Research Article

# Comparative Efficacy of *Eucalyptus globulus* Labill, Extracts Along With Their Green Synthesized Zinc Oxide Nanoparticles against Brassica Aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae)

**Muhammad Aqib Idrees[1]*, Ahmad Kamran Khan[2]*, Muhammad Shahid Nisar[2], Irfan Ahmed[3], Muhammad Irfan[2], Sadiqa Rukhsar[4] and Shujaat Hussain[5]**

*[1]Department of Agriculture Entomology & Pest Control, College of Plant Protection, Northwest, A&F University, Yangling, 712100, PR China; [2]Department of Plant Protection, Ghazi University, Dera Ghazi Khan, Punjab, Pakistan; [3]Department of Animal Nutrition, faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Punjab, Pakistan; [4]Department of Plant Breeding & Genetics, Ghazi University, Dera Ghazi Khan, Punjab, Pakistan.; [5]University of Agriculture, Faisalabad, Punjab, Pakistan.*

**Abstract** | *Brevicoryne brassicae* L. is the most destructive pest of brassica crops and causes huge yield losses. In this regard, experiments were conducted to determine the toxicity of *Eucalyptus globulus* extract as well as the zinc oxide nanoparticles mediated by this plant extract against *B. brassicase*. The Soxhlet apparatus was used for the extraction of plant extracts. Zinc oxide nanoparticles were synthesised by adding 0.082 g of zinc oxide and 10 ml of plant extracts to make a 1 mM solution in 1000 ml of distilled water at room temperature. The confirmation of nanoparticles was done by UV Spectrometry. The efficacy of plant extracts and *ZnO* nanoparticles the bioassay was performed by applying five concentrations of plant extracts (20, 10, 05, 2.5, and 1.25 percent) and ZnO nanoparticles (800, 400, 200, 100, and 50 ppm). Experiments were conducted under CRD with three replications of each concentration. Results indicated that *E. globulus* extract showed the highest mortality after 96 hr. While *ZnO* nanoparticles of *E. globulus* showed 78% and 65% mortality at 800 ppm, respectively, after 96 hours against *B. brassicae*, The *LC50* of *E. globulus*-mediated zinc oxide nanoparticles against *B. brassicae* was 386.110 and 271.599 ppm after exposure times of 96 hr, respectively. While the LC50 of *Eucalyptus globulus* extracts against *B. brassicae* was 5.48525 after an exposure time of 96 hr, respectively. It was found that synthesised nanoparticles zinc oxide are more effective at controlling aphids. The findings of this research will be useful in the managing *B. brassicae* infestations.

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***Correspondence** | Muhammad Aqib Idrees, Department of Agriculture Entomology & Pest Control, College of Plant Protection, Northwest, A&F University, Yangling, 712100, PR China; Ahmad Kamran Khan, Department of Agriculture Entomology & Pest Control, College of Plant Protection, Northwest, A&F University, Yangling, 712100, PR China; **Email:** aidrees925@gmail.com
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## Introduction

*Brevicoryne brassicae* (Hemiptera: Aphididae), commonly known as cabbage aphid, is a common pest in agricultural areas and has spread worldwide (Ahmad *et al*., 2020). It is a severe international pest and one of the most serious vegetable pests (Kanwal *et al*., 2023). It multiplies fast under favourable conditions on the leaves, stems, and inflorescences from which they suck the sap (Nematollahi *et al*., 2014, Rene *et al*., 2024). It causes direct as well as indirect quantitative and qualitative losses and the transmission of certain viruses to a specific crop (Roux *et al*., 2007). It has a strong impact on turnips, Brussels sprouts, and mustard, causing wrinkles and chlorotic markings on the leaves (Luria *et al*., 2016; Kroes *et al*., 2017; Soh *et al*., 2018). Because of the infestation, the growth of injured pods and seeds is slowed and affected seeds and pods were stunted (Aslam and Razzaq, 2007; Aslam *et al*., 2011; Syed *et al*., 2023).

