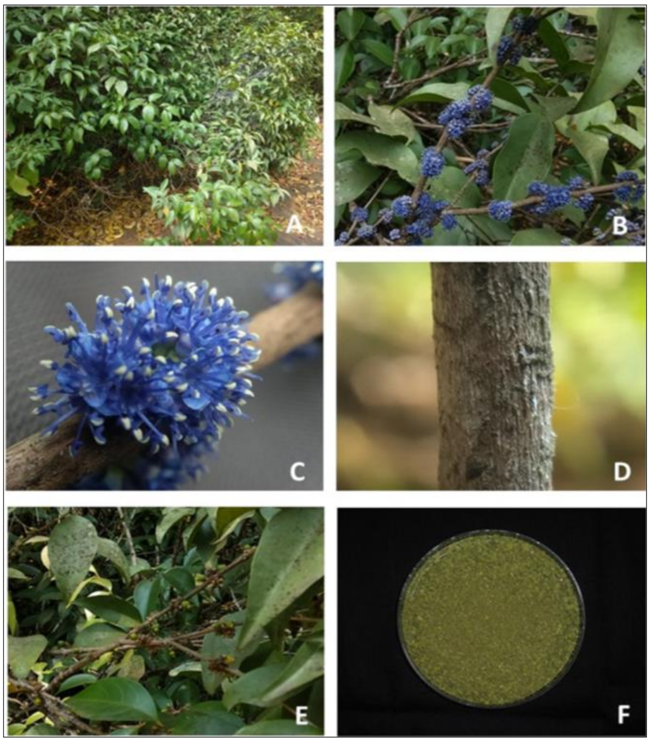

Fig 1: Memecylon talbotianum D. Brandis. A. Habitat, B. Inflorescence, C. Flower D. Stem, E. Fruit, F. Leaf Powder

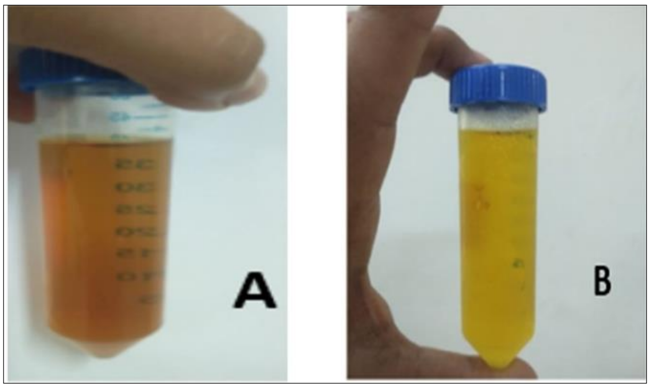

Fig 2: Green synthesis of M-ZnONPs from leaf extract *M.talbotianum*: Mixture of aqueous *M.talbotianum* leaf extract and ZnONPs solution A. Before incubation and B after incubation

***Characterization of Zinc Oxide Nanoparticles (ZnONPs)*:**
UV-visible spectroscopy analysis of *Memecylon talbotianum* (ZnO NP's)

The UV-visible spectra of synthesized nanoparticles of *Memecylon talbotianum* were recorded by configuring a double-beam (UV-9600A, Shanghai, China) placing the ZnO NP's from leaf extract in the cuvette and analyzing its optical density at wavelengths of between 300 and 600 nm. The graph was constructed by plotting wavelength (X-axis) against absorbance (Y-axis).

FTIR analysis of synthesized *Memecylon talbotianum* (ZnO NP's)

To investigate the bioactive compounds in the leaf of *Memecylon talbotianum* used to detect Fourier transforms infrared (FTIR) spectroscopy. Which use of an instrument FTIR spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA. Dried powder form of biosynthesized particles merged with potassium bromide (KBr) to produce a pellet, in KBr pellets was measured between 400 and 4000 cm^{-1} and which was then examined for the presence of IR spectral bands. The functional groups, present in the sample, spectral data between 400 and 4000 cm^{-1} in resolution were recorded.

Analysis of X-ray diffractometer of *Memecylon talbotianum* (ZnO NP's)

The dried powder form of biosynthesized *Memecylon talbotianum* leaf part was analyzed for XRD patterns of synthesized material by placing in a sample holder and then placing them in an X-ray diffractometer (Rigaku Miniflex 600, Smart-Lab SE, Tokyo, Japan) to record the spectral patterns by employing a current of 30 mA with Cu Kα radiation with an angle of 2 θ ranging from 20° to 80° operating at 40 kV.

