

## Synthesis of Nanoyttria Nanoparticles Using *Moringa oleifera* Seed as a Biological Factory and the Biocontrol Impact of the Nanoparticles on Houseflies (*Musca domestica*)

Rasha S. Nuaman, Raghad J. Fayyad, Rasha S. Hameed, Maan A.A. Shafeeq\*

Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

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## ABSTRACT

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Chemical pesticides are associated with several challenges, the most serious of which is the emergence of insect resistance and the negative effects of these chemicals on humans. Thus, there is a growing demand for the synthesis of natural insecticides, especially those based on plant materials. The aim of this study was to employ an aqueous *Moringa oleifera* seed extract to biologically synthesize nanoyttria (Mo-Y<sub>2</sub>O<sub>3</sub>NPs) and investigate their insecticidal effects on both larvae and adult houseflies (*Musca domestica*). Aqueous extract was prepared from *Moringa* seeds. Mo-Y<sub>2</sub>O<sub>3</sub>NPs were synthesized by mixing the seed extract with yttria oxide. UV-Visible Spectroscopy, Atomic Force Microscopy, and X-Ray Diffraction analyses were employed to characterize the biosynthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs. A preliminary phytochemical analysis was conducted on the *Moringa* seed extract. Also, the insecticidal activity of the Mo-Y<sub>2</sub>O<sub>3</sub>NPs was evaluated using both the larvae and adult houseflies. The results indicated that nanoyttria particles were synthesized successfully with a crystalline size of 28 nm and diameters ranging between 70 and 155 nm. The preliminary phytochemical analysis revealed the presence of phenols, tannins, alkaloids, flavonoids, resins, and saponins, which support nanoparticle synthesis and stabilization. The insecticidal activity of the Mo-Y<sub>2</sub>O<sub>3</sub>NPs against instar larvae and adult house flies indicated 100, 73.3, 70, and 93.3% mortality for 500 g/mL of the biosynthesized nanoyttria against 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> larvae, and adult flies, respectively. This finding presents the first study, which included an attempt to control houseflies using nanoyttria synthesized with *Moringa* seed extract as a safe, quick, and affordable biological factory.

**Keywords:** Alkaloids, Insecticide, *Moringa oleifera*, *Musca domestica*, Nanoparticles.

### Introduction

The housefly, *Musca domestica* L. is an insect belonging to the order Diptera, family Muscidae. This insect is globally distributed and strongly adaptable to various ecosystems. Houseflies are abundant examples of pests that can spread a variety of pathogens that threaten humans, dairy, and poultry.<sup>1</sup> Chemical pesticides are commonly employed to control houseflies, but their careless application has led to several problems, such as the emergence of insect resistance to these chemicals and adverse effects on non-target organisms, most notably humans. As a result, there is an increasing need to discover substitute materials that are both characterized by human safety and have considerable insecticidal characteristics.<sup>2</sup> *Moringa oleifera* is a plant belonging to the Moringaceae family. It is an edible plant cultivated in various countries of the Middle East, such as Iran, Iraq, and Pakistan.<sup>3</sup> Due to its economic importance and adaptability, this plant is conventionally termed a 'miracle tree'.<sup>4</sup> The moringa is greatly valued since virtually every part of the tree is used as a food with potent nutritive value.<sup>5</sup> Besides, the moringa seed has many medicinal properties.<sup>6</sup> Many researchers have attempted to prepare nano insecticides using green strategies.

