INSECTICIDAL TOXICITY POTENTIAL OF SYNTHESIZED COPPER NANOPARTICLES BY *Coffee arabica* PLANT EXTRACT AGAINST DENGUE VECTOR

POTENSI TOKSISITAS INSEKTISIDA DARY NANOPARTIKEL TEMBAGA YANG DISINTESIS DENGAN EKSTRAK BIJI KOPI ARABIKA TERHADAP VEKTOR DBD

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DISSERTATION

as one of the requirements for achieving a doctorate degree

Chemical Science Study Program

Prepared and submitted by

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TO:

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DISSERTATION APPROVAL PAGE

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STATEMENT OF DISSERTATION ORIGINALITY

AND COPYRIGHT TRANSFER

I hereby declare that the dissertation entitled "**Insecticidal Toxicity Potential of Copper Nanoparticles Synthesized by *Coffee arabica* Plant Extract Against Dengue Vector**" is the result of my own work under the guidance of the supervision committee, Prof. Dr. Ahyar Ahmad, as the Supervisor, dr. Isra Wahid as the Co-Supervisor 1, and Prof. Dr. Paulina Taba, M.Phil., as the Co-Supervisor 2. This scientific work has not been submitted and is not currently being submitted in any form to any other university. Sources of information derived from or cited from the published or unpublished works of other authors have been duly acknowledged in the text and listed in the References section of this dissertation. Some of the content of this dissertation has been published in the following journals: **1.** RASĀYAN J. Chem., Vol. 16 | No. 3 |1217-1228| July - September | 2023 (Green Synthesis of Copper Nanoparticles Mediated from *Coffee arabica* Seeds Extract) http://doi.org/10.31788/RJC.2023.1638417. **2.** Journal of Bioscience and Applied Research, 2023, Vol.9, No. 4, P.199-211. (Green Synthesis of Copper Nanoparticles Using *Coffee arabica*: larvicidal and biochemistry study.) http://doi.org/10.21608/jbaar.2023.326117. **3.** Journal of Advanced Biotechnology and Experimental Therapeutics. 2024 May; 7(2): 314-327, (*Coffee arabica*-derived copper nanoparticles: A potent larvicidal agent against *Aedes aegypti* mosquitoes) http://doi.org/ 10.5455/jabet.2024.d26.

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Despite the limitations of this dissertation, I hope it can contribute meaningfully to the advancement of applied biochemistry knowledge, particularly in the field of mosquito control.

Makassar, July 24, 2024

Yousef Abdulwahab Ahmed

ABSTRACT

YOUSEF ABDULWAHAB AHMED. **Insecticidal Toxicity Potential of Synthesized Copper Nanoparticles by *Coffee arabica* Plant Extract against Dengue Vector** (supervised by Ahyar Ahmad, Isra Wahid, and Paulina Taba).

Background: Mosquito-borne diseases pose significant health risks. This study investigated the larvicidal activity of *Coffee arabica*-mediated copper nanoparticles against *Aedes aegypti* mosquito larvae. Method: *Coffee arabica* extract was used to synthesize eco-friendly copper nanoparticles. The nanoparticles were then studied using UV, infrared, and XRD. Green, roasted *Coffee arabica* and nanoparticles were tested against *Aedes aegypti* mosquitoes. Microscopy and histopathology were utilized to assess nanoparticle-induced structural alterations in mosquito larvae. DNA fragmentation analysis was used to screen mosquito larvae for genetic toxicity. To determine how copper nanoparticles affect mosquitoes, glucose, total protein, and metabolic enzymes such as aspartate transferase, alkaline phosphatase, and lactate dehydrogenase were measured. Hemolysis and non-target organism tests showed CuNPs' toxicity. Results: Copper ions were successfully converted into CuNPs within 15 minutes using *Coffee arabica* beans. The synthesized CuNPs remained stable, with peak absorbance at 262 nm after 3 and 30 days. FTIR analysis confirmed the presence of bonds (OH, C=C, and C-H) crucial for CuNP formation. XRD results indicated monoclinic crystalline CuNPs with a mean size of 16.3 nm. Green *Coffee arabica* had LC_{50} and LC_{90} concentrations of 124.5 ppm and 456.5 ppm, while roasted *Coffee arabica* had concentrations of 73.5 ppm and 371.5 ppm. CuNPs at 2 to 100 ppm concentrations resulted in LC_{50} and LC_{90} concentrations of 5.796 ppm and 36.595 ppm. The synthesized CuNPs demonstrated concentration-dependent mortality, with lower LC_{50} values than Coffee extracts alone. Biochemical tests showed glucose, protein, and enzyme alterations. While glucose and aspartate transferase increased, total protein, alkaline phosphatase, and lactate dehydrogenase decreased. These studies show CuNPs' harmful effects on mosquito larvae and metabolic markers. Histological analysis further supported these effects, revealing disruptions in the tissues of mosquito larvae, particularly in the abdomen and thoracic regions. Molecular analysis at 100 ppm CuNPs showed DNA damage. CuNPs were found to be safe in hemolytic experiments. Copper nanoparticles also affected *Artemia salina* larvae, with higher concentrations causing increased mortality. The LC_{50} and LC_{90} values for *Artemia salina* were 344.3 ppm and 1073.2 ppm, respectively. Conclusion: The environmentally friendly copper nanoparticles derived from *Coffee arabica* seeds offer a promising solution for controlling *Aedes aegypti* mosquitoes.

Keywords: *Coffee arabica*, Copper nanoparticles, Insecticidal, Dengue Vector

ABSTRAK

YOUSEF ABDULWAHAB AHMED. **Potensi Toksisitas Insektisida Dary Nanopartikel Tembaga Yang Disintesis dengan Ekstrak Biji Kopi Arabika Terhadap Vektor DBD** (dibimbing oleh Ahyar Ahmad, Isra Wahid, dan Paulina Taba).