Scanning electron microscopic analysis and energy dispersive EDX analysis of *Memecylon talbotianum* (ZnO NP's)

The scanning electron microscopy (SEM) coupled with an energy dispersive X-ray (EDX) instrument, the topology and elemental compositions of *Memecylon talbotianum*-ZnONPs were determined using (JEOL, JSM IT 500LA, and Peabody, MA, USA). Briefly, plant leaf extract was placed on the stub using carbon tape, then fixed and covered with gold using sputtering, and the loaded stub was placed in the instrument chamber for analysis.

Determination of Larvicidal activity from plant extract and Memecylon talbotianum (ZnO NP's)

World Health Organization (WHO 2005) standard protocol guidelines were used for the larvicidal activity of the plant extract and the biosynthesized *Memecylon talbotianum* ZnO NPs. Live third instar mosquito larvae were collected, for the bioassay test six batches of 10 third instar larvae were transferred into the sterile glass Petri dishes (150ml capacity). Petri dishes containing the various concentrations of plant leaf extract 50 to 200mg/L and Zn ONP's 10 to 40mg/L were tested. These tests were carried out on the triplets and the mortality rate percentage was calculated after 24 and 48 hours. (Water temperature 24 ± 2ºC).

Results and Discussion

UV-visible spectroscopy analysis

In the current study, we have synthesized ZnO NPs from an aqueous extract of *M. talbotianum* leaf. A change in extract color confirmed the synthesis following the addition of Zn after 24 h of incubation at RT (27 °C), at pH 9.0, the suspension turned from brownish red to pale yellow (Fig. 02). In the range of between 200 to 800 nm, (Sasani Ghamsari M *et al.,* 2017) [15] the characteristic SPR absorption spectrum of the Biosynthesized particles was spotted at 365 nm, shown in (figure 03) confirming the Biosynthesis of *Memecylon talbotianum* NPs. It is reported that the intensity of absorption peak in the UV-visible spectrum is associated with particle size of nanoparticles.

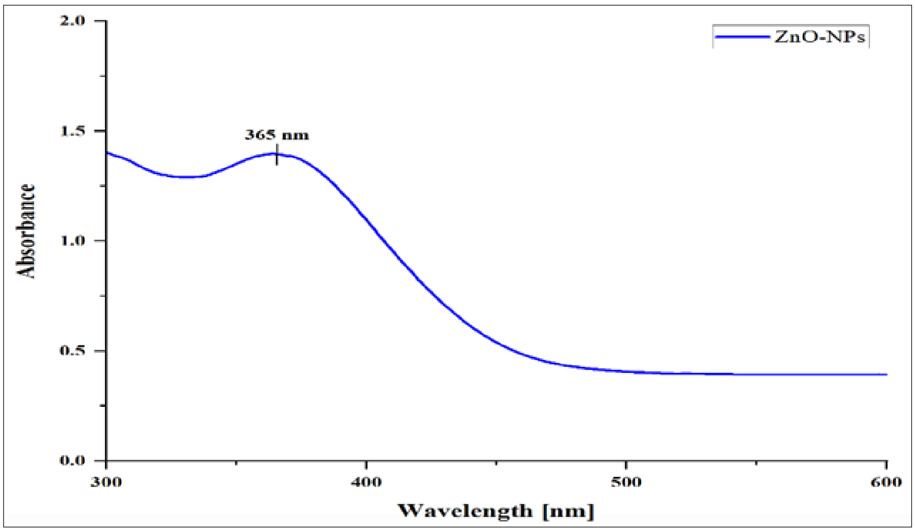

Fig 3: UV spectroscopy analysis of synthesized M-ZnONPS from *M.talbotianum* leaf extract

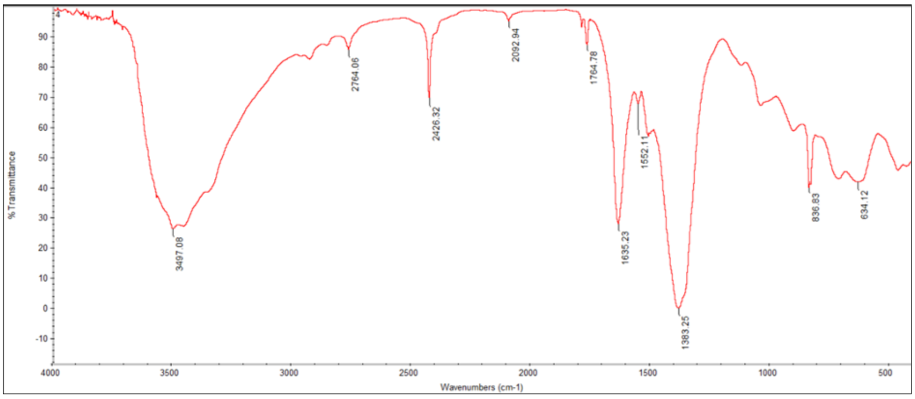

Fig 4: FT-IR Spectroscopy analysis of synthesized MMTAL-ZnONPS from *M.talbotianum* leaf extract

