



## **EFFECT OF BIOSYNTHESIZED NANOPARTICLES ON CONTROLLING NEMATODE *MELOIDOGYNE INCognITA* INFESTING *CORIANDRUM SATIVUM L.***

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### **Abstract**

The green synthesized nanoparticles: zinc nanoparticles (ZnNPs) and silver (AgNPs) were formulated using aqueous extracts of *Moringa oleifera* L. and *Azadirachta indica* L. leaves respectively, to investigate their nematicidal efficacy against *Meloidogyne incognita* infesting *Coriandrum sativum* L. The nanoparticles were characterized using UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). A comparative analysis was then done to evaluate the nematicidal activity of synthesized zinc and silver nanoparticles across a concentration range of 20-100 mg/L. UV-vis spectra confirmed the formation of ZnNPs and AgNPs with characteristic surface plasmon resonance peaks at 375nm and 435 nm respectively. FTIR analysis revealed the presence of various functional groups responsible for the reduction and stabilization of nanoparticles, while SEM analysis demonstrated their spherical morphology with the range of size 77.90-92.43 nm for ZnNPs and 26-62.2 nm for AgNPs. In pot experiments, both nanoparticles exhibited significant nematicidal activity against *M. incognita*, but ZnNPs showed better results in reducing gall formation and nematode population in coriander roots as compared to the AgNPs. Overall, this study demonstrates the substantial nematicidal efficiency of green-synthesized nanoparticles, particularly ZnNPs, as an eco-friendly alternative for managing root-knot nematode infestations in coriander cultivation. Additionally, it could provide a scalable model for managing other pests in economically significant crops.

**Keywords:** Green synthesized nanoparticles, *Moringa oleifera* L., *Azadirachta indica* L., Nematicidal efficacy, *Meloidogyne incognita* L., *Coriandrum sativum* L.

### **1. Introduction**

Spices have shaped human civilization through cuisine, trade, medicine, and culture. Derived from Latin 'species aromatacea' (Farrell, 2012), spices come from various plant parts including bark, buds, fruits,



seeds, and leaves. With 260 plant species used globally, they contain bioactive compounds valuable for traditional and modern medicine, food preservation, and reducing disease risk. Europeans historically categorized them by use in perfumes (cassia, cardamom, sweet marjoram, and cinnamon), incense (thyme, cinnamon, cassia, and rosemary), preservation (cassia, cumin, cinnamon, anise, clove, etc.), and antidotes (anise, coriander, garlic, and oregano) (Tapsell *et al.*, 2006; Ravindran, 2017; Arachchige *et al.*, 2021). Herbs, primarily from plant leaves, serve culinary, medicinal, and aromatic purposes, with examples like basil, rosemary, and coriander providing anti-inflammatory, antimicrobial and antioxidant benefits, earning designation as 'medicinal herbs' (Nath *et al.*, 2015; Ravindran, 2017). WHO recognizes 21,000 medicinal plants, with 2,500 native to India, the 'botanical garden of the world' (Mahima *et al.*, 2012).

Coriander (*Coriandrum sativum L.*), a widely cultivated annual herb, is native to the Mediterranean and Middle East and extensively grown in India, Morocco, France, Spain, Myanmar, Pakistan, Mexico, China, Bangladesh, and Australia (Small, 1997; Momin *et al.*, 2012; Sharma and Sharma, 2012). It is harvested for green leaves and dried seeds, with its essential oil used in cosmetics, fragrances, and pharmaceuticals. Rich in antioxidants and antimicrobial properties, coriander helps treat gastric disorders, urinary infections, and diabetes (Nath *et al.*, 2015). The chemical profile of *C. sativum* is made up of two major active chemical constituents, the essential oils, and fatty oils. The plant's essential oil contains linalool which is a principle constituent of coriander (Laribi *et al.*, 2015). Its adaptability to diverse climatic conditions (dry and cold to hot and humid) also makes it a valuable crop for farmers worldwide. For proper growth and development of *C. sativum*, the timing of planting is crucial. Environmental conditions vary throughout the growing season, so making the choice of sowing date a key factor in determining the plant's overall performance. When sown at the optimal time, its plants can best utilize available environmental resources. The planting date influences not just the crop's yield, but also its quality and disease resistance (Kuri *et al.*, 2015). With its increasing demand in the global market, coriander faces challenges such as susceptibility to pests and diseases like rot (stem, root, charcoal), stem gall, leaf blight, powdery mildew, phyllody, root knot nematode infections and viral diseases, which significantly impact its yield and quality (Uikey, 2022).

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive plant pathogens, inflicting severe damage to crops worldwide. These obligate endoparasites cause extensive damage to crops through root galling, vascular tissue disruption, and cellular changes, leading to reduced growth, yield,



and quality. The life cycle of root-knot nematodes is temperature-dependent, with females becoming sedentary and pyriform while males remain vermiform (Ravindra *et al.*, 2017; Eisenback *et al.*, 2020; Feyisa, 2021). *Meloidogyne incognita* (Kofoid and White), a key species affecting coriander, invades roots and forms galls, disrupting nutrient uptake and reducing growth and productivity. This nematode species has a wide host range and thrives in favourable conditions, making its management a persistent challenge. It displays year-round activity in hot climates and moist soils (Samojlik *et al.*, 2010; Wesemael *et al.*, 2011; Santos *et al.*, 2018; Subedi *et al.*, 2020). Conventional control methods, such as chemical nematicides, are often limited by environmental and health concerns. Therefore, integrated pest management strategies, including the use of biological agents and resistant cultivars, are being explored (Samojlik *et al.*, 2010). Moreover, the economic losses caused by these nematodes emphasize the urgent need for sustainable and effective management approaches to secure crop yields and food security. Addressing this issue requires not only innovative solutions but also a comprehensive understanding of nematode biology and its interactions with host plants.