\*Corresponding author. E mail: [maanalsalihi@uomustansiriyah.edu.iq](mailto:maanalsalihi@uomustansiriyah.edu.iq)  
Tel: 07903612411

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Sabbour *et al.*,<sup>2</sup> reported the advantages of these nanostructured insecticides as being more active, less luxurious, biodegradable, and harmless for humans and ecosystems than their chemically synthetic counterparts. Furthermore, plant-based nano synthesis is authenticated as advantageous because it does not involve elaborate processing such as intracellular preparations and multi-step purification or microbial culture maintenance.<sup>7</sup> Furthermore, many other efforts have been made to encourage the use of plant sources for nanoparticle (NP) synthesis, particularly to avoid environmental contamination. Another important reason for encouraging the use of plant machines is that plant cells contain active phytochemicals that act as both capping and reducing agents, such as vitamins, terpenoids, alkaloids, and tannins.<sup>8</sup> An example of an inorganic nanoparticle is yttrium oxide (Y<sub>2</sub>O<sub>3</sub>), which is one kind of valuable rare earth element. Owing to its higher thermal, chemical, and dielectric stability, nano yttrium is widely used in many technical applications, particularly those that require high-temperature processing, such as nuclear ceramics.<sup>9</sup> Many medical sectors, such as cancer therapy, biosensors, and nanoparticles, use Y<sub>2</sub>O<sub>3</sub> because of its antioxidant and antibacterial capabilities.<sup>10</sup> Nanoyttrium has been synthesized using a variety of methods, including plant-based.

The present study was conducted to develop a rapid, environmentally friendly, and easy methodology for the green synthesis of nanoyttria nanoparticles using an aqueous extract of *Moringa oleifera* seeds (Mo-Y<sub>2</sub>O<sub>3</sub>NPs). The insecticidal effects on both larvae and adult houseflies (*Musca domestica*) were also investigated.

### Materials and Methods

#### Source of plant materials

The seeds of *M. oleifera* were obtained from the MUST Herbarium, Iraq, with voucher number 301. They were collected in August 2021

and authenticated by a plant taxonomist, Asst. Prof. Dr. Hadeel Al-Newani of the Department of Biology, Mustansiriyah University, Iraq. Then it was processed into a fine powder.

#### Preparation of seed extract and Mo-Y<sub>2</sub>O<sub>3</sub>NPs

The aqueous extract was prepared by mixing 20 g of seed powder with 300 mL of deionized distilled water, and then heating the mixture to 80°C. After 30 min., the mixture was filtered using a Whatman No 1 filter paper. Y<sub>2</sub>O<sub>3</sub> (Sigma Aldrich, China) was prepared to obtain a 1 M concentration, and about 450 mL of the Y<sub>2</sub>O<sub>3</sub> solution was added to 50 mL of the seed filtrate. The pH was adjusted to 9. The mixing procedure was done on a hot plate at 70°C with a magnetic stirrer at 300 rpm for 30 min till a color change was observed.

#### Characterization of the prepared Mo-Y<sub>2</sub>O<sub>3</sub>NPs

The prepared Mo-Y<sub>2</sub>O<sub>3</sub>NPs were characterized using a variety of methods, the first of which was UV-visible spectroscopy with a Shimadzu 1601 spectrophotometer. For the second analysis, Atomic force microscopy (AFM; AA-3000, USA) was used. Another important analysis was the X-ray diffraction (XRD), which was carried out with the help of the XRD diffractometer (Shimadzu 6000, Japan).<sup>11</sup>

#### Phytochemical analysis of *Moringa oleifera* seed extract

The determination of the presence of active compounds (alkaloids, tannins, flavonoids, phenolic compounds, and saponins) in the test moringa seed extract was estimated by standard protocols.<sup>12</sup>

#### Insect rearing and insecticidal bioassay

Both adults and larvae of *M. domestica* were used as experimental models to study the insecticidal efficacy of the biosynthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs using the food incorporation method. The insect was raised and experimented on in the animal house of the Department of Biology, College of Science, University of Mustansiriyah, Baghdad, Iraq. The insecticidal efficacy of the Mo-Y<sub>2</sub>O<sub>3</sub>NPs was evaluated against newly hatched instar larvae (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup>) using the diet incorporation method. The larval artificial food (10 g) was prepared by replacing the water with 2 mL of each of the prepared concentrations (100, 300, and 500 µg/mL) of Mo-Y<sub>2</sub>O<sub>3</sub>NPs. In each concentration, 10 instar larvae were introduced. Concerning the adults, the bioassay was carried out by placing 10 adult houseflies on a cotton piece soaked in a 10% sucrose solution (1 g of sucrose and 9 mL of each concentration of the synthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs). The experiments were replicated four times in plastic cups. Simultaneously, controls using deionized water only were prepared. After 24 hours, the number of surviving larvae and adults was counted.<sup>13,14</sup>