The yield losses due to aphid infestation on brassica plants have been reported to be 30-35 percent, with losses exceeding 90 percent in some cases (Talekar and Shelton, 1993; Amer *et al*., 2010; Arif *et al*., 2012; Jabeen and Farooq, 2020). The current control of *Brevicoryne brassicae* infestations in the field is mostly based on the widespread use of chemical pesticides to reduce economic losses and maintain profitable crop production (Ali *et al*., 2015, Maqsood *et al*., 2023). Insecticide resistance is a widespread and public concern, so the use of natural pest management products is gaining traction (Moharramipour and Negahban, 2014). Biopesticides are pesticides generated from natural sources such as plants, animals, microbes, and minerals (Rai and Ingle, 2012; Fira *et al*., 2018; Aisvarya *et al*., 2021; Bahrun *et al*., 2025). In nature, many botanical insecticides can be biodegraded into harmless compounds (Kim *et al*., 2010). Botanical extracts have some insecticidal properties, and almost 2400 species of bioactive plants were identified. *Eucalyptus*, a member of the Myrtaceous family, is one of the most frequently cultivated genera in the world (Mousa *et al*., 2013). It contains bioactive terpenoids and polyphenols such as flavonoids, phloroglucinol derivatives, and tannins (Mohamed *et al*., 2007). The leaves of around 300 different species of the *Eucalyptus* genus produce toxic oils (Elaissi *et al*., 2012; Ghalem and Mohamed, 2014). Their leaves contain a variety of terpene compounds, namely 8-cineole (CIN), globulol, terpineol, and pinene (Lucia *et al*., 2012). The monocyclic monoterpene is the primary constituent of several *Eucalyptus* species (Mann and Kaufman, 2012). Several studies on *Eucalyptus* species have reported the separation of different phytoconstituents. In different solvent extracts of the bark, phytochemical components like fatty acids, polyphenolics, terpenes, alkaloids, glycosides, flavonoids, tannins, alkaloids, saponins, lignins, vitamin C, phenolics, flavones, and anthraquinone are found (Kumar and Laxmidhar, 2011; Godghate and Sawant, 2014). In contrast, leaf essential oils were discovered to contain flavonoids, glycosides, tannins, steroids, alkaloids, and terpenoids (Şen and Gençer 2023). A similar study on *Eucalyptus globulus* detected the presence of chemicals including Stigmasterol, Eucalyptone G, 7-Trimethoxy-kaempferol, Catechin, Epicatechin, and Octyl-β-D glucopyranoside (Beigh *et al*., 2023). Nanoparticles have unique properties due to their smaller size (10-9 m). Their small size provides them with exceptional surface-to-volume ratios, allowing them to limit electron movements inside boundaries while increasing optical characteristics. This makes them very valuable in a variety of applications such as agriculture (Rai and Ingle, 2012), medicine (Boisseau and Loubaton, 2011), water purification (Bhattacharya *et al*., 2013), solar cells (Stelzner *et al*., 2008), cosmetics (Raj *et al*., 2012), drug delivery (Suri *et al*., 2007), textiles (Wong *et al*., 2006), food (Duncan, 2011), and electronics (DeFranceschi and Kouwenhoven, 2002).

The biosynthesis of plant-mediated nanoparticles can be divided into three stages: reduction, growth, and stability and the most important step is the "reduction" phase (Kim *et al*., 2010; Malik *et al*., 2014). Nanomaterials create a safe and easy environment, and green processes have the potential to improve agriculture by enhancing the fertilisation process, the delivery of active components to the desired target sites, plant development and insecticides (Ghidan *et al*., 2016; Akhreim *et al*., 2024); the treatment of wastewater, and enhancing the absorption of nutrients in plants (Srilatha, 2011). Furthermore, reduce the number of toxic substances that destroy the environment. As a result, this technology contributes to the reduction of environmental pollutants (Huang *et al*., 2015; Aisvarya *et al*., 2021). Zinc oxide (*ZnO*) nanoparticles are the most well-known compound used in agriculture (Mohanpuria *et al*., 2008; Mitra *et al*., 2012; Aslam *et al*., 2023). Biological approaches, often known as green methods, include the use of microbes and plant extracts to synthesise NPs that are both cost-effective and environmentally friendly. Plant extracts use polyphenols, alkaloids, phenolic acids, and terpenoids as reducing and capping agents to convert metals into NPs (Duan *et al*., 2015).