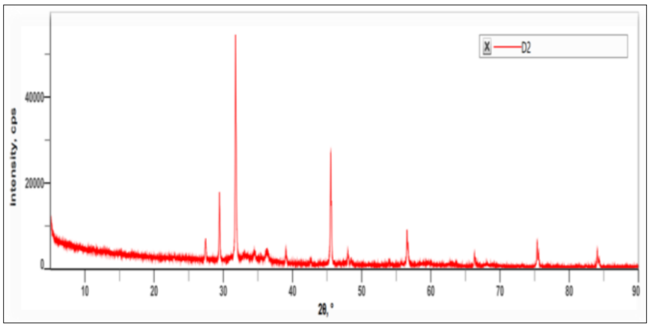

Fig 5: X-RD Graph of Zinc oxide nanoparticles

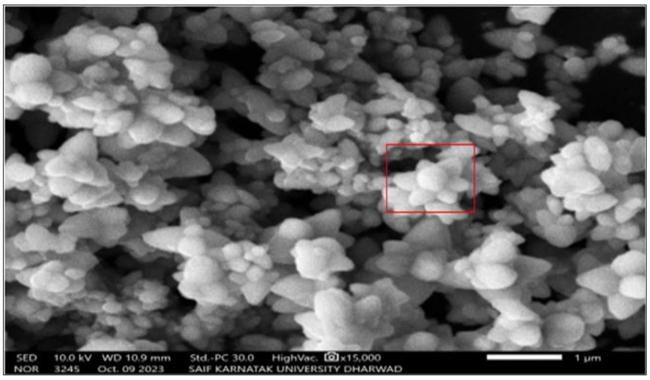

Fig 6: SEM image showing surface morphology

FTIR analysis

The zinc nanoparticles are synthesized through biomimetic methods via a molecular level of interaction with metallic surfaces. The character of the functional groups' interplay between synthesized materials was studied using FTIR analysis. The FTIR width of the *M. talbotianum* Leaf extract exhibited peaks shown in (fig. 04) at 3497.08 cm-1 appearances of Strong-broad which is an O-H Stretching group having compound class Alcohol it is Intermolecular Bonded, (Rai LF *et al.*,2015) 2764.06 cm-1Medium appearance group of C-H Stretching having compound class Aldehyde, 2426.32 cm-1showing Strong appearance & group of O=C=O Stretching compound class Carbon Dioxide, 2092.94 cm-1showing a Strong appearance with a group of N=C=S Stretching compound class were isothiocyanate 1764.78 cm-1 which is a strong appearance and group of C=O Stretching showing the compound class of Carboxylic acid which is Monomer 1635.23 cm-1 appearance of Strong, (Sangeetha G *et al.*,2011) [14] the group is C=C Stretching having a compound class of Alkene which is Mono-substituted 1552.11 cm-1.showing a strong appearance and group of N-O Stretching of Nitro compound 1383.25 cm-1 Medium appearance having a group of C-H Bending compound present is Alkane which is Gem dimethyl 836.83 cm-1 Medium appearance with a group of C=C Bending Alkene which is Tri-substituted,like these a similar result of shown in (Jayarambabu N *et* *al.*,2014) [7] whereas the showed major peaks as a result of bio-reduction, the FTIR width of the extract and the MMTAL-ZnO NP's exhibited minute shifts in peak positions. Table 01 shows the functional group of a compound.

Table 1: Showing the functional groups present in the compound through FTIR

Wave Numbers	Appearance	Groups	Compound Class	Comments
3497.08	Strong-broad	O-H Stretching	Alcohol	Intermolecular Bonded
2764.06	Medium	C-H Stretching	Aldehyde	Doublet
2426.32	Strong	O=C=O Stretching	Carbon Dioxide	Nil
2092.94	Strong	N=C=S Stretching	Isothiocyanate	Nil
1764.78	Strong	C=O Stretching	Carboxylic acid	Monomer
1635.23	Strong	C=C Stretching	Alkene	Mono-substituted
1552.11	Strong	N-O Stretching	Nitro-compound	Nil
1383.25	Medium	C-H Bending	Alkane	Gem dimethyl
836.83	Medium	C=C Bending	Alkene	Tri-substituted

