**International Journal of Entomology Research**
**www.entomologyjournals.com**
**ISSN: 2455-4758**
**Received: 12-03-2024, Accepted: 03-04-2024, Published: 25-04-2024**
**Volume 9, Issue 4, 2024, Page No. 152-157**

# Nymphicidal effect of Green synthesized ZnO Nanoparticle on *Dysdercus cingulatus*

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**Abstract**
Biologically produced nanoparticles are widely used in various fields, including agriculture and health. Nanotechnology research indicates that green chemistry approaches might provide technologically significant nanomaterials. Zinc oxide nanoparticles appear to have low effect on human cells and a significant toxicity to microbes. The advantages of ZnO Nanoparticles are their low cost, safety and easy of preparation. Zinc oxide (ZnO) is a metal oxide that is GRAS (Generally recognized as safe) according to the US FDA. The presence of illnesses and insect pests has caused the output of cotton to decline in recent years, instead of increasing. One of the most dangerous pests of cotton is the red cotton insect, also known as the cotton stainer *Dysdercus cingulatus*. It attacks cotton at an early stage of growth and causes significant harm by sucking the growing cotton bolls during harvest. Because of their extreme mobility. It is challenging to manage with insecticidal treatments. The goal of the current investigation was to examine the synthesis and analysis of ZnO NPs derived from leaf extract of *Azadirachta indica*, *Calotropis gigantea* and *Ricinus communis*. XRD and FESEM were used to characterize the ZnO NPs. The nymphicidal impact of different concentration of green produced ZnO NPs was assessed against *Dysdercus cingulatus*, a significant pest for the cotton industry. Each of the studied concentrations (125, 250 and 500) successfully produced spectrum of nymphal mortality. It is concluded that ZnO NPs can be biologically synthesized very quickly, easily, affordably, environmentally and without any side effects and also it has the nymphicidal effect on Cotton pest.

**Keywords:** Green synthesis, *Azadirachta indica*, *Calotropis gigantea*, *Ricinus communis*, ZnO NPs, Nymphicidal effect, *Dysdercus cingulatus*.

## Introduction

*Dysdercus cingulatus* (Fig.1) is a severe pest of cotton that is found across India's cotton growing regions. Because it is very mobile, polyphagous and polymorphic pest of several *Malvaceae* crops, it is challenging to manage with insecticides Sahayaraja *et al* [1]. Since insect pests and illness have taken over, cotton yields have not increased but rather decreased in recent years. A poor yield of cotton crop is caused by about 162 different kinds of insect pests. Aphids, jassids, cotton stainer, white flies and thrips were among the sucking pests that affected cotton Kannan *et al* [2]. It begins to infest cotton while it is still being harvested. This pest spreads fungus that grows in the immature lint and seeds, sucking the growing cotton bolls and ripe cotton seeds, which results in significant damage *Asha et al* [3] Additionally, it has been documented that pesticides used on cotton plants have adversely affected stream habitats, worsening water quality and reducing the diversity of aquatic life Hose *et al* [4]. Approximately 2,40,000 fish were killed by pesticide-polluted spillage from Alabama cotton field in 1995 Ramanathan *et al* [5]. Because using insecticides in rural areas causes biological imbalances in the environment, certain countries, like India, have restricted the use of certain insecticides Yadav *et al* [6]. In the most significant fields of biology, chemistry, physics and material sciences, nanotechnology is a significant subfield. Nanoparticles have several uses in a variety of industries, including pharmaceuticals, electronics, medicine and as analytical agents. Several techniques, such as chemical, physical, irradiation but also biological ones, can be used to create nanomaterials. Due to the high production of hazardous byproducts from the chemical processes used in the synthesis of nanomaterials, the development of novel chemical and physical approaches has led to environmental contaminations. Novel secondary metabolites found in plant crude extract include phenolic acid, flavonoids, alkaloids and terpenoids; these substances are primarily in charge of the decrease in of ionic into the production of bulk metallic nanoparticles Aromal *et al* [7]. These primary and secondary metabolites are continuously producing environmentally benign nanoparticles through the redox reaction Kim *et al* [8]. Green synthesis, which comprises a safe, clean, ecologically benign and non-toxic way of creating nanoparticles, is therefore necessary. Furthermore, high pressure, energy, temperature and hazardous chemicals are not required in this procedure Parthasarathy *et al* [9]. These days, a lot of researchers are interested in ZnO NPs because of its unique optical and chemical behavior, which is easily adjustable by altering the shape. Zinc oxide nanoparticles (ZnO NPs) are a member of the broad family of metal oxide nanoparticles and have found utility in a wide range of utilizing diverse fields, including biology, electronics, communication, sensing, cosmetics and environmental protection. Furthermore, due to its antibacterial, antifungal, acaricidal, pediculicidal, larvicidal and anti-diabetic properties, zinc oxide nanoparticles exhibit great potential in biological applications such as biological sensing, biological labelling, gene delivery, drug delivery and nanomedicine Rasmussen *et al* [10], Applerot *et al* [11], Sharma *et al* [12]. The goal of the current investigation was to determine if biosynthesized Zinc oxide nanoparticles made with *Azadirachta indica, Calotropis gigantea* and *Ricinus communis* leaf extract might effectively combat *Dysdercus cingulatus* insecticidal properties.