## Materials and Methods

*Preparation of plant extract*

Leaves of *Eucalyptus globulus* Labill (Safaida) were collected from the forestry area at the University of Agriculture, Faisalabad. To remove contaminants, the plant leaves were washed with tap water and then thoroughly cleaned with distilled water. Under shade, dried leaves were ground into powder through a grinder. Then, in a bottom flask, 50 g of plant powder was added to a Soxhlet apparatus with 300 mL of methanol. After completing 6 to 8 cycles, extracts were collected in a flask, and the sample was kept at 4°C as a stock solution (Barzinjy and Azeez, 2020; Ullah *et al*., 2021).

*Synthesis of ZnO nanoparticles*

ZnO nanoparticles were synthesized using a green synthesis approach with *Eucalyptus globulus* leaf extract as a reducing and stabilizing agent. The synthesis was carried out by dissolving 0.082 g of zinc oxide precursor in 1000 mL of distilled water to prepare a 1 mM ZnO solution (calculated as 82 mg/L or 82 ppm based on ZnO molecular weight). To this solution, 10 mL of *Eucalyptus globulus* leaf extract was added, and the mixture was stirred using a magnetic stirrer at room temperature for 10 minutes to ensure proper mixing. The reaction mixture was then incubated at 28°C, where chemical reduction reactions commenced after 48 hours of incubation, leading to the bio-reduction of $Zn^{2+}$ ions to ZnO nanoparticles. The appearance of a color change indicated the formation of ZnO nanoparticles, which remained stable in the aqueous medium. The final nanoparticle suspension was then concentrated through evaporation to achieve higher concentrations (up to 800 ppm) for subsequent bioassays.

*Confirmation of Zinc Oxide nanoparticles*

UV spectrometry was used for nanoparticle confirmation.

*Bioassay 1*

The extracts of two plants were used to make ZnO nanoparticles that killed *B. brassicae* L. Five concentrations (800, 400, 200, 100, and 50 ppm) of each plant extract mediated ZnO nanoparticles were prepared through serial dilution of the concentrated stock solution, and the toxic effect was evaluated by leaf dip bioassay. For this bioassay, brassica leaves were dipped in dilutions of nanoparticles. Then the leaves were dried and kept in Petri dishes. Ten nymphs of aphids were released in each petri dish and placed at room temperature. Each treatment was replicated three times along with the control treatment under a CRD (Completely Randomised Design). Mortality data was noted after 24, 48, 72, and 96 hr.

*Bioassay 2*

Biocidal effect of plant extract against *Brevicoryne brassicae* L. was observed under laboratory conditions.

*Data analysis*

The ANOVA test and mean comparison values were calculated using the Statistical software 8.1. Graphs were generated using Origin software 8.1.

## Results and Discussion

*UV–spectroscopy performance*

The UV-absorption spectrum of Tobacco extract-mediated Zinc oxide nanoparticles synthesised from various plant extracts showed different absorption peaks in UV spectrometry (Figure 1). In the range of 250-700 nm, Zinc oxide NPs synthesised with *Eucalyptus globulus* (Safaida) extract showed an absorption maximum at 310 nm. Furthermore, there was no extra peak observed in the spectrum, which indicated that the *ZnO* NPs were pure.

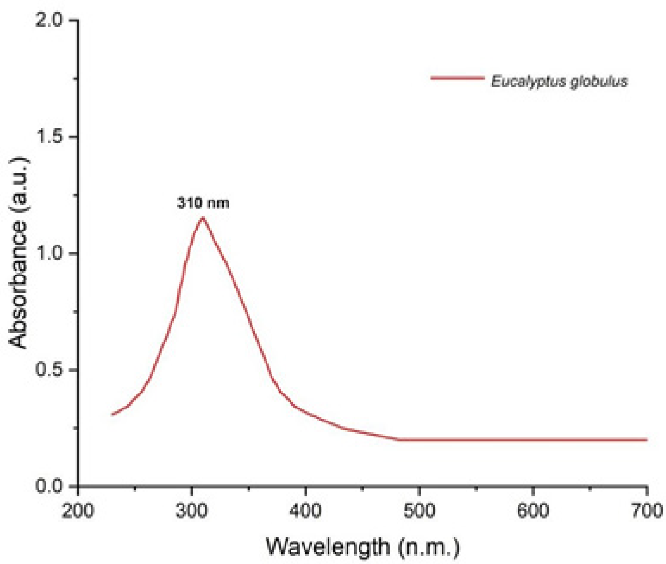


**Figure 1:** *UV-absorption spectrum of eucalyptus extracts mediated ZnO NPs*

**Table 1:** *$LC_{50}$ values of eucalyptus globulus mediated zinc oxide nanoparticles after different time intervals*

| Time | $LC_{50}$ | Fiduciallimits lower-upper | Slope | Chi-square |
|---|---|---|---|---|
| 24 h | 8988.92 | 2470.04 -998051 | 0.565981 | 0.966930 |
| 48 h | 2692.50 | 1180.81 -23861.4 | 0.418346 | 1.27470 |
| 72 h | 1341.44 | 790.348 -3916.38 | 0.483267 | 0.877317 |
| 96 h | 386.110 | 299.630 -523.265 | 0.558278 | 1.53829 |