Latar Belakang: Penyakit yang ditularkan oleh nyamuk memiliki risiko kesehatan yang signifikan. Penelitian ini menginvestigasi aktivitas larvasida nanopartikel tembaga yang disintesis menggunakan ekstrak kopi arabika terhadap larva nyamuk *Aedes aegypti*. Metode: Ekstrak kopi arabika digunakan untuk sintesis nanopartikel tembaga yang ramah lingkungan. Nanopartikel tersebut kemudian dianalisis menggunakan spektrofotometri, inframerah, dan sinar-X. Kopi arabika hijau, sangrai, dan nanopartikel tembaga diuji terhadap nyamuk Aedes aegypti. Mikroskopi dan histopatologi digunakan untuk menilai perubahan struktural pada larva nyamuk. Analisis fragmentasi DNA digunakan untuk skrining toksisitas genetik pada larva nyamuk. Glukosa, protein total, dan enzim metabolik seperti aspartat transferase, alkalin fosfatase, dan laktat dehidrogenase diukur untuk memahami pengaruh nanopartikel tembaga pada nyamuk. Uji hemolisis dan organisme non-target menunjukkan toksisitas CuNPs. Hasil: Ion tembaga berhasil diubah menjadi CuNPs dalam 15 menit menggunakan biji kopi arabika. CuNPs yang disintesis tetap stabil, dengan serapan puncak pada 262 nm setelah 3 dan 30 hari. Analisis FTIR mengkonfirmasi adanya ikatan (OH, C=C, dan C-H) yang penting untuk pembentukan CuNP. Hasil XRD menunjukkan CuNPs kristalin monoklinik dengan ukuran rata-rata 16,3 nm. Kopi arabika hijau memiliki konsentrasi LC_{50} dan LC_{90} masing-masing 124,5 ppm dan 456,5 ppm, sedangkan kopi arabika sangrai memiliki konsentrasi 73,5 ppm dan 371,5 ppm. CuNPs pada konsentrasi 2 hingga 100 ppm menghasilkan LC_{50} dan LC_{90} masing-masing 5,796 ppm dan 36,595 ppm. CuNPs menunjukkan kematian tergantung pada konsentrasi, dengan nilai LC_{50} lebih rendah dari ekstrak kopi saja. Tes biokimia menunjukkan perubahan glukosa, protein, dan enzim. Glukosa dan aspartat transferase meningkat, sedangkan protein total, alkalin fosfatase, dan laktat dehidrogenase menurun. Analisis histologis mendukung temuan ini dengan mengungkapkan gangguan pada jaringan larva nyamuk, terutama di perut dan dada. Analisis molekuler pada konsentrasi 100 ppm CuNPs menunjukkan kerusakan DNA. CuNPs aman dalam uji hemolitik. Nanopartikel tembaga juga mempengaruhi larva *Artemia salina*, dengan konsentrasi tinggi menyebabkan kematian. Nilai LC_{50} dan LC_{90} untuk Artemia salina masing-masing 344,3 ppm dan 1073,2 ppm. Kesimpulan: Nanopartikel tembaga yang ramah lingkungan dari biji kopi arabika menawarkan solusi yang menjanjikan dalam pengendalian nyamuk *Aedes aegypti*.

Kata Kunci: Kopi arabika, Nanopartikel tembaga, Insektisida, Vektor Demam Berdarah

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbols/abbreviations	Meaning and Description
nm	Nanometers
CuNPs	Copper Nanoparticles
DNA	Deoxyribonucleic Acid
XRD	X-Ray Diffraction
FTIR	Fourier - Transform Infrared
LC_{50}	Median Lethal Concentration
LC_{90}	Lethal Concentration Ninety
ppm	Parts Per Million
g/L	Gram Per Liter
0D	Zero-Dimensional
1D	One-Dimensional
2D	Two-Dimensional
3D	Three-Dimensional
NPs	Nanoparticles
CVD	Chemical Vapor Deposition
Ag	Silver
Au	Gold
ROS	Reactive Oxygen Species
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
AFM	Atomic Force Microscope
SPM	Scanning Probe Microscopy
BiNPs	Bismuth Nanoparticles
ZnO NPs	Zinc Oxide Nanoparticles
CAE	*Coffee arabica* Leaf
AgNPs	Silver Nanoparticles
SCGs	Spent Coffee Grounds
CuO NPs	Copper Oxide Nanoparticles
UV-Vis	Ultraviolet-Visible
MCF7	Breast Cancer Cells
WHO	World Health Organization
CuSNPs	Copper Sulphide Nanoparticles
Ni-MOFs	Nickel Metal Organic Frameworks
AST	Aspartate Aminotransferase
LDH	Lactate Dehydrogenase
ALP	Alkaline Phosphatase
$OH^{\bullet}$	Hydroxyl Radicals
UNHAS	Hasanuddin University
AAS	Atomic Absorption Spectrometry
QE	Quercetin Equates
GAE	Gallic Acid Equates
µL	Micro Liter
DPPH	2,2-Diphenyl-1- Picrylhydrazyl
RSA	Radical Scavenging Activity
IC_{50}	Half Maximal Inhibitory Concentration
PBS	Phosphate-Buffered Saline

Buffer ATL	Lysis Buffer
RNase A	Ribonuclease A
RPM	Revolutions Per Minute
TE buffer	Tris-EDTA Buffer
As	Absorbance Of the Standard
Ab	Absorbance Of Blank
4-NPP	4-Nitrophenol Phosphate
GOT	Glutamic-Oxaloacetic Transaminase
MDH	Malate Dehydrogenase
NAD	Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
RBCs	Red Blood Cells
SPR	Surface Plasmon Resonance
JCPDS	Joint Committee on Powder Diffraction Standards.
TPC	Total Phenolic Content
TFC	Total Flavonoid Capacity
p-value	Probability

CHAPTER I
INTRODUCTION

A. Background:

Mosquito-borne diseases pose a significant threat to global public health, with dengue fever being one of the most prevalent and debilitating diseases transmitted by mosquitoes. Dengue fever is caused by the dengue virus, which is transmitted to humans through the bites of infected Aedes mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus*. According to the World Health Organization, an estimated 390 million people are infected with dengue annually, leading to approximately 20,000 deaths (Togami et al., 2023).

Traditionally, chemical insecticides have been widely used to control mosquito populations and prevent the transmission of mosquito-borne diseases. These insecticides are effective in reducing mosquito populations; however, their pollution and excessive use have raised several concerns. One of the major concerns is the development of insecticide resistance in mosquito populations, making them less susceptible to the effects of the chemicals. As a result, the efficacy of conventional insecticides in controlling mosquito vectors has significantly decreased (Vale et al., 2023).

The use of chemical insecticides has been associated with negative environmental impacts. These insecticides can contaminate water sources, soil, and plants, leading to ecological disruption and posing risks to non-target organisms. Additionally, there are concerns regarding the potential adverse effects of chemical insecticides on human health, as exposure to these chemicals has been linked to various health issues (Zhang, et al., 2022). In recent years, there has been a growing interest in exploring alternative approaches for mosquito control that are effective, environmentally friendly, and sustainable. One promising avenue of research involves the use of nanoparticles synthesized from plant extracts. Plant extracts are rich in bioactive compounds, such as polyphenols and flavonoids, which have demonstrated insecticidal properties against mosquito vectors (Onen et al., 2023).