Nanotechnology has shown promising applications in controlling plant pests and diseases in recent years (Wang *et al.*, 2023). Nanotechnology represents a revolutionary path for technological development that concerns the management of material at the [nanometre](#) scale (one billion times smaller than a meter) (Nasrollahzadeh *et al.*, 2019). Engineered nanoparticles are materials between 1 and 100 nm and exist as metalloids, metallic oxides, nonmetals, and carbon nanomaterials and as functionalized dendrimers, liposomes, and quantum dots. Their small size, large surface area, and high reactivity have enabled them to be used as bactericides, fungicides, and nano fertilizers (Elmer and White, 2018). Nanotechnology offers a promising avenue for controlling plant-parasitic nematodes, including *Meloidogyne incognita*. Metal-based nanoparticles, such as silver and zinc oxide nanoparticles, have demonstrated potential as nematicides due to their small size, large surface area, and unique physicochemical properties (Dwivedi and Gopal, 2010; UlHaq and Ijaz, 2019; Tuncsoy, 2021; Elarabi *et al.*, 2022). Green synthesis of nanoparticles using plant extracts is gaining popularity as an environmentally friendly approach. Several studies highlight the effectiveness of green synthesized metal-based nanoparticles in disrupting nematode life cycles and enhancing plant defense mechanisms (Debnath *et al.*, 2020; Elarabiet *et al.*, 2022; Yadav *et al.*, 2023). This study aims to evaluate the nematicidal activity of green-synthesized nanoparticles against root-knot nematodes on coriander as a sustainable control strategy to address this agricultural challenge.

## 2. Materials and Methods



## 2.1. Materials

*Coriandrum sativum* L. plant, *Moringa oleifera* L. leaves, *Azadirachta indica* L. leaves, Zinc Nitrate ( $Zn(NO_3)_2$ ), Silver Nitrate ( $AgNO_3$ ), Distilled water, and Ethanol.

## 2.2. Collection of plant material

Three experimental plants, each belonging to a different species were collected from different areas in Jaipur, Rajasthan: *Coriandrum sativum* L. from Paota village area, Jaipur, and *Moringa oleifera* L. and *Azadirachta indica* L. from Campus of University of Rajasthan, Jaipur. Their identification and confirmation were carried out through comparisons with specimens kept in herbarium of Department of Botany, University of Rajasthan, Jaipur, having the following ID's RUBL21309 - *Coriandrum sativum* L., RUBL21310 - *Moringa oleifera* L., and RUBL21306 - *Azadirachta indica* L.

## 2.3. Preparation of *Moringa oleifera* L. and *Azadirachta indica* L. extract

The samples were prepared using modified method developed by Elumalai (2015). The leaves of *Moringa oleifera* L. and *Azadirachta indica* L. underwent a thorough cleaning process using tap water followed by distilled water. After shed drying, the leaves were ground into a fine powder. To prepare the aqueous extract, 10 g of each powdered leaves were mixed with 100 ml of distilled water. This mixture was subjected to continuous stirring and heating on a magnetic stirrer for 2 hrs, after which it was filtered using Whatman's filter paper.

## 2.4. Synthesis of nanoparticles from plant extracts

### 2.4.1. Synthesis of ZnNPs using *M. oleifera* extract

The zinc (Zn) nanoparticles were synthesized by heating 50 ml of *M. oleifera* aqueous extract at 80°C for 10 min in a beaker under constant stirring. After adding 5 g of zinc nitrate ( $Zn(NO_3)_2$ ), the mixture was stirred at 600 rpm for a total of 10 min, during which 20% NaOH was added to adjust the pH to 12, creating an alkaline medium. The resultant then underwent a purification process involving two washes with distilled water and one with ethanol, followed by centrifugation. The mass obtained was then dried in an oven at 80°C until a powder form was achieved. Finally, the obtained dried powder was calcined at 450°C for 2 hrs (Elumalai *et al.*, 2015).

### 2.4.2. Synthesis of AgNPs using *A. indica* extract



Silver nanoparticles (AgNPs) were synthesized using silver nitrate ( $\text{AgNO}_3$ ) as a precursor. The synthesis began by preparing a 6 mM  $\text{AgNO}_3$  solution, where the appropriate mass of  $\text{AgNO}_3$  was dissolved in 100 mL of distilled water. To initiate the reduction process, different volumes of plant extract were individually added to separate aliquots of 10 mL  $\text{AgNO}_3$  solution, while maintaining the 6 mM concentration of  $\text{AgNO}_3$ . The reaction mixtures were then incubated at room temperature in a dark chamber to prevent photochemical activation of  $\text{AgNO}_3$  (Ahmed *et al.*, 2016).

## 2.5. Characterization of synthesized nanoparticles

### 2.5.1. UV-vis spectra analysis

UV-vis spectrophotometry was used to assess the maximum absorbance of samples, confirming the bio-reduction of  $\text{Zn}^{2+}$  and  $\text{Ag}^+$  ions in aqueous solutions. Spectral analysis was conducted on a spectrophotometer [GENESYS 180, USA], across a wavelength range of 300 to 700 nm, having an interval time of 2 nm.

### 2.5.2. Fourier Transform Infrared Spectroscopy [FTIR]

The FTIR spectroscopy of the samples was analysed in the wavelength range 600 to 4000  $\text{cm}^{-1}$ . This exercise was done to know the information about the functional groups present over the surfaces of the materials.