X-RD Differactometric analysis

The powdered sample was used by a Cu KαX-ray diffraction (XRD), to confirm the presence of Zn and analysis of the structure. The peak appeared at 2θ values ranging from 27.3°, 31.73°, 34.4°, 36.22°, 47.70°, 56.53°, 62.84°, 66.24°, 68.10°, 72.51°, 75.32° values correspond to pure Zn shown in (Fig 05). These graph peaks are good with the literature (Devi RS *et al.*, 2014) [3]

SEM analysis

SEM analysis showed the morphology of zinc oxide nanoparticles. The SEM images displayed agglomerations of individual Zn oxide nanoparticles. A closer look at the lump showed the presence of several nanoparticle aggregates. Looking at the agglomerated lump in more detail, the presence of the aggregate of some nanoparticles was remarkable. When the SEM images were estimated, some nanoparticle particles were seen one by one while others were clustered, some nanoparticles were seen as flower-like bundles of average size 10.9mm seen in (figure 6) as that of similar flower-shaped ZnO NPs are reported in the literature (Lai Y, Meng M *et al.*,2011)as that aspected ratio of flower shaped structure of ZnO nanoparticles maximum at lower temperature and decreases towards high temperature were discussed in (Khan MF *et al.*,2014) [8]

Elemental Detection by X-Ray Diffraction Method of Analysis (EDX)

Further analysis of the Zn oxide nanoparticles by EDX spectrum in (Figure 07) confirmed only the signal characteristic of zinc and oxygen. The EDX spectrum confirmed the synthesised zinc and oxygen signal characteristics of zinc oxide nanoparticles. The absence of different signals among all the resulting peaks showed that these biosynthesised Zinc oxide Nanoparticles were pure. The stoichiometric mass per cent of Zn and O are 70.43% and 21.73% respectively. the peaks are comparable to those described earlier for the synthesis of Zinc oxide nanoparticles. (Shnawa BH *et al.*,2022) [16]

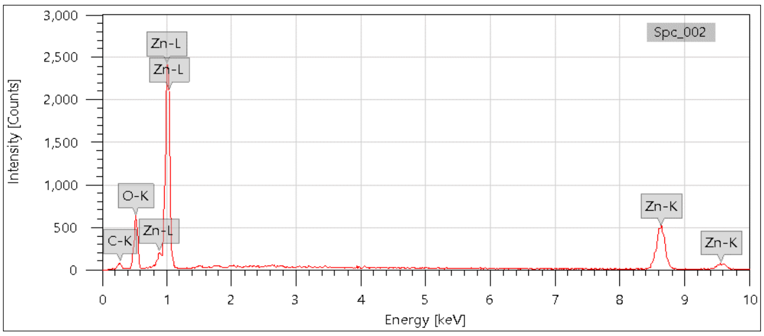

Fig 7: EDX analysis of synthesized M-ZnONPs from *M.talbotianum* leaf extract EDX spectrum showing the presence of Zinc

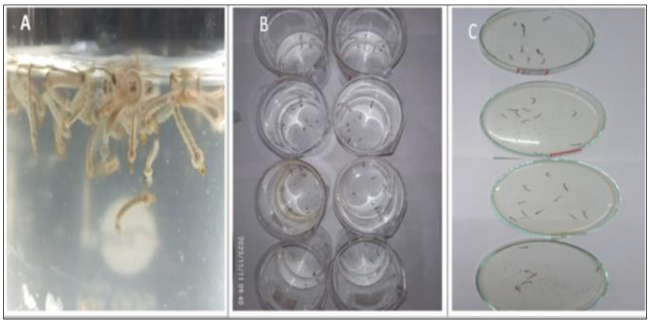

Fig 8: 3rd instar larvae A. Control Culex species B. Treated larvae with different solvents. C. Treated with Zn oxide nanoparticles

Larvicidal activity

In the present Investigation, The toxicity of different solvent extracts of an *M.talbotianum.* was tested against Culex *species* shown in (Figure 8), and test against extract and Zn nanoparticles were shown (Figure 09) the data were recorded and statistical data regarding LC50 values were calculated. The LC50 values for the Pet ether, methanol, and Zn nanoparticles extract of *M.talbotianum* against at 24 and 48h post-treatment were 229.08mg/L, 128.82mg/L, 205.11mg/L, 307.6 mg/L, 98.17mg/L, 21.72 mg/L. (Table 2), respectively. and (Figure 10) shows the induced toxic effect and damages caused by the larvae. (larvicides MO WHO 2005) [18]