Among the various plant sources, *Coffee arabica* has gained attention in insecticidal research due to its diverse bioactive compounds. *Coffee arabica*, a species of coffee plant, is widely cultivated for its beans, which are used to produce coffee beverages. Apart from its economic significance, *Coffee arabica* has been recognized for its medicinal and therapeutic properties. The plant extract derived from *Coffee arabica* contains a range of bioactive compounds, including polyphenols and flavonoids, which have been reported to exhibit insecticidal activity against mosquito vectors (Drago et al., 2021).

In addition to the insecticidal properties of plant extracts, the use of nanoparticles has shown promise in various fields. Nanoparticles are particles with dimensions in the range of 1 to 100 nanometers. They possess unique physicochemical properties that differ from their bulk counterparts, making them highly useful in various applications. Copper nanoparticles, in particular, have received considerable attention due to their potent antimicrobial and insecticidal activities (El-Saadony et al., 2020).

The combination of plant extracts and nanoparticles offers a potential solution for mosquito control. By synthesizing copper nanoparticles using *Coffee arabica* plant extract, it is possible to create a novel insecticide that connects the insecticidal properties of both components. The synthesis of nanoparticles using plant extracts, often referred to as green synthesis, provides an eco-friendly and sustainable alternative to conventional methods (Alghamdi, 2021).

The potential of synthesized copper nanoparticles from *Coffee arabica* seed extract as an insecticide against dengue vectors has not been extensively explored. While there is existing research on the insecticidal properties of plant extracts and nanoparticles individually, there is a research gap in evaluating the insecticidal toxicity potential of synthesized copper nanoparticles using *Coffee arabica* plant extract specifically against dengue vectors.

Addressing this research gap is crucial to further our understanding of the efficacy and safety of these synthesized copper nanoparticles as an alternative for controlling dengue vectors. By investigating the insecticidal properties of the nanoparticles, their mode of action, and their potential impact on non-target organisms and the environment, we can gain valuable insights into the development of sustainable strategies for dengue vector control.

In summary, the background of this research highlights the significant threat of mosquito-borne diseases, the limitations and concerns associated with conventional chemical insecticides, and the potential of nanoparticles synthesized from plant extracts as eco-friendly alternatives. The focus on *Coffee arabica* plant extract and copper nanoparticles presents a novel approach that requires further investigation to determine its effectiveness, mode of action, and potential benefits for controlling dengue vectors.

B. Problem Formulation:

The research objectives outlined in this study aim to address the following questions:

1. What are the physicochemical properties of copper nanoparticles synthesized using *Coffee arabica* plant extract?
2. What is the larvicidal activity of the synthesized copper nanoparticles against *Aedes* mosquito larvae?
3. How do synthesized copper nanoparticles affect dengue vectors?
4. What is the potential impact of the synthesized copper nanoparticles on non-target organisms and the environment?

5. What are the qualitative and quantitative analyses of phytochemicals in both green and roasted *Coffee arabica* plant extracts?

C. Research Objectives:

1. Synthesize copper nanoparticles using *Coffee arabica* plant extract and characterize their physicochemical properties.
2. Evaluate the larvicidal activity of the synthesized copper nanoparticles against *Aedes* mosquito larvae.
3. Investigate the mode of action of the synthesized copper nanoparticles on dengue vectors.
4. Determine the potential impact of the synthesized copper nanoparticles on non-target organisms and the environment.
5. Perform qualitative and quantitative analysis of phytochemicals in both green and roasted *Coffee arabica* plant extracts.

D. Benefits of Research:

- Contributes to knowledge on green synthesis methods and eco-friendly approaches for nanoparticle synthesis.
- Provides insights into effective and safe alternatives to chemical insecticides for dengue vector control.
- Enhances understanding of the mode of action of the synthesized copper nanoparticles against dengue vectors.
- Evaluates the possible hazards and environmental effects to ensure the safe deployment of the nanoparticle.

CHAPTER Ⅱ
A COMPREHENSIVE REVIEW ON THE EFFECTIVENESS OF COFFEE PLANT EXTRACTS AND GREEN NANOPARTICLES FOR MOSQUITO CONTROL OF THE DENGUE VECTOR *Aedes aegypti*.

2.1 Abstract:

This comprehensive review examines the efficacy and environmental impact of coffee plant extracts and green nanoparticles as biocontrol agents for mosquitoes. Mosquitoes pose a significant public health threat by transmitting diseases such as malaria, dengue, Zika, and yellow fever. However, the use of insecticides for mosquito control raises concerns about the development of resistance and environmental and human health impacts. As an alternative, biocontrol strategies that utilize natural predators, parasites, and pathogens have gained attention. Coffee plant extracts have shown larvicidal, ovicidal, and repellent properties against mosquitoes, whereas green nanoparticles, particularly copper and silver nanoparticles, exhibit insecticidal activity. These alternatives offer advantages such as environmental friendliness, reduced resistance risks, and targeted mosquito species control while minimizing harm to non-target organisms. However, challenges exist in the mass production and distribution of biocontrol agents as well as their susceptibility to environmental factors. Ongoing research is aimed at developing more effective biocontrol agents for mosquito-borne disease prevention. This review provides valuable insights into the potential of coffee plant extracts and green nanoparticles for mosquito control, their efficacy, and the environmental considerations associated with their use.

Key words: coffee plant, green nanoparticles, copper nanoparticles, mosquito control, environmental impact.

2.2 Introduction

Mosquitoes belong to the family Culicidae and are known to transmit various diseases, including malaria, dengue fever, Zika virus, and Chikungunya. *Aedes aegypti* and Aedes albopictus are the two most common mosquito species in Indonesia and are responsible for transmitting diseases such as chikungunya, Zika, and dengue fever. Vector control plays a crucial role in preventing the spread of these diseases (Song et al., 2020). Mosquito larvae primarily breed in standing fresh water, although some species can utilize brackish water or specific conditions in flowing streams. Different mosquito species have different habitat preferences, including stream pools, rainwater pools, tree holes, and plant leaf axils (Ali et al., 2021).The behavior of Aedes mosquitoes, particularly Aedes aegypti, has been extensively studied. They are highly anthropophilic and tend to bite daily. *Aedes aegypti* is adaptable and can breed in various habitats, including urban areas. These mosquitoes are attracted to humans and exhibit multiple bites before egg-laying. The behavior of *Aedes aegypti* and Aedes albopictus mosquitoes can vary, with differences in resting behavior, feeding activity, and activity patterns (Ahebwa et al., 2023).