**Table 2:** *Mean comparison test for percent mortality of B. brassicae after24hr treatment of E. globulus mediated Zinc oxide nanoparticles*

| Concentrations(ppm) | Mean±S.E. |
|---|---|
| 800 | 20.67 ± 3.33a |
| 400 | 15.67± 3.46ab |
| 200 | 10.00± 0.33ab |
| 100 | 6.67± 3.33ab |
| 50 | 3.33± 3.04b |
| Control | 0.00± 0.00b |

**Table 3:** *Mean comparison test for percent mortality of B. brassicae after 48 hr treatment of E. globulus mediated Zinc oxide nanoparticles*

| Concentrations(ppm) | Mean ± S.E. |
|---|---|
| 800 | 36.67 ± 2.23a |
| 400 | 30.33± 0.21b |
| 200 | 16.67± 3.33b |
| 100 | 12.81± 3.58b |
| 50 | 6.33± 3.67b |
| Control | 0.00 ± 0.00c |

*Efficacy of different concentrations of Eucalyptus globulus-mediated Zinc oxide nanoparticles against Brevicoryne brassicae after 24, 48, 72, and 96 hr of treatment*

The analysis of variance demonstrated that different concentrations of *E. globulus*-mediated ZnO nanoparticles had significant toxic effects on *B. brassicae* nymphs under laboratory conditions. with mortality rates increasing substantially over time and with higher concentrations. At the 24-hour treatment interval, a clear dose-dependent response was observed (Figure 2A; Table 2). The 800 ppm concentration produced the highest mortality at 20.67%, followed by decreasing rates at lower concentrations: 400 ppm (15.67%), 200 ppm (10%), 100 ppm (6.67%), and 50 ppm (3.33%). Extended exposure to 48 hours enhanced the nanoparticles' efficacy across all concentrations (Figure 2B; Table 3). Mortality rates nearly doubled, with 800 ppm reaching 36.67%. The progressive reduction in mortality continued with 400 ppm (30.33%), 200 ppm (16.67%), 100 ppm (12.81%), and 50 ppm (6.33%). The 72-hour treatment period demonstrated markedly increased toxicity (Figure 2C; Table 4). The highest concentration achieved 63.67% mortality, while intermediate concentrations showed substantial increases: 400 ppm (48.33%), 200 ppm (24.83%), 100 ppm (17.50%), and 50 ppm (14.67%). Maximum efficacy was reached after 96 hours of treatment (Figure 2D; Table 5). The 800 ppm concentration achieved 77.67% mortality, with other concentrations following the established pattern: 400 ppm (68.62%), 200 ppm (48.10%), 100 ppm (24.10%), and 50 ppm (12.8%). Control treatments showed no mortality through 72 hours, with minimal mortality (6.67%) observed only at the 96-hour interval.

**Table 4:** *Mean comparison test for percent mortality of B. brassicae after 72 hr treatment of E. globulus mediated Zinc oxide nanoparticles*

| Concentrations(ppm) | Mean±S.E. |
|---|---|
| 800 | $63.67 \pm 4.00^{a}$ |
| 400 | $48.33\pm 4.33^{ab}$ |
| 200 | $24.83\pm 4.00^{ab}$ |
| 100 | $17.50\pm 3.33^{ab}$ |
| 50 | $14.67\pm 2.50^{ab}$ |
| Control | $0.00\pm 0.00^{b}$ |

**Table 5:** *Mean comparison test for percent mortality of B. brassicae after 96 hr treatment of E. globulus mediated Zinc oxide nanoparticles*

| Concentrations(ppm) | Mean±S.E |
|---|---|
| 800 | $77.67 \pm 4.67^{a}$ |
| 400 | $68.62\pm 3.67^{ab}$ |
| 200 | $48.10\pm 3.33^{abc}$ |
| 100 | $24.10\pm 2.23^{bc}$ |
| 50 | $12.8\pm 1.25^{bc}$ |
| Control | $6.67 \pm 0.67^{c}$ |