## Materials and Methods

### Collection and Preparation of plant extract

The leaves were collected in and around Vellore district. Fresh plant materials were dried after being cleaned under flowing tap water. *Azadiracta indica, Calotropis* and *Ricinus* leaves were crushed separately. Then using 100ml of each distilled water, these coarse particles 10g were extracted one at a time. After being stored, the extract was used for further purpose.

### Synthesis of Zinc oxide Nanoparticle

Zinc oxide nanoparticle preparation: to create ZnO nanoparticles, modified methods from earlier research on green synthesis have been used in the study, which is published in Vidya *et al* [13] using a stirrer heater, 50ml of plant leaf extract were heated to 60-80 degree Celsius in order to synthesis nanoparticles. Once the temperature hit 80 $^{o}$ c when the color changes, 5g of zinc nitrate hexahydrate was added to the mixture. After that the combination was brought to a boil and turned into a suspension with deep yellow color. This was kept in a hot air oven at 100$^{o}$c for 1hr to get yellow color paste and dry. This paste was the gathered in a ceramic crucible and heated for 2 hours at 400$^{o}$c in an air fired furnace. After obtaining a powder with light white color, it was carefully collected and submitted for various characterizations.

### Collection of Insect and Nymphicidal activity

The adult (Fig.1) and nymphs of *Dysdercus cingulatus* were collected from the cotton fields. Using a cleaned plastic container with a perforated cover, the insect was reared in a laboratory condition and daily fed with a leaf of *Ricinus communis* and honey-soaked cotton. The nymphs were randomly selected for insecticidal activity. 5 insects were kept in plastic containers and brushed them with the various concentration of biosynthesized zinc oxide nanoparticles (125,250,500 ppm) Every test was conducted in triplicate (n=15) (Fig 2). The control was sterile, nanoparticle free distilled water. The mortality was determined 24 and 48 hrs.' of exposure. After 24 and 48 hrs.' of exposure period, the average mortality of three duplicates of the insect was reported. The EPA was used to derive the LC 50 value. Version 1.5 of the probit analysis.

### Percent nymphal mortality

The experimental and control nymphs of *D. cingulatus* were studied in order to determine the % mortality using the formula proposed by Thangam and kathiresan (1992).

$$\%\ \text{Mortality} = \frac{\text{No. of dead nymphs}}{\text{No. of treated nymphs}} \times 100$$

## Results and Discussion

XRD gives a crystalline nature of ZnO NPs. The average crystalline size of the generated nanoparticles was calculated using Scherrer's formula and it was discovered to be 26nm (Fig. 3), 19nm (Fig 4) and 27nm (Fig 5). The 2 theta values of 31.62, 34.70,36.30, 47.60, 56.65, 62.82, 66.03. These results are consistent with those that demonstrated the crystalline structure of nanoparticles exhibiting comparable peak indices for zinc oxide nanoparticles produced utilizing various extract of *Cassia fistula, Melia azedarach, Calotropis gigantea* Sesime *et al* [14].