2.2.1 Mosquitoes

There are around 3,000 species of mosquitoes in the Diptera order. The mosquito family Culicidae comes from the Latin word "culex" meaning gnat. The term "mosquito" comes from "Mosca" and "Ito"—"little fly" in English. Mosquitoes have a thin, segmented body, two wings, three pairs of long legs that resemble hair, feathery antennae, and extended mouthparts (Figure 2.1). About 226 million years ago, these insects appeared.

Their life cycle comprises four stages: egg, larva, pupa, and adult. The eggs are laid on the water surface and hatch into mobile larvae that feed on aquatic algae and organic matter, as shown in Figure 2.1. Female mosquitoes possess tube-like mouthparts, known as proboscis, which can penetrate the host's skin (commonly referred to as a bite) to consume blood. Blood serves as a source of protein and iron, which are necessary for egg production. (Swart et al., 2023).

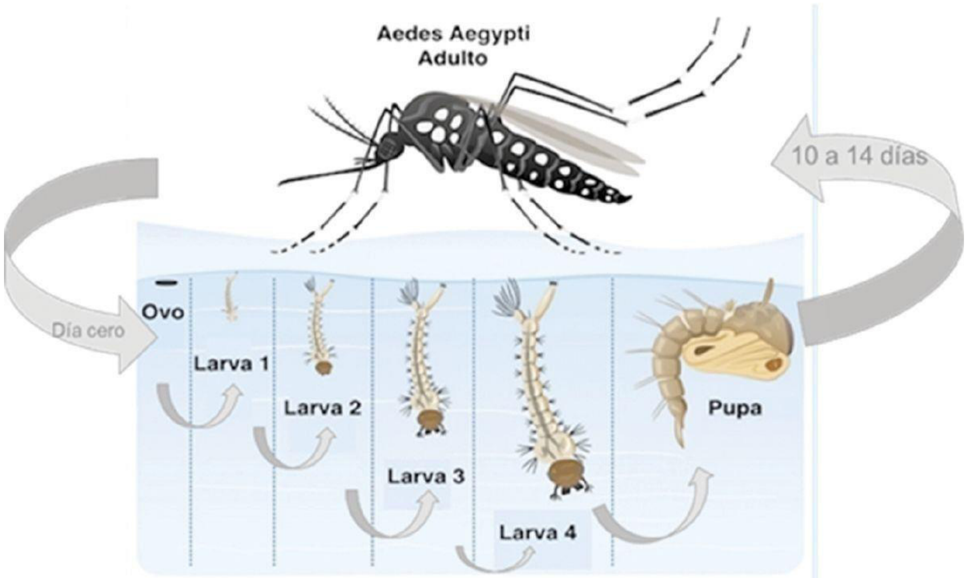


Figure 2-1 Morphology and life cycle *Aedes aegypti* (Koehler & Pereira, 2020)

Mosquitoes transmit diseases to the host through their saliva, causing itching and a rash. Additionally, many species of mosquitoes carry disease-causing organisms and act as vectors for diseases like malaria, yellow fever, Chikungunya, West Nile virus, dengue fever, filariasis, Zika virus, and other arboviruses. It is estimated that mosquitoes are responsible for over 700,000 deaths annually, making them the deadliest animal taxon in terms of human fatalities (Hribar et al., 2022).

Table 2-1 The classification of mosquitos

Taxonomic Rank	Scientific Name
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Diptera
Suborder	Nematocera
Family	Culicidae
Subfamily	Culicinae
Genus	*Aedes*
Species	*aegypti, albopictus*

The two most common mosquito species in Indonesia are *Aedes aegypti* and *Aedes albopictus* (Table 2.1). These mosquitoes can transmit chikungunya, Zika, and dengue fever. There is currently no approved vaccine for chikungunya or Zika, and the dengue vaccine is still being tested. There is also no specific treatment for any of these three diseases (Carreto et al., 2022).

Vector control is an important way to prevent the spread of these diseases. The most important vector is *Aedes aegypti*, which transmits dengue fever. This mosquito is found in tropical and subtropical regions all over the world. The incidence of dengue fever has increased fourfold since 1970, and nearly half of the world's population is now at risk. In 1990, almost 30% of the world's population, or 1.5 billion people, lived in areas where the risk of dengue transmission was greater than 50% (Song et al., 2020).

In 2005, an outbreak of chikungunya virus infection began in the southwest Indian Ocean islands. The outbreak spread to India and has resulted in an ongoing epidemic that has affected more than 1.5 million people, including travelers who have visited these areas (Khongwichit et al., 2021).

In other words, the two most common mosquito species in Indonesia can transmit serious diseases for which there is no cure. Vector control is an important way to prevent the spread of these diseases (Organization, 2021).

2.2.1.1 Mosquito larval habitats

According to Ali et al. (2021), the breeding sites of mosquito larvae predominantly consist of standing fresh water, with only a few species capable of utilizing salty water or specific conditions in flowing streams. It was observed that unused polythene sheets in rubber plantations, when left undisturbed, can retain water and serve as breeding grounds for many larvae. (Ali et al., 2021). Generally, different mosquito species prefer different habitats. Some common habitats include stream pools, slow-flowing streams, rainwater pools, sandy pools, spring pools, fallow fields, bamboo stumps, tree holes, and plantain leaf axils.

In other words, there are two main types of environmental factors that affect whether a place is suitable for mosquitoes to breed; physical and chemical factors. Physical factors include things like temperature, humidity, and the availability of water and shelter. Chemical factors include things like the pH of the water and the presence of chemicals that can kill mosquitoes or their larvae.

2.2.1.2 Behavior of *Aedes* mosquitoes

Egid et al. (2022) reviewed the ecology and behavior of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Western Africa and their implications for vector control. They found that both species are highly anthropophilic and bite predominantly during the day. They also found that *Aedes aegypti* is more likely to rest indoors than *Aedes albopictus* (Egid et al., 2022). Facchinelli et al. (2023) studied the biology and behavior of *Aedes aegypti* mosquitoes in the human environment, and the opportunities for vector control of arbovirus transmission. They found that *Aedes aegypti* is a highly adaptable mosquito that can breed in a variety of habitats, including urban areas. They also found that *Aedes aegypti* mosquitoes are attracted to humans and bite multiple times before laying eggs (Facchinelli et al., 2023). Ahebwa et al. (2023) investigated the behavior of *Aedes aegypti* and *Aedes albopictus* mosquitoes in northeastern Thailand. They found that both species exhibit indoor resting behavior, *Aedes aegypti* exhibits a stronger feeding activity during hot season while *Aedes albopictus* is an all-round biter. They also found that *the first kind of* mosquitoes are more active during the day than *the later kind of* mosquitoes, *Aedes aegypti* is adapting to zoophagic feeding while *Aedes albopictus* is becoming endophagic (Ahebwa et al., 2023).