### 2.5.3. Scanning Electron Microscopy [SEM]

The actual sizes and agglomeration state of the nano-material was studied by using Scanning electron microscope [ZEISS, Germany], operating at an extra high tension or accelerating voltage [EHT] of 20 kV, where working distance [WD] was 10.5 mm. Minute amount of the test materials were loaded one by one on the sample discs, and then the surface topology, shape, and size of green synthesized Zinc and Silver nanoparticles were examined.

## 2.6. Nematicidal activity of green synthesized nanoparticles

The experiment was conducted in 8 inches mud pots under outdoor screen-house conditions. Certified *C. sativum* seeds were sown in pots (10 seeds per pot) containing sterilized sandy loam soil. After 2 weeks, seedlings were seen in experimental pots. Then 1000  $\text{J}_2$  / eggs of *M. incognita* were added to the seedlings and 50 ml of AgNPs solution of concentrations 0, 20, 40, 60, 80, 100 ppm were made and added to those



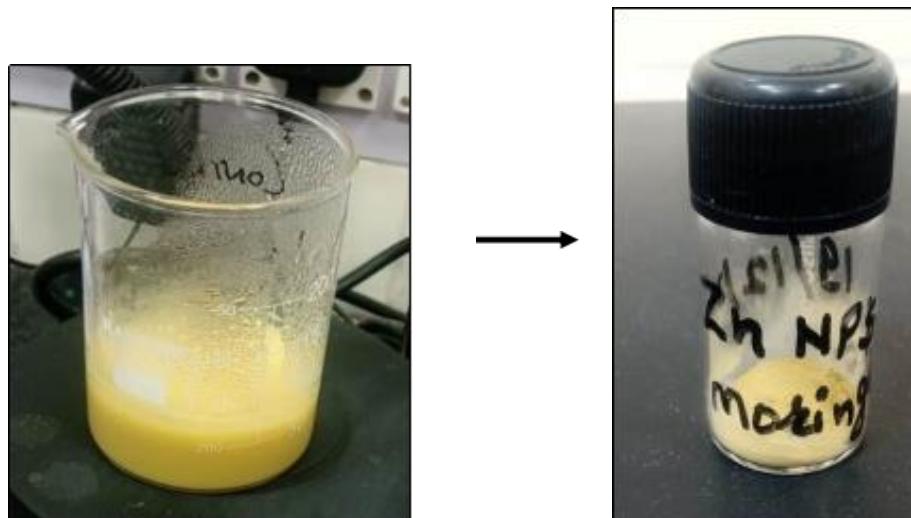
pots at seedling stage. The experiment was ended after 14 days when the gall no. was counted. Same procedure was followed for Zn NPs.

### 3. Result and Discussion

#### 3.1. Visual observation of green synthesized nanoparticles

The successful synthesis of zinc nanoparticles (ZnNPs) and silver nanoparticles (AgNPs) was confirmed through visual observation of the color change in the reaction mixture.

The synthesized ZnNPs solution exhibits a yellow coloration, which is a typical indicator of nanoparticle formation (Fig. 1). This yellow color suggests the reduction of zinc precursor ions and the subsequent stabilization of ZnNPs. Similarly, Jaya Chandran *et al.* (2021) and Wouters *et al.* (2023) used plant extracts of *Cayratia pedata* and *Eucalyptus grandis* respectively to act as reducing agents, which resulted in yellow-colored Zn NPs.

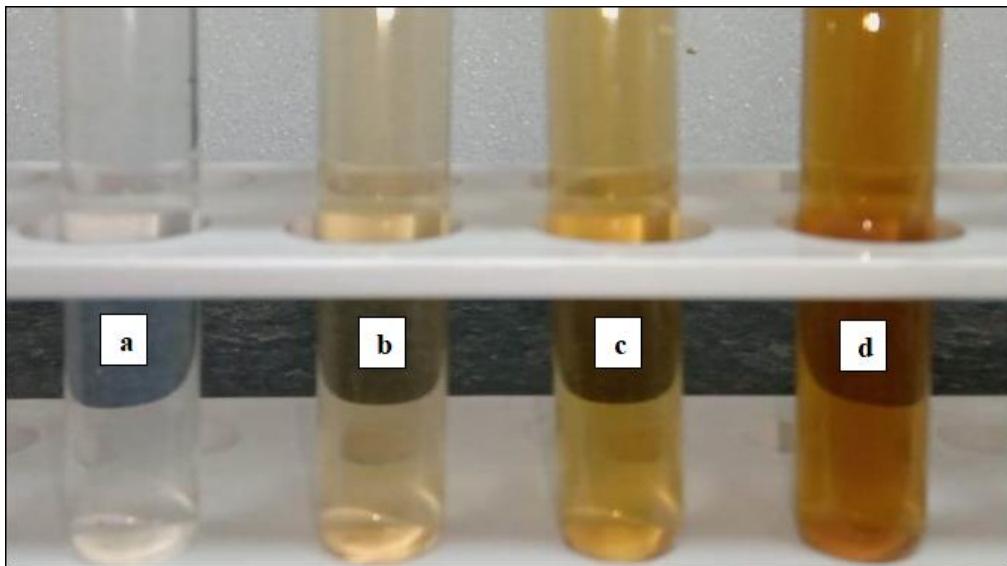


**Figure 1: Green synthesized Zinc Nanoparticles (ZnNPs); (a) Zinc Nanoparticles solution, (b) Powdered Zinc Nanoparticles**

Silver nanoparticles (AgNPs) synthesis was confirmed by a progressive shift in solution color from colourless to light yellow, followed by an increase in intensity to deep brown (shown in Fig.2). The successful transformation of the solution from colorless to brown, indicating the reduction of silver ions