### FESEM analysis of Zinc oxide NPs

The agglomerated shape of nanoparticles is confirmed by the surface morphology. The zinc oxide nanoparticle size and shape were found to be 40-70nm (Fig 6a) in *Azadirachta indica* leaf extract, 89-129nm (Fig 7a) in *Calotropis gigantea* leaf extract and 55-106nm (Fig 8a) in *Ricinus communis* leaf extract according to the FESEM analysis.

### EDX analysis

The presence of zinc in the oxide form is confirmed by the strong signal for zinc and oxygen seen in the EDX analysis. The EDX provides the composition of each element present in the analyte; significant peaks measuring 66.2 % for zinc and 33.8 % oxygen are obtained (Fig. 6b) in *Azadirachta indica* leaf extract. 31.84 % zinc and 68.16 % oxygen were obtained (Fig. 7b) in *Calotropis gigantea* leaf extract. 16.66 % zinc and 83.34 % oxygen were obtained (Fig. 8b) in *Ricinus communis* leaf extract. These weight percent peaks are similar to those previously reported for the nanoparticle synthesis. these results are unique to oxygen and zinc, confirming the elemental makeup for the produced molecule.

### Nymphicidal activity of *Dysdercus cingulatus*

We selected *D. cingulatus* nymphs in their third instars to assess their mortality. During a 24-hour observation period, the maximum mortality percentage of 86% was noted at 250 and 500 ppm of ZnO NPs produced by *C. gigantea* and 500 ppm of ZnO NPs synthesized by *A. indica*. Over the course of a 48-hour observation period, the highest death rate of 93% was recorded at 500 ppm of ZnO NPs produced by *C. gigantea*, 100% at 500 ppm of ZnO NPs produced by *R. communis*, and 250 and 500 ppm of ZnO NPs produced by *A. indica*.

### One-way ANOVA analysis of Nymphicidal effect of ZnO NPs in 24 hrs of Treatment

The obtained F value in Table (2) is 3.154. Their p-value, or significant value, was attained at 0.061. P value and 0.05 are compared. The obtained p value is found to be greater than 0.05 upon comparison. Consequently, there isn't a statistically significant variation in the group means. The significant (p) values exceed the alpha value ($p<0.05$) in each of the three scenarios. It is clear from the data that R1, C2, and AZ do not differ statistically significantly. The magnitude of the mean for each group is shown by the height of each bar or the location of each point along this axis in figure (9). It is clear from the graph that C2 has a low magnitude and AZ has a high magnitude.

### LC 90 and LC 50

In (Table- 1) *Calotropis gigantea* leaf extract synthesized ZnO NPs showed the mortality of LC 50 = 19.563 ppm with Chi square value of 0.246. *Ricinus communis* leaf extract synthesized ZnO NPs showed the LC 50 value = 2.795ppm with Chi square value of 0.065. *Azadirachta indica* synthesized ZnO NPs showed the highest mortality rate of

LC 50 value = 79.321 ppm with Chi square 0.046.

In (Figure - 10) comparison to the other treatments, treatment *R. communis* exhibits the lowest LC50 (2.795) and LC 90 (11128.565) values, suggesting a higher level of toxicity. In comparison to the other treatments, treatment *A. indica* exhibits the greatest LC 50 (79.321) and LC 90 (2892467.0) values, indicating a lower level of toxicity. Slope and intercept differ between treatments, indicating that *Dysdercus* nymphs respond differently to various treatments. A better match to the observed data is shown by lower $\chi 2$ values (0.046 for treatment *A. indica*). *Dysdercus* nymphs seem to respond most toxically to treatment R. communis, treatment *C. gigantea*, and treatment *A. indica*.