2.2.1.3 Mosquito control

Methods used for mosquito control include the elimination of breeding sites and the control of mosquito larvae and adults.

2.2.1.4 Emerging mosquito control strategies

Mosquito breeding site elimination is a critical component of integrated vector management programs to reduce mosquito populations and control the transmission of mosquito-borne diseases. A recent study found that environmental modification and larviciding were effective in reducing mosquito populations by up to 90%. The study found that biocontrol was effective in reducing mosquito populations by up to 70%. These findings highlight the importance of mosquito breeding site elimination for reducing mosquito populations and controlling the transmission of mosquito-borne diseases (Onen et al., 2023).

In addition to these traditional mosquito control strategies, new and advanced approaches are emerging. A recent study (Steele & McDermott, 2022), found that a new type of mosquito trap that uses sound to attract mosquitoes was effective in reducing mosquito populations by up to 95%. This study suggests that sound-based mosquito traps could be a valuable new tool for mosquito control programs.

Overall, the research on mosquito control is rapidly evolving, and new and effective strategies are emerging all the time. By integrating traditional and innovative approaches, we can make significant progress in reducing mosquito populations and controlling the transmission of mosquito-borne diseases.

2.2.1.5 Insecticide on the market

Currently, the agrochemical engineering offers a diverse array of insecticides that effectively combat agricultural pests and disease vectors. However, in the past, alternative methods were employed to control insect pests, including the use of plant extracts, wood, sulfur, and chalk. As the 19^{th} century emerged, botanical insecticidal compounds such as pyrethrum emerged as viable options, alongside the utilization of sulfur, arsenic, fluorides, soaps, and kerosene to tackle insect infestations and outbreaks (Nasir, 2021).

The rational advancement of synthetic insecticides commenced during World War II, leading to significant steps in the creation of reasonable and efficient chemical insecticides. This period witnessed a surge in insecticide development, facilitated by the synthesis of novel compounds and the establishment of standardized screening techniques and bioassays. These efforts concluded with the identification of diverse chemical structures with potent biological activity against pest insects, marking an essential advance in the field of insecticide research and development (Umetsu & Shirai, 2020).

Regrettably, certain chemicals within this category pose risks to mammals, beneficial arthropods, and the overall environment. However, in the 1970s, the advent of pyrethroids brought about a substitution of other hazardous and less ecologically friendly compounds. Pyrethroids underwent thorough screening to identify compounds that specifically target insects while minimizing impacts on non-target organisms and ecosystems (Ravula & Yenugu, 2021).

Emerging compounds are now required to adhere to more stringent criteria, including novel modes of action coupled with reduced resistance risks, environmental friendliness, and targeted control. The introduction of innovative insecticide classes, such as uncouplers, oxadiazines, diacylhydrazines, and compounds derived from natural sources like Neem extract, Rotenone, spinosyns and avermectins, holds promising prospects for future management of lepidopteran pests. These advancements aim to alleviate the strain on existing resistance mechanisms while providing new avenues for effective control measures (Mingbo et al., 2022).

2.2.1.6 Chemical insecticides

Chemical insecticides are a type of pesticide that is specifically designed to kill insects. They are used in a wide variety of settings, including agriculture, forestry, and urban areas, to control insect pests. Chemical insecticides can be classified into different groups based on their mode of action, which is the way in which they kill insects.

Chemical insecticides play an important role in agriculture and other industries, but they must be used carefully to minimize their negative impacts on human health and the environment. A 2017 review of the effects of chemical insecticides on four common butterfly families found that all of the insecticides studied had negative effects on butterfly survival, feeding, and oviposition behaviour (Mulé et al., 2017). A 2022 review of the mixture toxicity of pesticides to aquatic organisms found that mixtures of pesticides can be more toxic to aquatic organisms than individual pesticides (Kadiru et al., 2022). A 2021 study found that the use of neonicotinoids is associated with a decline in bee populations (Stuligross & Williams, 2021).

These studies highlight the need for careful and thoughtful use of chemical insecticides. It is important to balance the benefits of using insecticides to control insect pests with the potential risks to human health and the environment. Chemical insecticides should be used only when necessary and at the lowest effective dose. They should also be applied in a targeted manner to avoid harming non-target organisms.

2.2.1.7 Insecticide Resistance

Insecticide resistance refers to the ability of certain insect populations to survive exposure to insecticides that are designed to kill them. This resistance can occur due to genetic mutations or adaptations in the insect's physiology, rendering the insecticide less effective in controlling their population. The problem of insecticide resistance has become widespread and poses a significant challenge in mosquito control (Ahmed et al., 2022).

To combat this issue, it is crucial to regularly monitor mosquito populations for resistance to different types of insecticides. By implementing integrated management strategies that include mechanical control, sanitation, and educational actions to reduce breeding sites, we can lessen the reliance on chemical control methods. By diversifying the types of insecticides used and regularly rotating them, we can also lessen the development of resistance (Arifin et al., 2022).

There are several mechanisms through which mosquitoes develop resistance to insecticides. These mechanisms include target site insensitivity, increased detoxification enzyme activity, and reduced penetration of the insecticide into the mosquito's body (Xu et al., 2022). Insecticide resistance not only affects mosquito control efforts but also has significant implications for agriculture. Insecticide resistance in agricultural pests can lead to reduced crop yields and increased economic losses for farmers (Liu, et al., 2022).

2.2.1.8 Biocontrol strategies

Mosquitoes are a major public health threat, transmitting a wide range of diseases, including malaria, dengue, Zika, and yellow fever. The widespread use of insecticides to control mosquitoes has led to the development of resistance, as well as environmental and human health concerns. Biocontrol strategies offer a promising alternative to insecticides, using natural predators, parasites, and pathogens to suppress mosquito populations.