#### Statistical analysis

The Statistical Analysis System- SAS (SAS, 2012) program was used to detect the effects of different factors on study parameters. The least significant difference (LSD) test and analysis of variance (ANOVA) were used to compare the level of significance between the means in this study.<sup>15</sup>

## Results and Discussion

#### Physical characteristics of the biologically synthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs

The successful synthesis of nanoyttria was confirmed by several observations, among which was a colour change. The visual observation of colour change was detected during the synthesis, as shown in Figure 1.

#### UV-visible spectra of the biologically synthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs

The absorbance value of Mo-Y<sub>2</sub>O<sub>3</sub>NPs was initiated at 0.83 in the UV-visible spectra measurement (Figure 2). At a wavelength of 209 nm, the transmittance was 14.4%. In addition, there was a reflection factor of 0.016. The degree of absorbance was then reduced until it reached 0.21 at a length of 306 nm, while the values for both the transmission and reflection constancy increased to 60% at 231 and 0.2 at 216 nm, respectively. Starting at 400 nm and above, these variations ceased, and values remained constant (within the visible region).

#### Atomic force microscopy (AFM) of Mo-Y<sub>2</sub>O<sub>3</sub>NPs

According to AFM results, the diameters of synthesized NPs ranged from 70 to 155 nm, with volume percentages ranging from 0.43-10.87% and an average diameter of 86.38 nm, while the roughness average (RA) and root mean square were 7.2 and 8.8 nm, respectively. The granularity volume distribution of these NPs is depicted in Figure 3.

#### X-ray diffraction (XRD) of Mo-Y<sub>2</sub>O<sub>3</sub>NPs

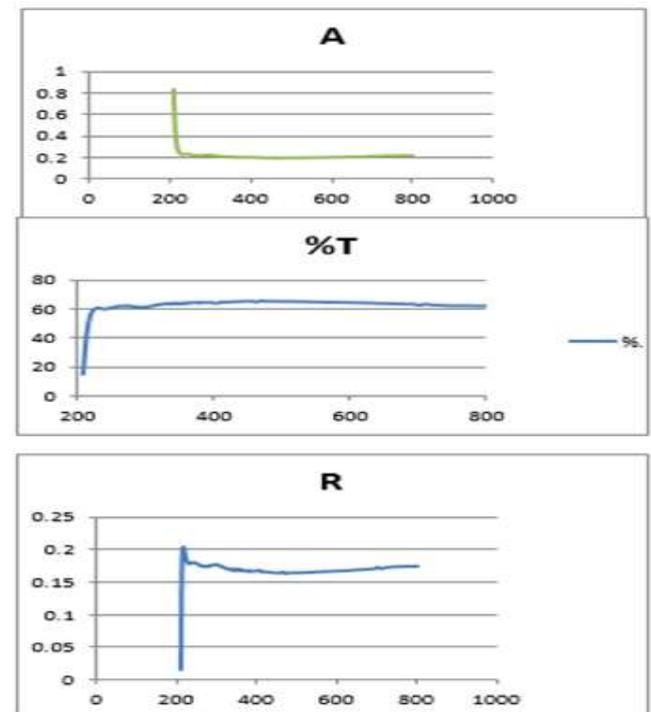
Table 1 shows the X-ray diffractometer pattern of the biologically synthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs. The diagram indicates the presence of three peaks: strong diffraction peaks (2-theta 28.13°), (2-theta 31.42°), and (2-theta 50.22°), while the average crystallite size of the Mo-Y<sub>2</sub>O<sub>3</sub>NPs was 28 nm.