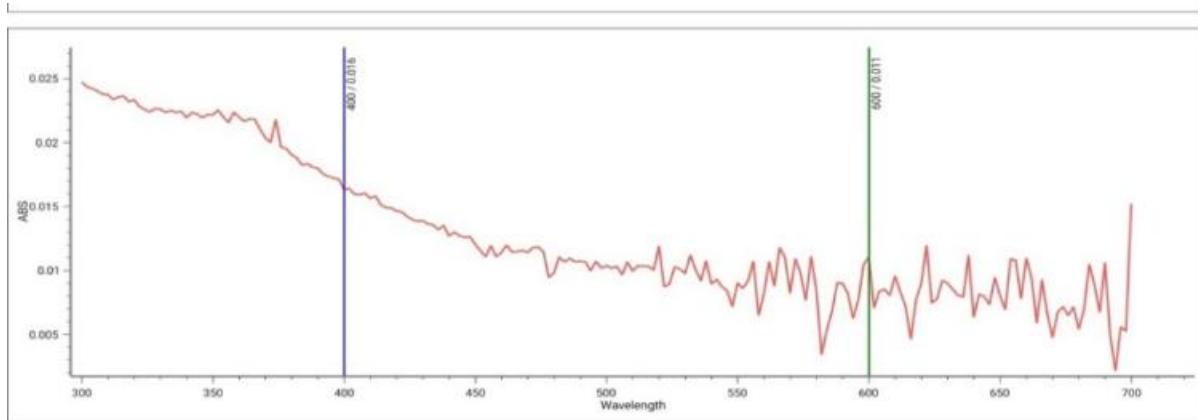
( $\text{Ag}^+$ ) to metallic silver nanoparticles ( $\text{Ag}^0$ ). This was a common observation across studies of Saranyaadevi *et al.* (2014) and Jegadeeswaran *et al.* (2012) and where they green synthesized Ag NPs from plant extracts of *Capparis zeylanica* and *Padina tetrastromatica*.



**Figure 2: Green synthesized Silver Nanoparticles (AgNPs); (a) colourless solution, (b) light buff solution, (c) light yellow solution, (d) deep brown solution**

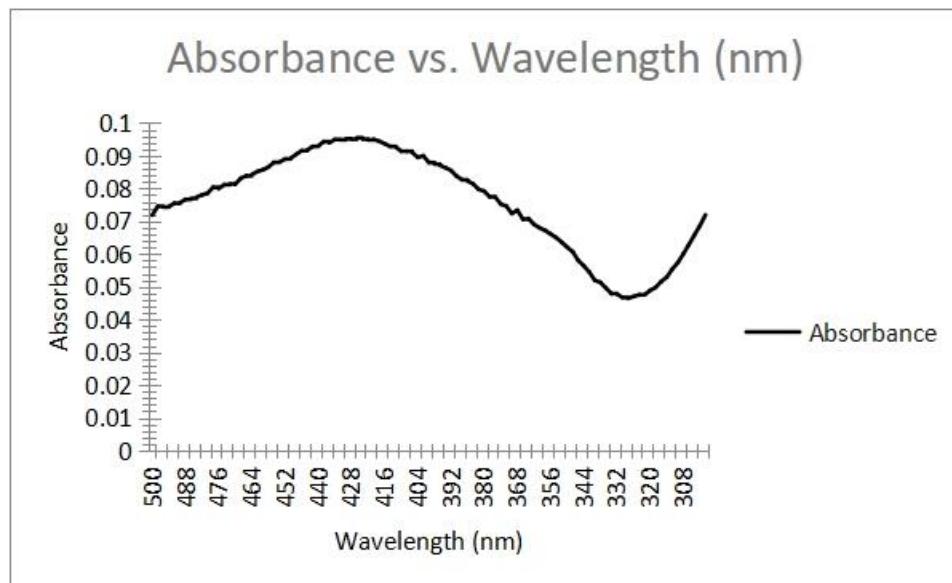
### 3.2. UV-Vis Spectra Analysis

The UV-vis Spectra of Zn NPs was confirmed by the surface plasmon resonance (SPR)peak, that was observed at 375 nm (Fig. 3). Talam *et al.*, 2012 also found absorbance maxima at 355 nm of zinc nanoparticles. Fakhari *et al.* (2019) and, Manokari and Shekhawat (2016) have also reported absorption peaks around 350 nm and between 290-302 nm respectively, with some variation depending on synthesis methods and precursors.



**Figure 3: UV-Vis spectra of Zn NPs**

The formation of Ag NPs was attributed to obtaining a peak at 435 nm as shown in UV-Vis spectra (Fig.4). This confirmed the SPR peak that was observed in the range of 428–448 nm in the studies of Banerjee (2014) and Tripathy *et al.* (2010) and validated the formation of AgNPs.