*$LC_{50}$ values of different concentrations of Eucalyptus globulus-mediated Zinc oxide nanoparticles against Brevicoryne brassicae at different intervals of time*

Table 1 showed that the $LC_{50}$ of *E. globulus*-mediated *ZnO* nanoparticles after 24 hr was 8988.92 ppm, the slope line was 0.57, the fiducial limit at the 95% confidence interval was 2470.04-998051, and the chi-square value was 0.97. The LC50 of *E. globulus*-

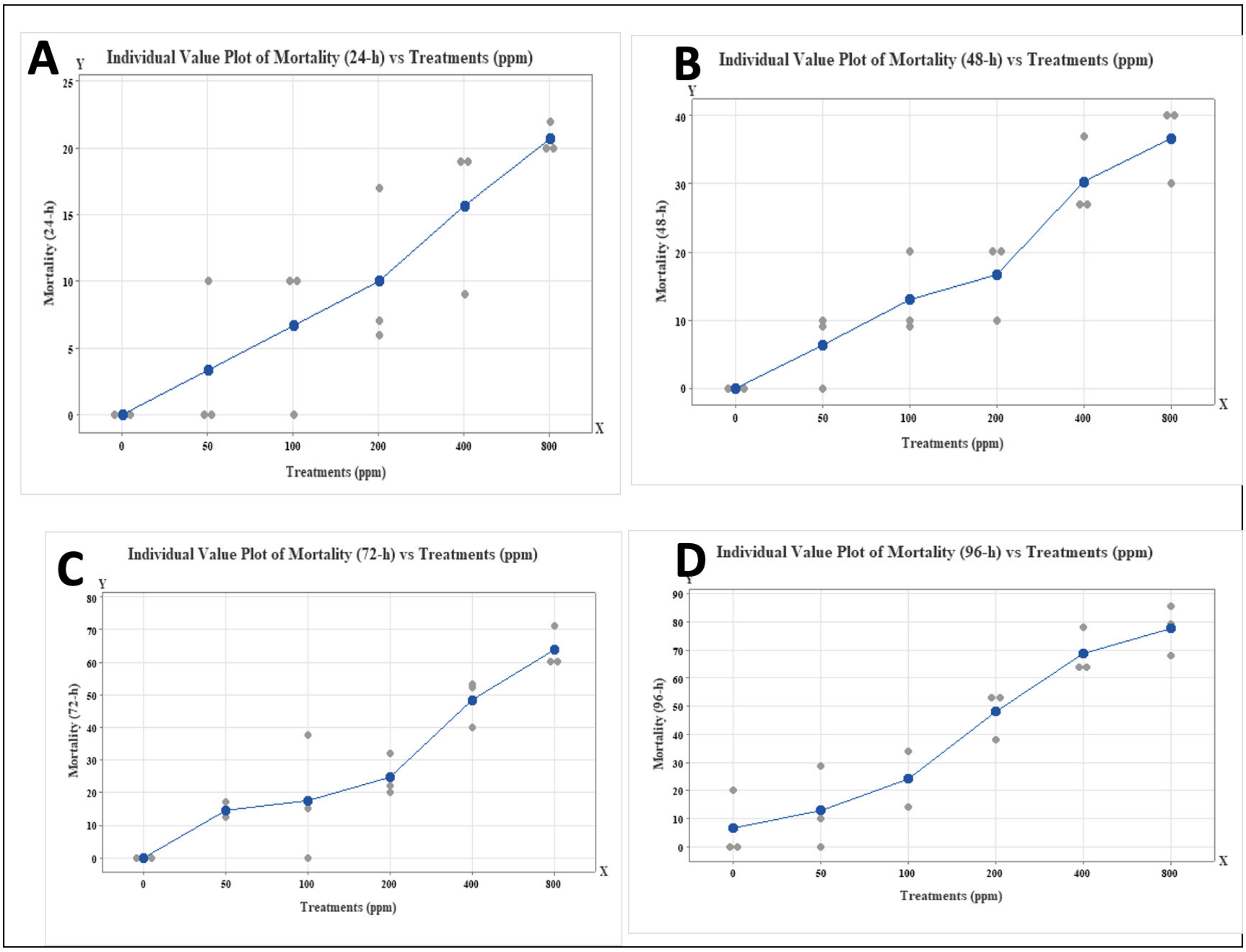


**Figure 2:** *Individual value plot of percent mortality of Brevicoryne brassicae after 24, 48 hr of Eucalyptus globulus-mediated Zinc oxide nanoparticles*