A variety of biocontrol strategies have been developed, including predators, parasites and pathogens. Predators: Predatory fish, such as *Gambusia affinis* and *Poecilia reticulata*, are widely used to control mosquito larvae in aquatic habitats. Other predators, such as bats, birds, and dragonflies, also play a role in mosquito control (Singh et al., 2022). Parasites: A number of parasitic organisms can infect and kill mosquitoes, including nematodes, fungi, and bacteria. For example, the nematode *Romanomermis culicis* is a highly effective parasite of mosquito larvae (Dahmana & Mediannikov, 2020). Pathogens: Certain bacteria and viruses can also be used to control mosquitoes. For example, the bacterium *Bacillus thuringiensis* var. israelensis (Bti) is a selective mosquito larvicide that is widely used in public health programs (Ferreira et al., 2019). Biocontrol strategies have a number of advantages over insecticides. They are generally more environmentally friendly and less likely to lead to resistance. Biocontrol agents can also be targeted to specific mosquito species, which can help to reduce the impact on non-target organisms.

However, biocontrol strategies also have some challenges. One challenge is that some biocontrol agents can be difficult to mass-produce and distribute. Another challenge is that biocontrol agents can be affected by environmental factors, such as temperature and pH (Dey et al., 2023). Despite these challenges, biocontrol strategies are an increasingly important part of mosquito control programs. A number of research projects are underway to develop new and more effective biocontrol agents.

2.2.1.9 Mosquito-controlling plants and derivatives

Plant extracts can be used to develop effective mosquito control products, including repellents, larvicides, and adulticides. Repellents prevent mosquitoes from biting, while larvicides and adulticides kill mosquitoes at different stages of their life cycle. Some common mosquito-repellent plants include citronella, eucalyptus, lavender, lemongrass, peppermint, and thyme. These plants can be used in a variety of ways as repellents, such as applying the essential oils to the skin, burning the dried leaves or stems, or growing the plants around homes and gardens (Anwar & Siringo-Ringo, 2020; Bava et al., 2023; Lee, 2018).

Some common mosquito larvicidal plants include neem, garlic, chili pepper, turmeric, and ginger. Plant extracts from these plants can be applied to water bodies where mosquito larvae are breeding. Some common mosquito adulticidal plants include pyrethrum, chrysanthemum, lantana, catnip, and mint. Plant extracts from these plants can be used to develop sprays, coils, and other products that can be used to kill adult mosquitoes (Chetri et al., 2022; Gouri Sreepriya, 2022).

Researchers are also developing new mosquito control products from plant extracts. For example, a study found that a combination of neem oil and citrus oil was effective in repelling and killing mosquitoes (Boanyah & Boakye, 2022). Another study found that a compound derived from the garlic plant was effective in killing mosquito larvae (Dusi et al., 2022).

Plants offer a promising alternative to synthetic insecticides for the control of mosquitoes. Plant extracts can be used to develop effective mosquito control products that are safe and environmentally friendly.

2.2.2 *Coffee arabica* plant

The *Coffee arabica* plant is a native of Ethiopia and is considered to be the oldest and most popular species of Coffee in the world (figure 2.2). It is responsible for producing about 60% of the world's coffee supply. *Coffee arabica* plants are typically grown in high altitudes, between 3,000 and 6,000 feet above sea level, and require a mild climate with moderate rainfall. The plants are relatively slow-growing and take about three to four years to reach maturity.

Coffee arabica beans are known for their high quality and complex flavor profile. They are often described as having a smooth, chocolatey flavor with notes of caramel, fruit, and nuts. The flavor of *Coffee arabica* beans can vary depending on the growing conditions, the processing method, and the roast level.

Coffee arabica beans are a good source of antioxidants and other beneficial compounds. Antioxidants can help to protect the body against damage caused by free radicals. Free radicals are unstable molecules that can damage cells and lead to diseases such as cancer and heart disease. *Coffee arabica* beans also contain Caffeine, which is a stimulant that can improve alertness and energy levels.

Figure 2-2 *Coffee arabica* plant (Coffea arabica) | Rafael Medina | Flickr is licensed under CC BY-NC-ND.

In addition to its use as a beverage, *Coffee arabica* has also been used for medicinal purposes. Here is a brief overview of some of the key findings from the literature on the *Coffee arabica* plant:

Coffee arabica beans are a good source of antioxidants and other beneficial compounds. *Coffee arabica* extract has anti-inflammatory and antimicrobial properties. *Coffee arabica* extract has been shown to be effective in treating skin conditions such as eczema and psoriasis. Table 2.2 lists some health benefits of *Coffee arabica*, with references:

Table 2-2 Summarizes some medical benefits *Coffee arabica*

Key finding	**References**
Coffee arabica beans are a good source of antioxidants	(Jung et al., 2021)
Coffee arabica extract has anti-inflammatory and antimicrobial properties.	(Artusa et al., 2022)
Coffee arabica extract has insecticide and antimicrobial properties.	(Hussein et al., 2022)
Coffee arabica extract has been shown to be effective in treating skin conditions such as eczema and psoriasis.	(Chang et al., 2023)
Reducing the risk of type 2 diabetes	(Kolb et al., 2021, p. 2)

Coffee arabica may also have other health benefits, such as reducing the risk of diabetes, Parkinson's disease, and Alzheimer's disease. However, more research is needed to confirm these findings. Overall, the *Coffee arabica* plant is a valuable resource with a wide range of potential applications. It is a popular drink crop that produces high-quality coffee beans with a complex flavor profile. In addition, *Coffee arabica* extract has been shown to have a number of medicinal properties.

2.2.2.1 *Coffee arabica* in Yemen

Yemeni coffee is regarded worldwide for its rich flavor and great quality. This unusual flavor comes from its cultivation in Yemen's mountains at up to 6,000 feet and traditional natural processing. Yemeni coffee is roasted to a medium or light roast and brewed in jebenas. Yemen exports coffee, which coffee lovers worldwide enjoy. Yemenis provide coffee to guests as a show of hospitality. Small cups of Yemeni jebena coffee with sugar and cardamom are provided (Mannaa & Benlarabi, 2021).

2.2.2.2 Biochemical Composition

Coffee arabica is a complex mixture of over 80 biochemical compounds, including caffeine, Chlorogenic acid and Phenylindanes as antioxidants, and other beneficial compounds (figure 2.3).