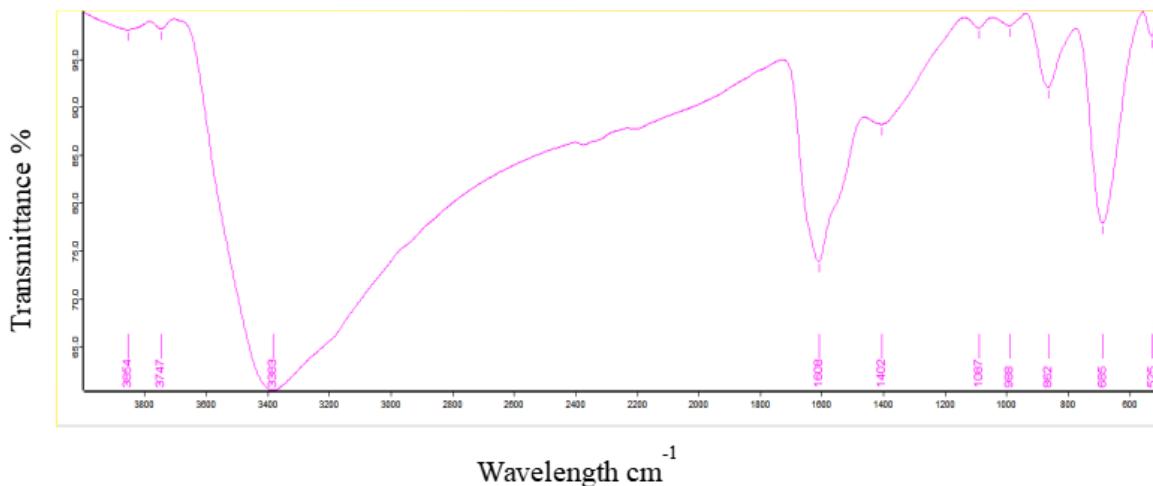
**Figure 4: UV-Vis spectra of Ag NPs**

### 3.3. FTIR Analysis

The FTIR spectrum of Zn NPs showed wide band at  $3383\text{ cm}^{-1}$  correlated with -OH vibration in a water molecule ( $\text{H}_2\text{O}$ ). The shrinkage of the -OH peaks indicate the better removal of water molecules from the

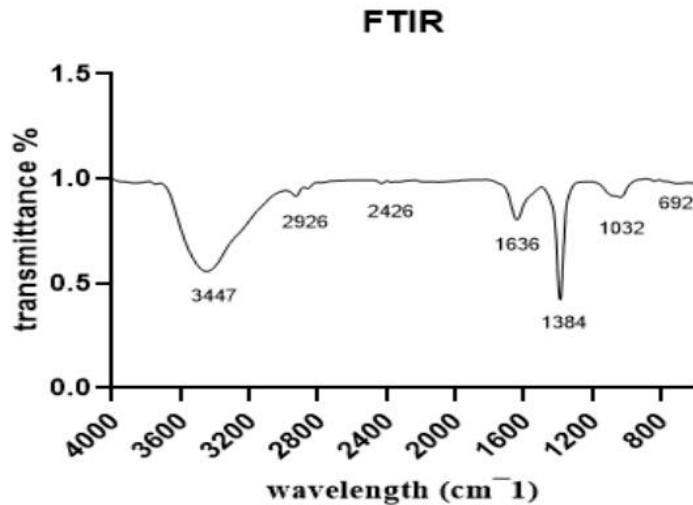


nanostructure at the higher annealing temperature. Wideband at  $1608\text{ cm}^{-1}$  correspond to C-H bonding in the alkene compound similar peak was shown in Elumalai *et al.* (2015) study. Such vibration may also come from heterocyclic molecules, for example, from alkaloid or flavonoid molecules and amide (I) bonding from protein and enzyme come from the extract or powdered leaves (Rashid *et al.*, 1972). The absorption peak at  $685\text{ cm}^{-1}$  corresponds to metal-oxide bonding as shown in Figure 5 also observed in Pal *et al.* (2018) and in Poovizhi and Krishnaveni (2015) study the FTIR peaks of Zn NPs from *C. procera* was near  $621.08$  to  $692.44\text{ cm}^{-1}$ .



**Figure 5: FTIR spectra of Zn NPs**

The dual role of the plant extract as a reducing and capping agent and presence of some functional groups was confirmed by FTIR analysis of silver nanoparticle. A broad band between  $3447\text{ cm}^{-1}$  is due to the N-H stretching vibration of group  $\text{NH}_2$  and OH the overlapping of the stretching vibration of attributed for water and leaf extract molecules. The band at  $1636\text{ cm}^{-1}$  corresponds to amide  $\text{C}=\text{O}$  stretching. The observed peaks are mainly attributed to flavonoids and terpenoids excessively present in plants extract (Banerjee *et al.*, 2014; Prathna *et al.*, 2011). On the other hand, the extract sample prepared shows a strong peak with maximum intensity at  $1384\text{ cm}^{-1}$ . The results are in good agreement with those found in literature of Mahdi *et al.* (2015). From FTIR results it can be concluded that Ag nanoparticles were formed.



**Figure 6: FTIR spectra of Ag NPs**

### 3.4. SEM Analysis

The aggregation in the green synthesized Zn NPs and Ag NPs was analysed by scanning electron microscopy as shown in Figure 7 and 8. The SEM micrographs showed that the synthesized Zn NPs and Ag NPs were found to be spherical particles in clusters and aggregated (irregular shape) in smaller clusters where the observed sizes of the NPs were in the range of 77.90-92.43 nm for ZnNPs and 26-62.2 nm for AgNPs. Biosynthesis of the Zn NPs and Ag NPs is due to the results of electrostatic interactions and bonding of the bio-organic capping molecules. Fakhari *et al.* (2019) and Srivalli *et al.* (2023) also green synthesized Zn NPs and had reported similar observation, where the size range was between 21-25 nm and 80-130 nm respectively. While in Arunachalam *et al.* (2012) and Mukunthan *et al.* (2011) SEM analysis revealed that green synthesized AgNPs were in the range of 20-30 nm and 67-48 nm respectively.