**Table 1:** Nymphicidal activity of green synthesized Zinc oxide nanoparticle on *Dysdercus cingulatus*

| **Descriptives** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Test** | **N** | **Mean** | **Std. Deviation** | **Std. Error** | **95% Confidence Interval for Mean** | | **Minimum** | **Maximum** |
| | | | | | **Lower Bound** | **Upper Bound** | | |
| C2 | 9 | .8889 | .78174 | .26058 | .2880 | 1.4898 | .00 | 2.00 |
| R1 | 9 | 1.2222 | .83333 | .27778 | .5817 | 1.8628 | .00 | 2.00 |
| AZ | 9 | 2.1111 | 1.45297 | .48432 | .9943 | 3.2280 | .00 | 4.00 |
| Total | 27 | 1.4074 | 1.15223 | .22175 | .9516 | 1.8632 | .00 | 4.00 |

| **ANOVA** | | | | | |
|---|---|---|---|---|---|
| Test | | | | | |
| | Sum of Squares | df | Mean Square | F | Sig. |
| Between Groups | 7.185 | 2 | 3.593 | 3.154 | .061 |
| Within Groups | 27.333 | 24 | 1.139 | | |
| Total | 34.519 | 26 | | | |

**Table 2:** One-way ANOVA Analysis of Nymphicidal activity of *D. cingulatus* after 24 hrs

| ***Dysdercus nymphs*** | **$LC_{50}$** | **95% Confidence Limit** | | **$LC_{90}$** | **95% Confidence Limit** | | **Slope ± SE** | **Intercept ± SE** | **$\chi^2$** |
|---|---|---|---|---|---|---|---|---|---|
| | | **LL** | **UL** | | **LL** | **UL** | | | |
| C2 | 19.563 | - | - | 621.871 | - | - | 0.85±0.91 | 3.89±2.16 | 0.246 |
| R1 | 2.795 | - | - | 11128.565 | - | - | 0.35±0.83 | 4.84±1.99 | 0.065 |
| AZ | 79.321 | - | - | 2892467.0 | - | - | 0.28±0.76 | 4.46±1.83 | 0.046 |