*globulus* mediated *ZnO* nanoparticles after 72 hours was 1341.44, the slope line was 0.48, the fiducial limit at the 95% confidence interval was 790.34-3916.38, and the chi-square value was 0.88. $LC_{50}$ of *E. globulus*-mediated ZnO nanoparticles after 96 hr was 386.11, slope line 0.56, fiducial limit at 95% confidence interval was 299.63-523.26, and chi-square value was 1.54. Green synthesised *ZnO* nanoparticles of *Eucalyptus globulus* showed 78% mortality at 800 ppm after 96 hr under laboratory conditions (Table 5), 400 ppm showed 67% mortality, 200 ppm showed 48% mortality, 100 ppm showed 24% mortality, and 50 ppm showed 12% mortality of aphids after 96 hours. The death rate of *Brevicoryne brassicae* L. decreased with decreasing concentrations and time.

Similar results have been obtained by different workers, like Amjad *et al.* (2022), who reported that silver nanoparticles revealed 93% mortality of *Sitobion avenae* at 600 ppm after 2 days. Gogate *et al.* (2018) showed that green-synthesised zinc oxide nanoparticles revealed 83% mortality of *Corcyra cephalonica*.

Similarly, *ZnO* nanoparticle applications under laboratory conditions on *B. brassicae* under 24 hr, 48 hr, 72 hr, and 96 hr treatment periods have given significant, highly significant, significant, and highly significant results. So, the application periods of 48 hours and 96 hours of *ZnO* nanoparticle treatment to control *B. brassicae* are observed as best.

In accordance with Khoshraftar *et al.* (2019) determination of the effect of nano capsules for pest control. Botanical pesticide nanocapsules offer a viable option for this target. To assess the insecticides of the nanocapsule, eucalyptus extract was pretreated for 12, 24, and 48 hours after exposure at concentrations of 0, 10, 15, 25, 35, and 50 mg/ml. The results showed that the greatest time encapsulation exposure (LC50 = 14.93 mg/ml) was considerably more vulnerable from 12 to 48 hours. Our results were similar to those of Al-Antary *et al*. (2018), *who* investigated the aphicidal potential of several green synthesised nanoparticles on green peach aphids. The synthetic nanoparticles, magnesium hydroxide bio-nanoparticles, provided the best control for *M. persicae*. The nanoparticles mediated by *M. spersicae* showed the highest mortality, and the $LC_{50}$ value was 0.09. Our results were in accordance with Al-Dhabi and Arasu, (2018), who used pure ZnO nanoparticles as a capping and stabilising agent as well as *Scadoxus multiflorus* liquid extract. On *A. aegypti* eggs and larvae, the $LC_{50}$ value was 34.04 ppm. At 120 ppm, the egg-laying capacity shows a death rate of 96.4 ± 0.24. Our results were similar to Buhroo *et al*. (2017) reported that ZnO nanoparticles showed 100% mortality of 4th instar larvae of *Mythimna separate* at 500ppm under laboratory conditions, 400 ppm, 300 ppm and 200 ppm concentrations showed 93%, 83% and 46% mortality of 4th instar larvae of *M. separate* respectively. Our results were in accordance with Siripireddy and Mandal (2017), who carried out an experiment with *Eucalyptus globulus* leaf extract that induced the formation of circular zinc oxide nanoparticles (ZnO NPs). The creation of ZnO nanoparticles was confirmed by UV-visible examinations of the produced nanoparticles, which displayed a distinctive peak at 361 nm. ZnO NPs demonstrated effective photocatalytic activities in degrading Methylene blue and Methyl orange at 30 mg catalyst doses, with a maximum biological degradation of 98.3%. *Eucalyptus globulus* leaf extract showed 65% mortality at 20% concentration after 96 hours under laboratory conditions; at 10%, 5%, 2.5%, and 1.25% concentration, the extract showed 61%, 54%, 42%, and 24% mortality of aphids after 96 hr, respectively. The mortality rate of aphids decreased with decreasing concentrations and time.