Fig 9: Stereomicroscopic view of Third instar larvae treated with control (H, head; T, thorax; A, Abdomen; LH, Lateral hairs; RS, Respiratory siphon; TG, Tracheal gills; VB, Ventral brush)

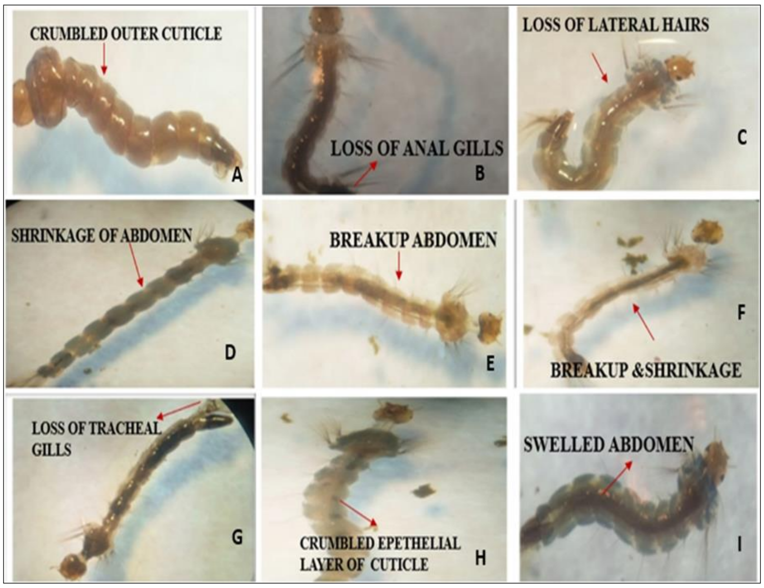

Fig 10: Stereomicroscopic view of the 3rd instar larvae, the treatment induced toxic effects on the larvae (red arrows) A: Crumbled Epithelial layer of cuticle B: Loss of anal gills C: Loss of lateral hairs D: Shrinkage of abdomen E: Breakup abdomen F: Breakup&Shrinkage G: Loss of Tracheal gills H: A crumbled epithelial layer of cuticle I: Swelled abdomen after treatment with plant extract and with ZnONPs extract.

Table 2: Showing the LC50 value of a particular extract of the compound and ZnONPs

Plant extract	Exposure period	Concentration	Log Concentration	Mean Mortality (%Dead)	Probit of Kill	LC50 (mg /L)
Petroleum ether	24 Hours	50	1.698	20	4.16	**229.08**
		100	2	30	4.48	
		150	2.176	50	5	
		200	2.301	40	4.75	
	48 Hours	50	1.698	30	4.48	**128.82**
		100	2	50	5	
		150	2.176	60	5.25	
		200	2.301	50	5	
Methanol	24 Hours	50	1.698	10	3.72	**205.116**
		100	2	30	4.48	
		150	2.176	50	5	
		200	2.301	30	4.48	
	48 Hours	50	1.698	20	4.16	**307.6**
		100	2	50	5	
		150	2.176	50	5	
		200	2.301	30	4.48	
ZnO NP's	24 Hours	10	1	20	4.16	**98.174**
		20	1.301	20	4.16	
		30	1.477	30	4.48	
		40	1.602	40	4.75	
	48 Hours	10	1	30	4.48	**21.727**
		20	1.301	40	4.75	
		30	1.477	50	5	
		40	1.602	80	5.84	

Conclusions

This study outlines an Eco-friendly method of Zn oxide Nanoparticles synthesized using Methanol, Pet ether, and an Aqueous extract of *Memocylon talbotianum* leaf. The structure, morphology, and size of Zn Nanoparticles were examined by UV–vis, XRD, SEM, EDX, and FTIR—analysis Uv–vis spectra of *M. talbotianium* ZnO NP's nanoparticles Absorption peak at 365nm. XRD and SEM images imply the flower structure of Zn nanoparticles with an average size ranged 10.9mm. EDX assessment established the purity of the synthesized Zn nanoparticles without any other impurity peaks. FTIR analysis shows the different functional groups.in larvicidal activity extract might be used directly as a larvicidal agent in small volumes in aquatic habitats these results could uplift the search for new active natural compounds offering an alternative approach to synthetic repellents and multi-drug insecticides from plants. However, in this research NPs biological efficiency on a large scale for identification in the therapeutic mechanisms.

Ethical Approval: (for research involving animals or humans) Not applicable

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript

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