## Figures

**Fig 1:** *Dysdercus cingulatus* adult

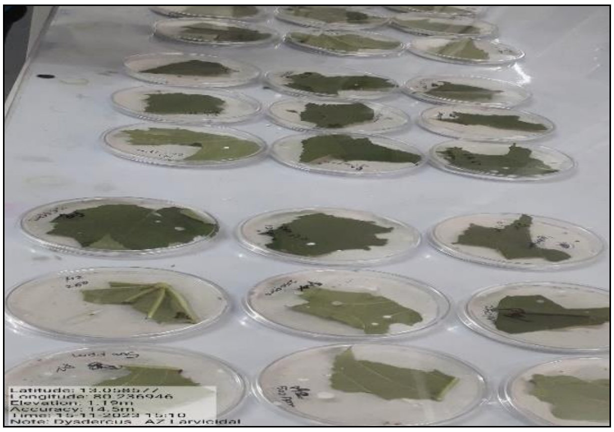


**Fig 2:** Nymphicidal activity treatment using ZnO NPs

## XRD analysis of ZnO NPs

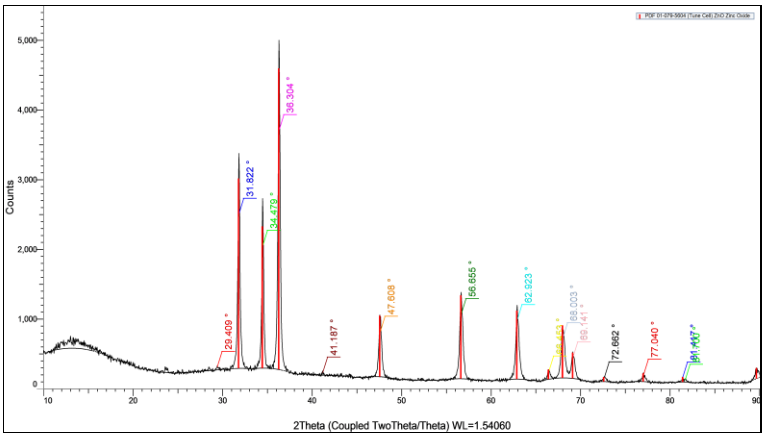



**Fig 3:** XRD Spectrum of Zinc oxide NPs by *Azadirachta indica* leaf

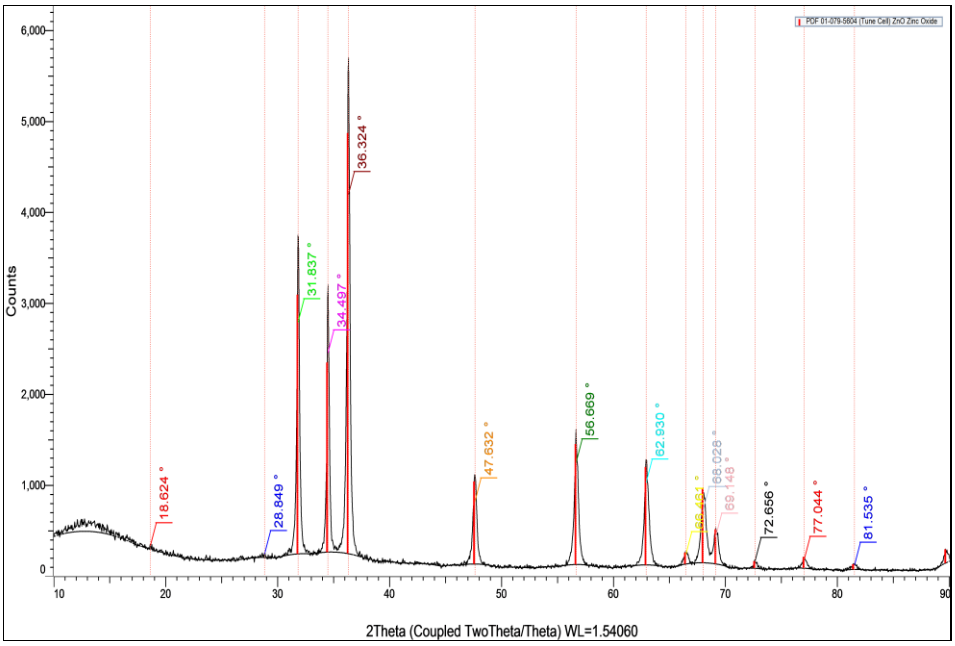



**Fig 4:** XRD Spectrum of Zinc oxide NPs by *Calotropis gigantea* leaf

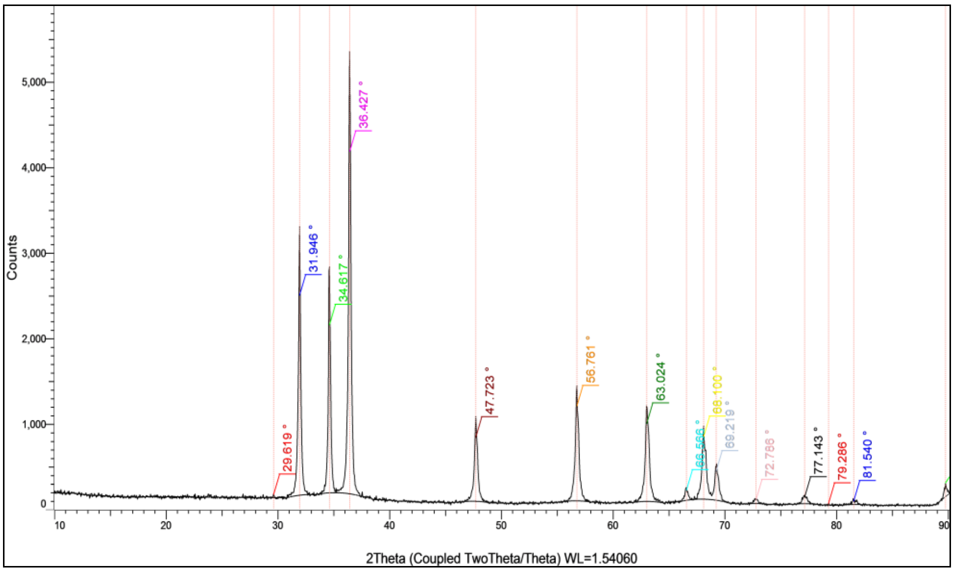
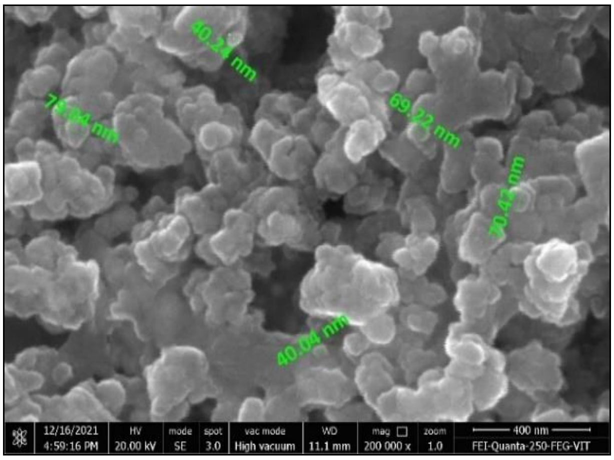