Singh and Lal (2012) assessed the effectiveness of some different plant leaf extracts against mustard aphids on Brassica crops. The effect of botanical leaves (*Tagetes erecta, Azadirachta indica*, *Parthenium hysterophorus*, *Lantana camera*, *Cymbopogon citrates,* and *Allium sativum)* on mustard aphids was evaluated in the field. The botanical extracts had varying effects on the aphid population, with neem leaf extract (T1) consistently inflicting the highest level of aphid death rates (77.33 percent and 71.76 percent), followed by garlic leaf extract (73.19 percent and 62.17 percent) and punch phuli leaf extract (74.35 percent and 70.96 percent) on the 7th day after spray in both 2009-2010 and 2010-2011.

*Comparative efficacy of crude extract versus nanoparticles*

Statistical analysis revealed significantly enhanced insecticidal activity of *E. globulus*-mediated ZnO nanoparticles compared to crude plant extract under identical laboratory conditions. At the 96-hour treatment period, nanoparticles at 800 ppm achieved 78% mortality, substantially outperforming the crude *E. globulus* leaf extract which reached maximum mortality of 65% at 20% concentration. The dose-response relationship demonstrated superior potency of nanoparticles, with 400 ppm nanoparticles (67% mortality) exceeding the efficacy of crude extract at its highest tested concentration. Statistical significance testing confirmed highly significant differences ($p \leq 0.001$) for nanoparticle treatments at 48 and 96 hours, while crude extract showed comparatively modest effects with mortality rates declining more rapidly at lower concentrations (61%, 54%, 42%, and 24% at 10%, 5%, 2.5%, and 1.25% concentrations, respectively). The enhanced bioactivity of nanoparticles can be attributed to improved penetration, sustained release properties, and increased surface area-to-volume ratio, resulting in more efficient delivery of active compounds to target sites in *B. brassicae* nymphs.

## Conclusions and Recommendations

This experiment was conducted to evaluate the effectiveness of an alternate source to control pests, like green synthesised nanoparticles and plant extract. Nanoparticles and plant extracts are cheaper than pesticides and show no toxicity to plants. Green-synthesised Zinc oxide nanoparticles and plant extracts were used against *Brevicoryne brassicae* L. at the University of Agriculture, Faisalabad, under laboratory conditions. Five concentrations of nanoparticles and botanical extracts were used as a treatment. All treatments with nanoparticles and botanicals exhibited significant mortality related to their treatments. Field-applicable doses showed a 60-80% death rate after 96 hr, and the death rate decreased with decreasing doses and time. *ZnO* nanoparticle applications under laboratory conditions on *B. brassicae* under 24 hours, 48 hours, 72 hr, and 96 hr treatment periods have given significant, highly significant, significant, and highly significant results. So, the application periods of 48 hours and 96 hours of *ZnO* nanoparticle treatment to control *B. brassicae* are observed as best.

Green-synthesised Zinc Oxide nanoparticles of *Eucalyptus* showed 77% mortality at 800 ppm after 96 hr under laboratory conditions; 400 ppm showed 68% mortality; 200 ppm showed 48% mortality; 100 ppm showed 24% mortality; and 50 ppm showed 12% mortality of aphids after 96 hr. The death rate of *Brevicoryne brassicae* L. decreased with decreasing concentrations and time.

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## Novelty Statement

This study demonstrates for the first time that *Eu-calyptus globulus*-mediated zinc oxide nanoparti-cles achieve superior aphicidal activity (78% mortality) against *Brevicoryne brassicae* compared to crude plant extracts, providing an eco-friendly nanopesticide al-ternative for brassica crop protec-tion.

## Author's contribution

**Muhammad Aqib Idrees:** Made by data curation, Methodology, Writing and editing the original draft.
**Ahmad Kamran Khan:** Made by data curation, Methodology, Software, Writing review and editing.
**Muhammad Shahid Nisar:** Made by formal analysis and Methodology.
**Irfan Ahmed:** Made by data curation, Software, Writing the original draft, Review and editing.
**Muhammad Irfan:** Made by Methodology and Writing the original draft.

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The authors declare that no genrative AI was used in the creation of this manuscript.

*Conflict of interest*
The authors declare that there was no conflict of interest or reported personal relationships that could influence the results reported in this paper.

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