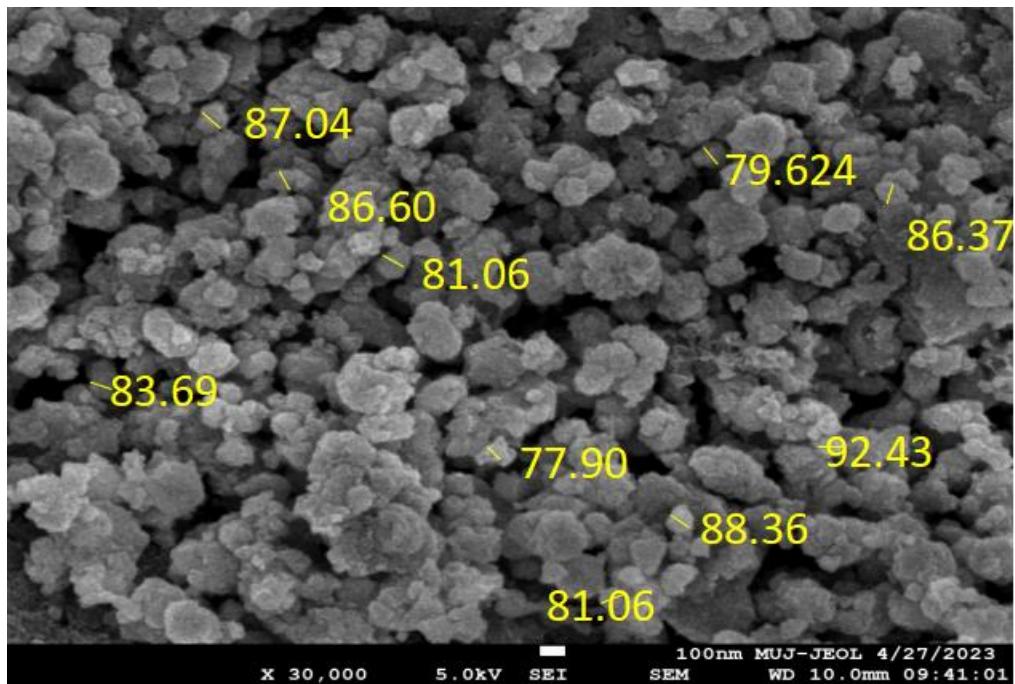
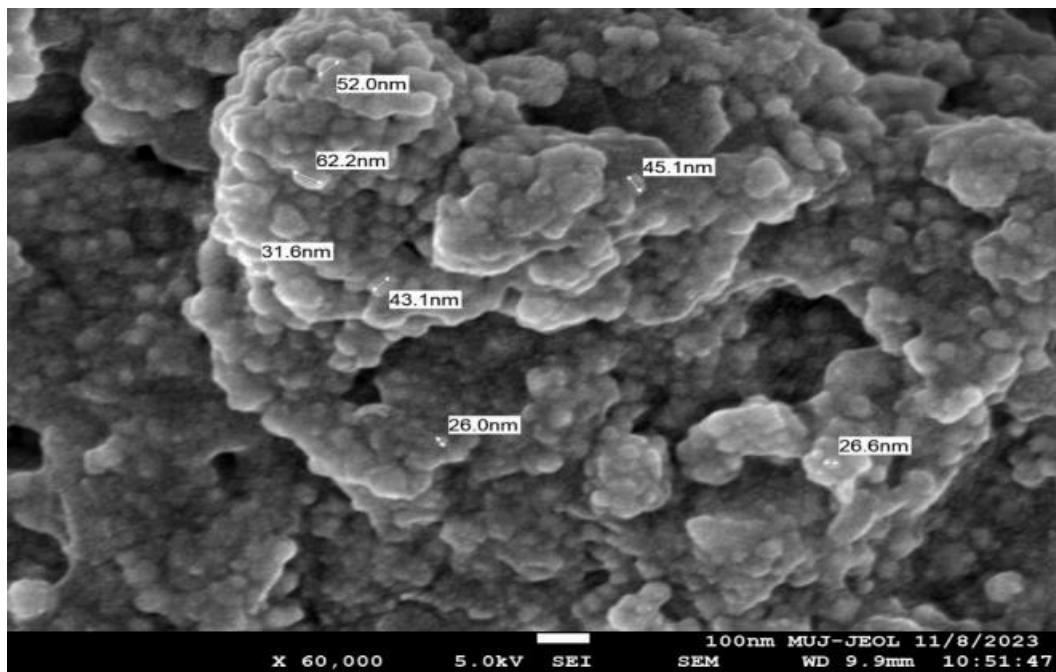


Figure 7: Scanning electron micrograph of Zn NPs solution (100 nm micrograph)