**Fig 5:** XRD Spectrum of Zinc oxide NPs by *Ricinus communis* leaf

## FESEM Analysis of ZnO NPs



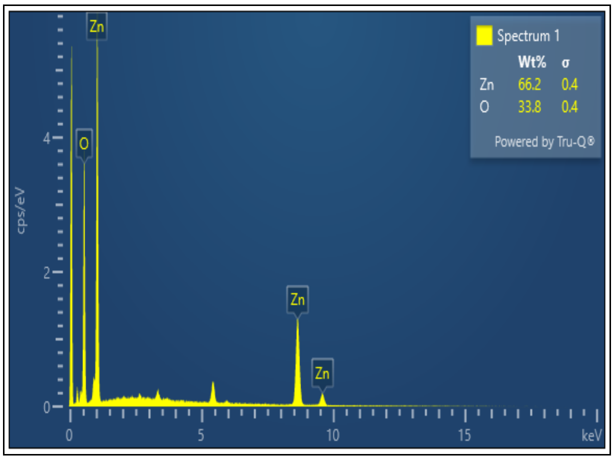
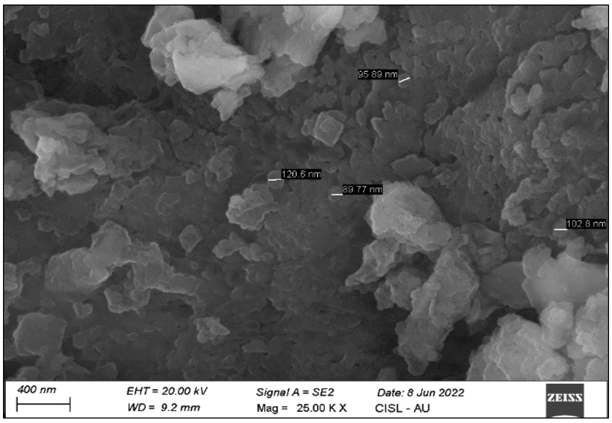