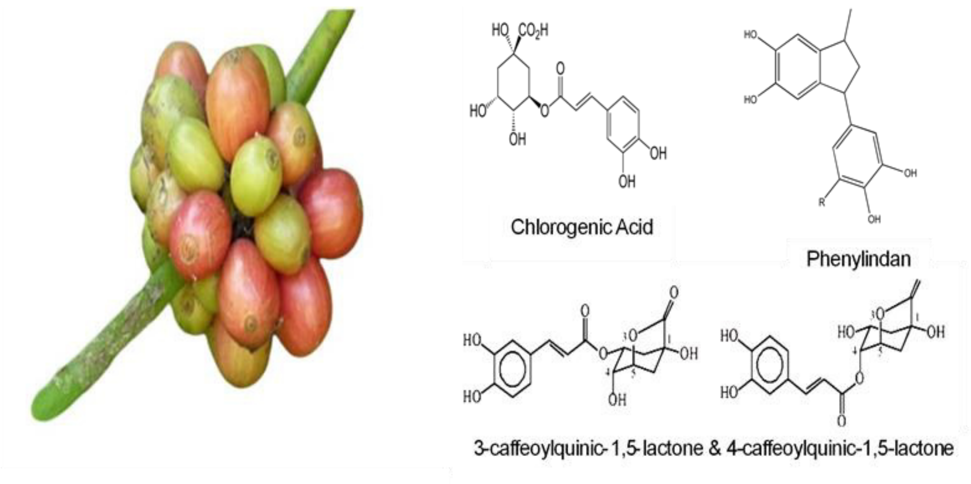


Figure 2-3 Coffee arabica seeds and their important compounds
https://www.compoundchem.com/2014/01/30/why-is-coffee-bitter-the-chemistry-of-coffee/

The biochemical composition of *Coffee arabica* can vary depending on the variety of coffee bean, the growing conditions, and the processing method. One of the most important biochemical compounds in *Coffee arabica* is Caffeine. Caffeine is a stimulant that can improve alertness and energy levels. Caffeine is also known to have a number of other health benefits, such as reducing the risk of type 2 diabetes and Parkinson disease.

Some of the other beneficial biochemical compounds found in *Coffee arabica* include: Chlorogenic acids: Chlorogenic acids are a type of antioxidant that has been shown to have a number of health benefits, such as reducing blood pressure and improving blood sugar control. Trigonelline: Trigonelline is a compound that gives coffee its characteristic aroma. Trigonelline has also been shown to have a number of health benefits, such as improving cognitive function and reducing the risk of type 2 diabetes. Melanoidins: Melanoidins are compounds that are formed during the roasting process. Melanoidins have been shown to have a number of health benefits, such as reducing inflammation and improving gut health.

Here are some recent research findings on the biochemical composition of *Coffee arabica*: A study published in the journal Trends in Food Science & Technology found that *Coffee arabica* beans contain a variety of phenolic compounds, including flavonoids, hydroxycinnamates, and lignans (Bondam et al., 2022). A study published in the journal Molecules found that *Coffee arabica* beans contain a variety of other bioactive compounds, including caffeine, trigonelline, and quinic acid (Saud & Salamatullah, 2021). A study published in the journal Food Science & Nutrition found that the roasting process of *Coffee arabica* beans leads to the formation of new compounds, such as melanoidins and pyrazines. These compounds contribute to the flavor and aroma of coffee (Wu et al., 2022).

2.2.2.3 Mosquito larvicidal and repellent activity

Coffee plants have been shown to have mosquito larvicidal and repellent activity. This is due to the presence of various compounds in coffee plants, such as Caffeine, Quinic acid, and Chlorogenic acids. Coffee grounds, which are typically considered waste in coffee production, have demonstrated their effectiveness as a bio-larvicide against *Aedes aegypti* larvae. Several studies have investigated the larvicidal properties of coffee grounds against *Aedes aegypti* larvae, yielding promising results. Table 2.3 summarizes the key information from each study.

Table 2-3 Lists mosquito larvicide activity and Coffee plant

Study	Title	Results	LC_{50}
(Thanasoponkul et al., 2023)	Spent Coffee Grounds and Novaluron Are Toxic to *Aedes aegypti* (Diptera: Culicidae) Larvae	Complete mortality was observed after 48 h of exposure to 50 g/L	50 g/L
(S. E. Sharawi, 2023)	Larvicidal effect of some traditional Saudi Arabian herbs against *Aedes aegypti* larvae, a vector of dengue fever	Positive results with inhibiting the larvae survival	4.34 g/L
(Tangtrakulwanich et al., 2022)	The Comparative Study of Arabica Used Coffee Grounds and Temephos in Controlling the *Aedes aegypti* Larvae	the light roasted used coffee grounds at the concentration of 125 mg/mL was the best in inhibiting the larvae survival.	Not reported
(Satho et al., 2015)	Coffee and its waste repel gravid Aedes Albopictus females and inhibit the development of their embryos	Coffee grounds repelled gravid female mosquitoes and inhibited larval development	Not reported
(Monteiro Guirado et al., 2016)	Attractiveness of bioinsecticides caffeine and used coffee grounds in the choice of oviposition site by *Aedes aegypti* (Diptera: Culicidae)	caffeine and coffee grounds blocks development and causes death of *Aedes aegypti* in the larval stage.	Not reported
(Aditama & Zulfikar, 2019)	Efficacy of coffee grounds as a bio-larvicide against *Aedes aegypti* (L.)	Coffee grounds were effective in killing *Aedes aegypti* larvae and inhibiting their development	33.66 g/L
(Nakano, 2019)	Larvicidal and Ovicidal Activity of Roasted and Unroasted Coffee Grounds Against *Aedes aegypti* (Diptera: Culicidae)	The fumigant formulation was found to effectively control 100% of the adult mosquitoes	Not reported

- Thanasoponkul et al., 2023: Exposure to 50 g/L of spent coffee grounds resulted in complete mortality of *Aedes aegypti* larvae after 48 hours. LC_{50} = 50 g/L.
- Sharawi, 2023: Traditional Saudi Arabian herbs exhibited larvicidal activity against *Aedes aegypti* larvae, with an effective concentration of LC_{50} = 4.34 g/L.
- Tangtrakulwanich et al., 2022: Light roasted used coffee grounds at a concentration of 125 mg/mL showed the best inhibition of larvae survival. LC_{50} = Not reported.
- Satho et al., 2015: Coffee grounds repelled gravid Aedes Albopictus females and inhibited larval development. LC_{50} = Not reported.
- Monteiro Guirado et al., 2016: Caffeine and coffee grounds blocked larval development and caused death in Aedes aegypti. LC_{50} = Not reported.
- Aditama & Zulfikar, 2019: Coffee grounds effectively killed *Aedes aegypti* larvae and inhibited their development. LC_{50} = 33.66 g/L.
- Nakano, 2019: The fumigant formulation derived from coffee grounds effectively controlled 100% of adult mosquitoes. LC_{50} = Not reported.