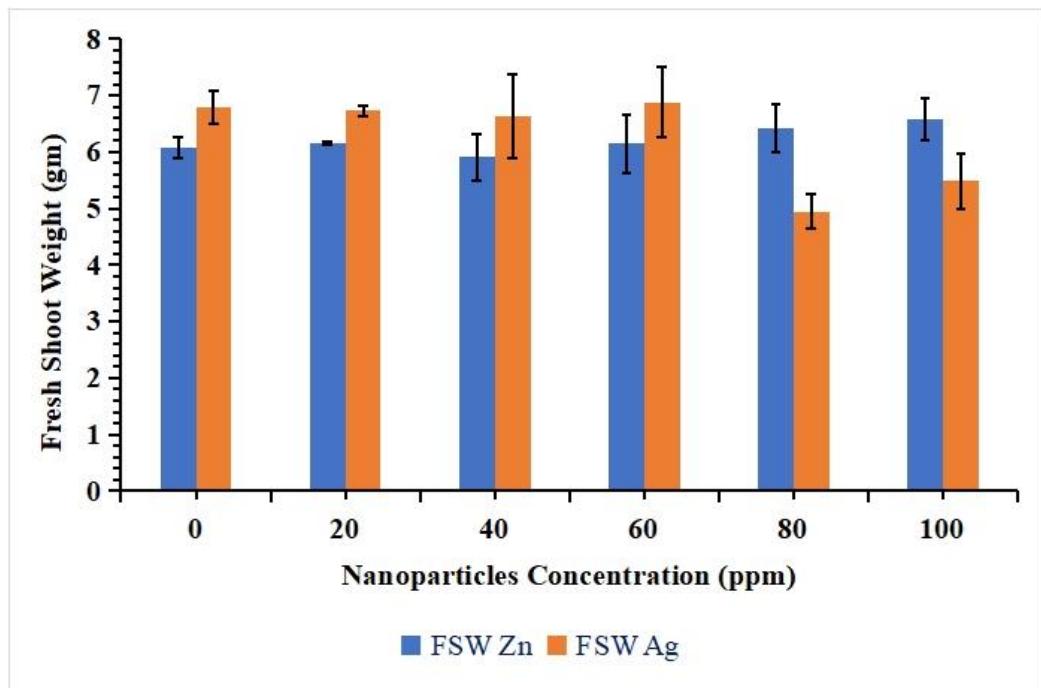


**Figure 8: Scanning electron micrograph of Ag NPs solution (100 nm micrograph)**

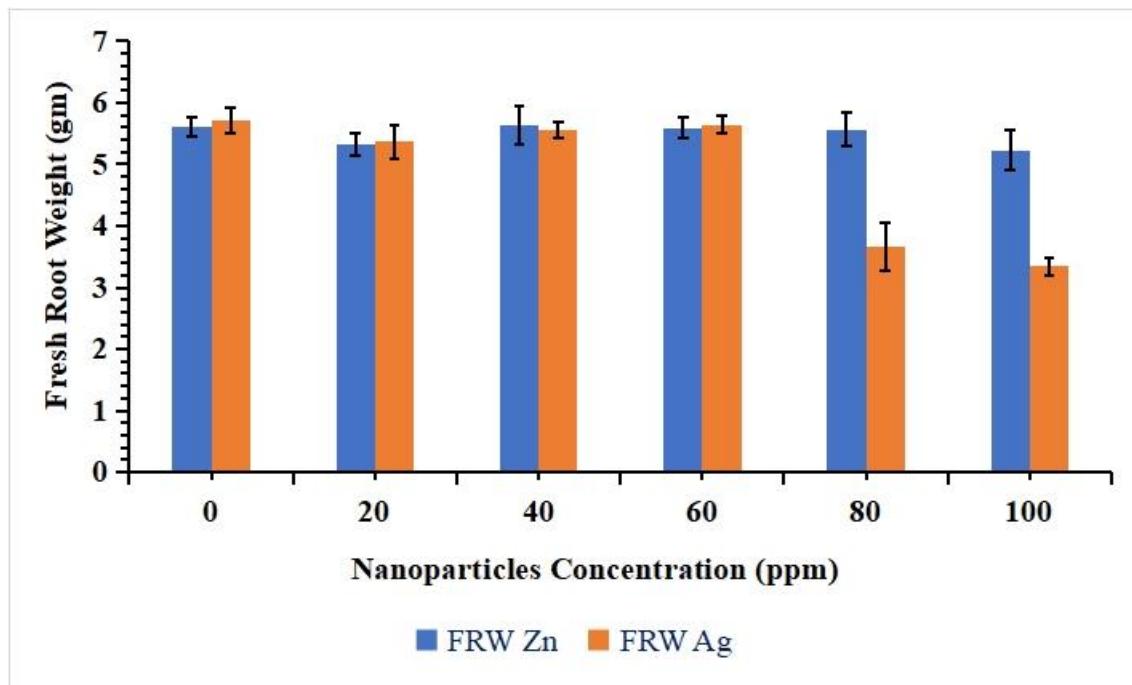
### 3.5. Nematicidal activity of green synthesized nanoparticles analysis

The results of the treatment given to the infected *C. sativum* plants, of green synthesized Zinc and Silver nanoparticles (to check their nematicidal activity) were comparatively analysed on the following basis: Fresh Shoot weight (FSW), Fresh Root weight (FRW), Plant Height (PH), and Galls Intensity (as shown in the Figures 9-12). In case of fresh shoot weight, the highest weight with Zn NPs was seen at 100 mg/L (ppm) concentration and with Ag NPs at 60 ppm. The maximum fresh root weight with Zn NPs was seen at 40 ppm and 60 ppm with Ag NPs. The plant height with Zn NPs was found to be highest at 40 ppm and with Ag NPs at 20 ppm.

The effectiveness against *M. incognita* was mainly measured by examining root gall formation in *C. sativum* plants, where both zinc and silver nanoparticles showed a consistent reduction in gall intensity as their concentrations increased (with 0 ppm concentration taken as control), and it was found that Zn NPs had better nematicidal activity than Ag NPs at all concentrations. In a similar study Ghareeb *et al.* 2022 reported 22.25 d $\pm$ 2.72 no. of galls in tomato plant after treatment with green synthesized Ag NPs with reduction percentage of almost 75.95%. In Hussain *et al.* (2024) treatment of infected cowpea plants with 100 ppm Zn NPs resulted in no. of galls decreased to 3.01  $\pm$  0.1 with a reduction percentage of 76.13.