#### Phytochemical constituents of *Moringa oleifera* seed extract

The presence or absence of the active phyto-components is displayed in Table 2. Many active phytochemicals are detected in the extract of moringa seed, and these phytochemicals support the formation of NPs. These phytochemicals act as reducing and capping factors for metal oxide as well as stabilize the created nanoparticles by avoiding agglomeration.<sup>16,17</sup>

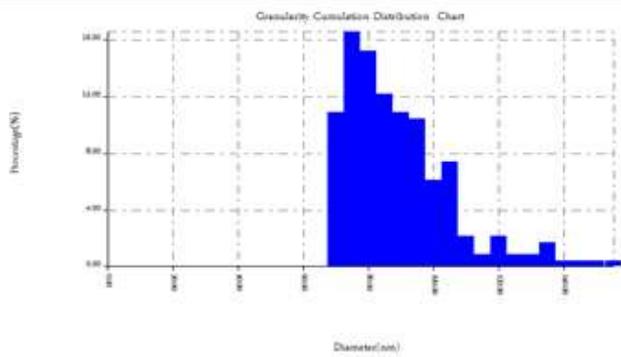


**Figure 1:** A scheme explaining the total procedure of nanoyttria synthesis using *Moringa oleifera* seeds



**Figure 2:** UV-visible spectra of nanoyttria synthesized with *Moringa oleifera* seeds.

A: absorbance; T: transmittance; R: reflection



**Figure 3:** Granularity volume distribution of Mo-Y<sub>2</sub>O<sub>3</sub>NPs

**Table 1:** X-ray diffractometer values of synthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs

2 Theta (deg)	FWHM (deg)	D (nm)	$\eta \times 10^{-4}$ lines <sup>-2</sup> .m <sup>-4</sup>	$\sigma \times 10^{14}$ lines/m <sup>2</sup>
28.13	0.45	36.4023191	9.518624323	7.546436037
31.42	0.41	40.24187515	8.61043375	6.175094117
50.22	0.7	25.06726202	13.82280999	15.91425083

**Table 2:** Preliminary detection of active compounds in *Moringa oleifera* seed extract

Chemical compound	Saponins	Phenols	Tannins	Alkaloids	Flavonoids	Resins
Present	+	+(hydrolyzed)	+	+	+	+

**Table 3:** Percentage mortality of moringa seed extract, Y<sub>2</sub>O<sub>3</sub>, and Mo-Y<sub>2</sub>O<sub>3</sub>NPs on larvae and adults of a housefly.

Applied material	Mortality percentage%		
	100 $\mu$ g/mL	300 $\mu$ g/mL	500 $\mu$ g/mL
Seed extract solution	10	16.6	26
1 <sup>st</sup> larval stage			
2 <sup>nd</sup> larval stage	3.3	13.3	20
3 <sup>rd</sup> larval stage	0	7	13.3
Adults stage	13.3	20	26.6
L.S.D.= 1 <sup>st</sup> larval stage = 0.14; 2 <sup>nd</sup> larval stage = 0.373; 3 <sup>rd</sup> larval stage = 0.171; Adults stage = 0.037			
Bulk Y2O3solution			
1 <sup>st</sup> larval stage	20	36.6	36.6
2 <sup>nd</sup> larval stage	10	26.6	30
3 <sup>rd</sup> larval stage	3.3	13.3	20
Adults stage	16.6	26.6	30
L.S.D.= 1 <sup>st</sup> larval stage = 0.050; 2 <sup>nd</sup> larval stage = 0.184; 3 <sup>rd</sup> larval stage = 0.014; Adults stage = 0.154			
Prepared Mo-Y2O3NPs solution			
1 <sup>st</sup> larval stage	96.6	96.6	100
2 <sup>nd</sup> larval stage	56.6	63.3	73.3
3 <sup>rd</sup> larval stage	50	53.3	70
Adults stage	76.6	83.3	93.3
L.S.D.= 1 <sup>st</sup> larval stage = 0.630; 2 <sup>nd</sup> larval stage = 0.033; 3 <sup>rd</sup> larval stage = 0.066; Adults stage = 0.033			
Control (untreated larvae)		0.6	
1 <sup>st</sup> larval stage			
2 <sup>nd</sup> larval stage		0.3	
3 <sup>rd</sup> larval stage		0	
Adults stage		0	