**Fig 6a):** 40-70nm **Fig 6b):** EDX revealed the peak of ZnO NPs

**Fig 6:** FESEM image and EDAX peaks of ZnO NPs synthesized by *Azadirachta indica* leaf extract



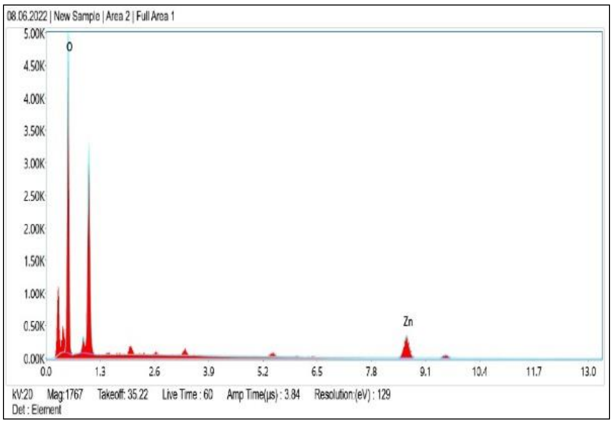
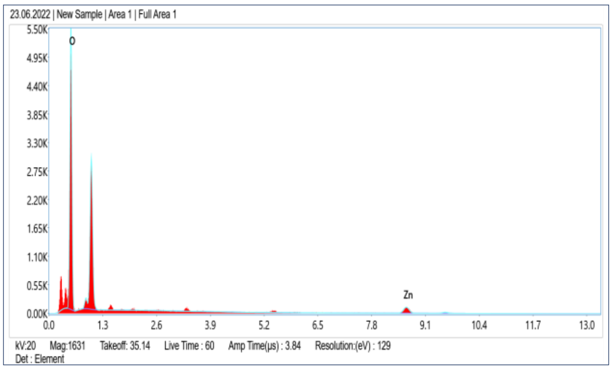



**Fig 7a):** 89-120nm **Fig 7b):** EDX revealed the peak of ZnO NPs

**Fig 7:** FESEM image and EDAX peaks of ZnO NPs synthesized by *Calotropis gigantea* leaf extract



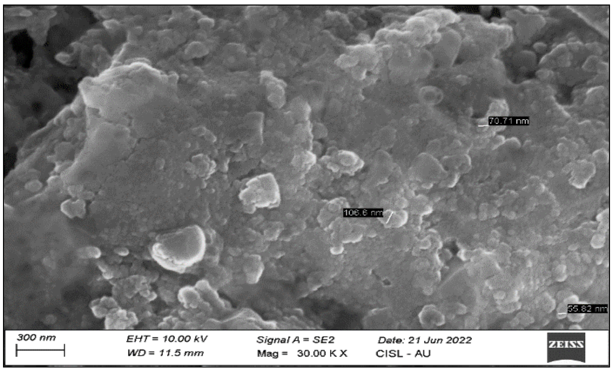
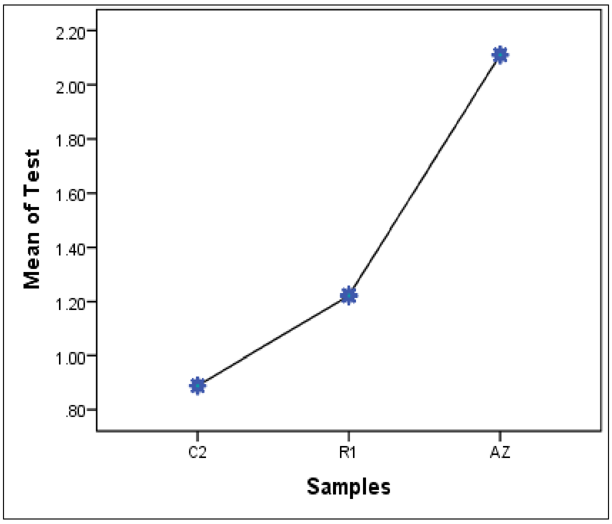