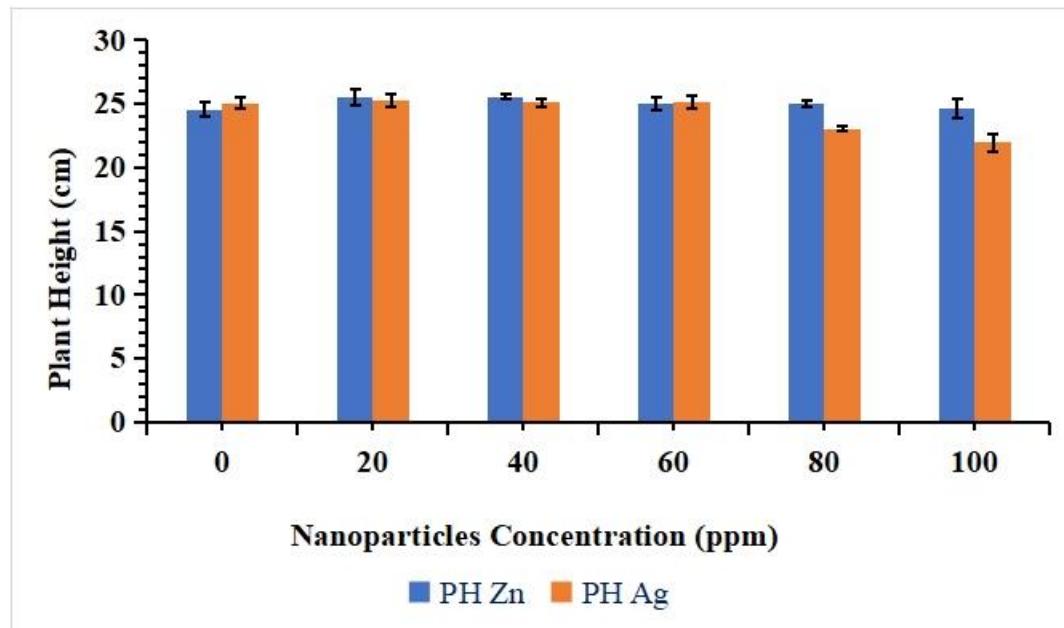


**Figure 9: Comparative analysis of fresh shoot weight (FSW) of infected *C. sativum* plants after treatment with Zn NPs and Ag NPs different concentrations**

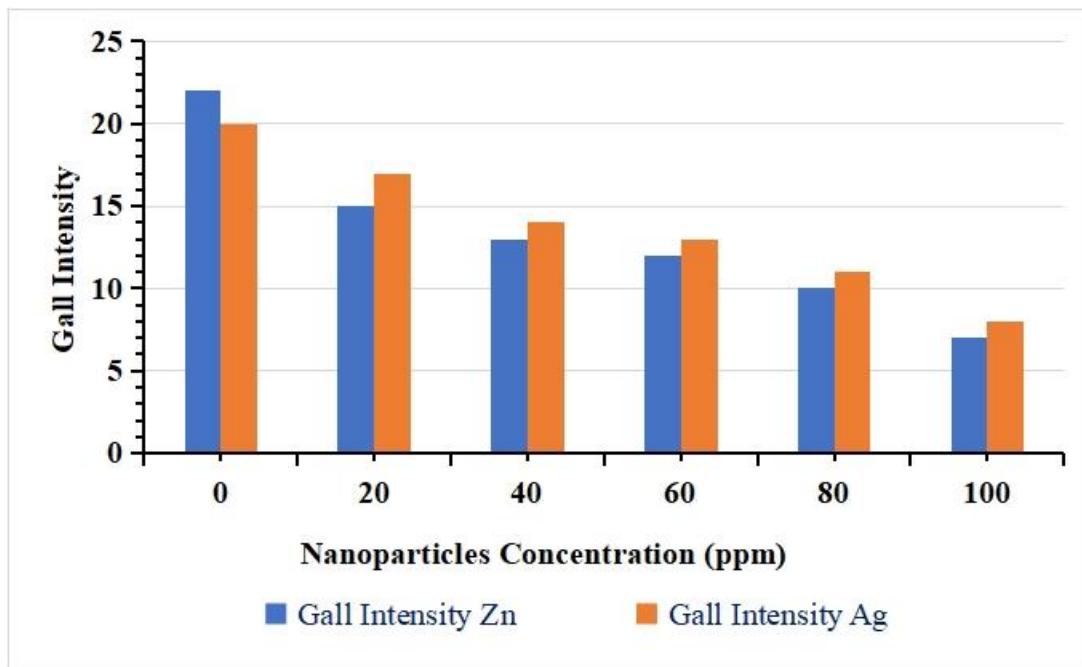




**Figure 10: Comparative analysis of fresh root weight (FRW) of infected *C. sativum* plants after treatment with Zn NPs and Ag NPs different concentrations**



**Figure 11: Comparative analysis of plant height (PH) of infected *C. sativum* plants after treatment with Zn NPs and Ag NPs different concentrations**



**Figure 12: Comparative analysis of gall intensity in infected *C. sativum* plants after treatment with Zn NPs and Ag NPs different concentrations**

#### 4. Conclusion:

In this research, the synthesis of zinc nanoparticles (ZnNPs) and silver (AgNPs) using aqueous extracts of *Moringa oleifera* L. and *Azadirachta indica* L respectively, generated a scope for green technology and in agricultural sectors for managing pests in economically significant crops. UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) results confirmed the formation of green synthesized nanoparticles. Then they were evaluated for nematicidal activity in *M. incognita*-infected *C. sativum* plants by analysing fresh shoot weight (FSW), fresh root weight (FRW), plant height (PH), and gall intensity. Optimal results for Zn NPs were observed at 100 ppm for FSW, 40 ppm for FRW, and 40 ppm for plant height. For Ag NPs, peak performance was noted at 60 ppm for FSW, 60 ppm for FRW and 20 ppm for plant height. Both nanoparticles demonstrated concentration-dependent reduction in root gall formation, with Zn NPs exhibiting superior nematicidal activity across all tested concentrations.



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