### Insecticidal activity of Mo-Y<sub>2</sub>O<sub>3</sub>NPs

In the present study, the insecticidal activity of Mo-Y<sub>2</sub>O<sub>3</sub>NPs against instar larvae and adult forms of *M. domestica* is presented as mortality percentages (Table 3). Since low mortality percentages revealed weak efficacy of seed extract and yttrium core solutions, significant percentages of 100% against 1<sup>st</sup> larvae, 73.3% against 2<sup>nd</sup> larvae, 70% against 3<sup>rd</sup> larvae, and 93.3% against adults were recorded for 500 g/mL of the biosynthesized Mo-Y<sub>2</sub>O<sub>3</sub>NPs. The total protein content as well as the activities of several metabolic enzymes, particularly acetylcholine esterase and glutathione S-transferase, were significantly lower when housefly larvae were fed a diet containing Mo-AgNPs and Mo-ZnONPs compared with when they were fed a control diet.<sup>13</sup> Furthermore, total levels of the essential enzyme phosphatase were shown to be lower in individual insects treated with biologically synthesized titanium oxide nanoparticles.<sup>14</sup> Different toxicity pathways have been examined by many researchers to elucidate the potential effect of NPs on insects, especially those made with plant-based materials.

According to a study by Nagrare,<sup>18</sup> the high mortality rate observed after copper nanoparticles (Cu-NPs) were applied to *Phenacoccus solenopsis* may be the result of stress damage to the cuticle, which was brought on by the influence of Cu-NPs on lipid bilayers or in proteins embedded in the cuticle membrane. Another suggested toxicity pathway reported by other studies corresponds with the production of reactive oxygen species (ROS) in insects exposed to silver nanoparticles.<sup>19,20</sup> This exposure resulted in a significant decline in insect vitality. The results obtained from Table 3 indicate that the recorded mortality percentages are dose-dependent. The sizes, forms, and applications of biologically produced NPs are determined by the source from which they were synthesized.<sup>8</sup> *M. oleifera* seeds are a good option for NP synthesis since they have a high ability to accumulate metals and have previously been employed for heavy metal removal in aqueous solutions and flocculation investigations.<sup>7</sup> This observation agrees with the findings which indicated that nanoparticles can affect various physiological parameters in the exposed organisms. This effect was caused by oxidative stress, which resulted in dose-dependent cell death.<sup>21</sup>

The influence of nanoparticles on insect antioxidants and detoxifying enzymes could cause oxidative stress.<sup>22</sup> On the other hand, metal nanoparticles can cause insect death by adhering to sulfur and phosphate groups in proteins and nucleic acids, resulting in a reduction in cellular and organelle membrane permeability and enzyme denaturation.<sup>23</sup> Insect pests that have been treated with nanoparticles are unlikely to develop resistance to this mechanism of action, either genetically or physiologically. In addition, as compared to chemical insecticides, nanoparticles are relatively safe for humans and ecosystems. For these reasons, nanoparticles have been proposed as a possible alternative to insecticides.<sup>24</sup>

### Conclusion

The present study is the first to show that Y<sub>2</sub>O<sub>3</sub> nanoparticles can be synthesized with a simple, low-cost, and environmentally friendly plant-assisted approach using only an aqueous extract of *M. oleifera* seeds. This study produced yttrium particles with significant nano properties, due to the use of *Moringa* seeds that supply a rich reduction and chemical stabilizing agent and the optimized preparation conditions. Additionally, by observing a high mortality ratio against both adults and larvae of an experimental housefly, the efficacy of these nanoparticles as an insecticide was proven. All of these discoveries have offered a new insight into using these NPs in numerous biocontrol fields and against various pests.

### Conflict of Interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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