**Fig 8a):** 55-106nm **Fig 8b):** EDX revealed the peak of ZnO NPs

**Fig 8:** FESEM image and EDX peaks of ZnO NPs by *Ricinus communis* leaf extract



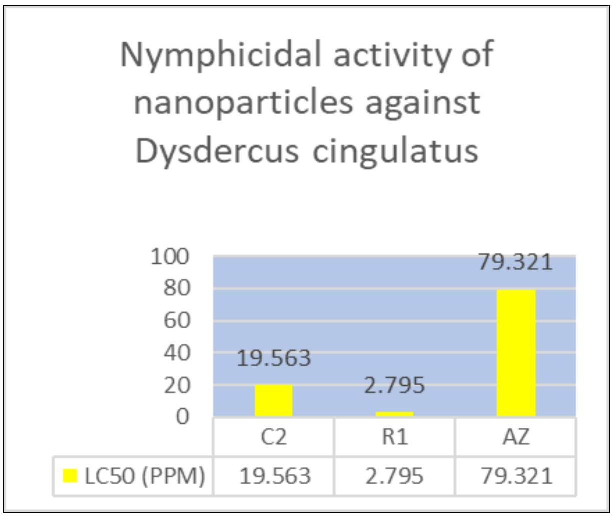




**Fig 9:** Means Plots

**Fig 10:** Nymphicidal activity of ZnO NPs against *Dysdercus cingulatus*

The seaweed *Padina pavonica* has been shown by Sahayaraj and Kalidas (2011) to have nymphicidal and ovicidal effects on the cotton pest *D. cingulatus*. Asha *et al* [15] conducted tests on *D. cingulatus* third instar nymphs to determine the biological effect of hexane, chloroform, methanol and water extract of *Ulva fasciata* and *Ulva lactuca* Linnaeus at varying concentrations (100-800 ppm). At 96 hours there was greater nymphicidal activity in the methanol extraction of *Ulva fasciata* (LC 50 = 313.59 ppm) and *Ulva lactuca* (LC 50 = 399.27 ppm). The evaluation of the nymphicidal effects of two indigenous plant extracts, such as *Adathoda vasica* and *Vitex negundo* crude methanolic leaves, on the cotton pest *Dysdercus cingulatus*, was reported by Ranilalitha *et al* [16]. The findings showed that, at concentrations of 0.8 and 1.0%, *Vitex negundo* caused more nymphal fatalities than the other plant antifeedant. According to Ramanathan *et al* [17] the plant *Azadirachta indica* leaf extract was used to biosynthesized silver nanoparticles and the results of the dose-dependent test indicated that the LD 50 value was recorded as 1.64 mg/l in 48hrs. 100% of insecticidal activity was seen in 25 mg/l and 20mg/l. In the present findings revealed that the *Azadirachta indica* leaf synthesized ZnO NPs showed the highest mortality in nymphs of *Dysdercus cingulatus* in 24 hrs of treatment.

**Conclusion**

In this work, leaf extract from *Azadiracta indica, Calotropis gigantea* and *Ricinus communis* was used to produce ZnO NPs. When the nanoparticles were for nymphicidal action, they proved to be successful. FESEM and XRD were used to analyse the nanoparticles. The FESEM revealed the size of the ZnO NPs to be between 40-70nm, 89-120nm and 55-106nm and the EDX demonstrated the presence of ZnO NPs by highlighting their peaks. The XRD patterns revealed the precise peaks of zinc oxide nanoparticles as well as the crystallite size of 26nm, 19nm and 27nm. This work made it clear that *Azadirachta indica, Calotropis gigantea* and *Ricinus communis* plant leaves may be utilised to create ZnO nanoparticles for a variety of uses by employing green chemical techniques. In the present study *Azadirachta indica* synthesized ZnO NPs of 40-70nm showed the highest nymphicidal activity compared with others.

**Financial support and Sponsorship**

Nil

**Conflict of Interest**

There are no conflicts of interest

**Acknowledgement**

We are grateful to the Administrator and Director of ERI Dr. J. Merline Shyla, Dr. V. Duraipandiyan, Scientist and Dr. M. Muthupandi, Scientist of ERI, Loyola college, Chennai for the laboratory facilities and guidance. And also, thanks to VIT university, Vellore for characterizing samples by XRD and FESEM Instruments.

**Abbrevations**

AZ – *Azadirachta indica*
C2 – *Calotropis gigantea*
R1 – *Ricinus communis*
ppm – Parts per million
XRD- X-Ray diffractometry
FESEM – Field Emission Electron Microscope
ZnO – Zinc oxide
NPs – Nanoparticle
nm – Nano-meter
LC 50 & LC 90 – Lethal concentration

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