2.2.3 Nanotechnology

The method of substance at the atomic and molecular levels is known as nanotechnology. Many industries, including healthcare, energy, and manufacturing, stand to benefit from it (Joachim et al., 2023). Nanomaterials have unique properties compared to their bulk counterparts, due to the high percentage of atoms at the surface. These unique properties can be exploited to develop new drugs and therapies, diagnostic tools and devices, energy sources and storage technologies, and materials and manufacturing processes (Tovar-Lopez, 2023). Here are some examples of nanotechnology in use today:

- In healthcare, nanoparticles are being used to deliver drugs directly to cancer cells, to develop new diagnostic tools, and to create artificial organs and tissues (Raj et al., 2021).
- In the energy sector, nanotechnology is being used to develop new solar cells, batteries, and fuel cells (Yang, 2022).
- In manufacturing, nanotechnology is being used to create new materials with unique properties, such as strength, lightness, and conductivity (Ayub, 2023).

Here are some potential future applications of nanotechnology:

- Nanobots could be used to perform surgery inside the body, deliver drugs to specific cells, or even repair damaged (Singh & Deshmukh, 2023).
- Molecular computers could be millions of times faster than current computers and could be used to solve complex problems that are currently intractable (Khan, 2023).

- Self-healing materials could repair themselves when damaged, increasing the lifespan of products and reducing waste (Mashkoor et al., 2022).

Nanotechnology is a rapidly developing field with the potential to have a major impact on our world. It is important to stay informed about the latest advances in nanotechnology and to consider the potential benefits and risks of this technology.

2.2.3.1 Classification of Nanomaterials

Nanomaterials have gained significant attention in various scientific disciplines due to their unique properties and potential applications. To better understand and utilize these materials, researchers have developed classification systems based on their origin, dimensions, and structural configuration. This literature review aims to provide an overview of existing research on the classification of nanomaterials and discuss the significance of categorizing them according to these criteria.

Origin-Based Classification: This type of classification divides nanomaterials into groups according to where they come from or how they were synthesized. One type of nanomaterial is natural, such as those found in biological systems. Nanoparticles made from bacteria, plants, or animals are a few examples. Conversely, engineered nanomaterials are created using a variety of techniques, including physical vapor deposition, sol-gel synthesis, and chemical vapor deposition. Knowing where nanoparticles come from enables scientists to take advantage of their special qualities and modify them for certain uses (Lahir, 2020).

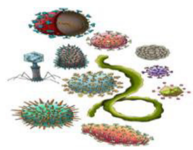
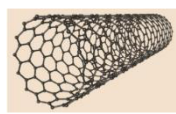
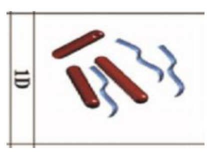
Dimension-Based Categorization: The size range and morphology of nanomaterials are the main topics of this dimension-based classification. The nanoscale dimensions of nanomaterials are commonly defined as those that fall between 1 and 100 nanometers. One-dimensional (1D) nanowires or nanotubes, two-dimensional (2D) nanosheets, three-dimensional (3D) nanostructures, and zero-dimensional (0D) nanoparticles are all included in this categorization. Because of the enhanced surface-to-volume ratio and quantum confinement effects, each dimension has unique characteristics and behaviors. Understanding and forecasting the characteristics and uses of nanoparticles requires an understanding of this taxonomy (Jana et al., 2020).

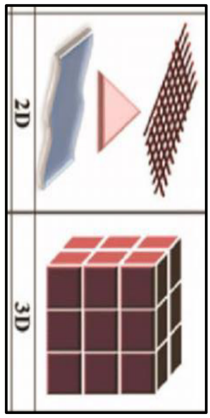
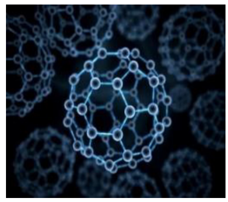
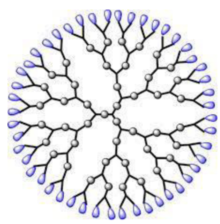
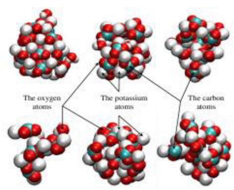
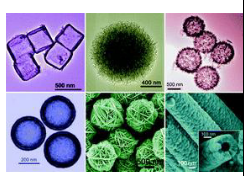
Structural Configuration-Based Classification: Nanomaterials are categorized using this method according to their composition and organization. Crystalline nanomaterials possess distinct optical, electrical, and mechanical capabilities due to their well-defined periodic arrangement of atoms. Conversely, amorphous nanomaterials have distinct properties and lack long-range order. To provide better functionality, core-shell structures are composed of a core material encircled by a shell made of a different material. Composite nanomaterials combine the features of two or more separate materials to accomplish particular goals. Comprehending the structural arrangement is essential to customizing nanomaterials for intended uses (Halim et al., 2022; Kumar et al., 2022).

2.2.3.2 Significance and Applications:

The classification of nanomaterials is crucial in various sectors for their effective application. It helps in the design of targeted drug delivery systems in medicine by engineering nanoparticles to transport medications to specific locations in the body. In the field of electronics, the categorization aids in the construction of nanoscale devices with accurate measurements and optimal electrical characteristics. Additionally, the classification of nanomaterials plays a significant role in energy generation and storage, contributing to the development of highly effective solar cells, supercapacitors, and batteries. Furthermore, it advances materials science and industrial processes by facilitating the development of new materials and improved production methods. Overall, the categorization of nanomaterials is vital for their successful utilization in various fields (Anand, 2023; Nyffeler, 2016; Rodríguez et al., 2021). Class of Nanomaterials: As indicated in Table 2.4, nanomaterials are divided into groups according to their origin, size, and structural makeup.

Table 2-4 The classification of nanomaterials

Classification	Type	The form	Examples	Photo
Based on origin	Natural Nanomaterials	Nanomaterials which are present naturally in nature	Virus, Clay, Natural Colloids, Fullerenes, Graphene	
Based on origin	Artificial Nanomaterials	Engineered nanomaterials prepared by well-defined mechanical	Carbon Nanotubes, Quantum Dots	
Based on dimension	0-D	Nanomaterials have dimensions in all the 3 directions	Metallic Nanomaterials (Silver, Gold, Copper)	
Based on dimension	1-D	structures one dimension of nanomaterials is outside the nanometre range.	Nanowires, Nanotubes, Nanorods	

Based on dimension	2-D	These nanostructures have two dimensions outside the nanometer range.	Nanofilms, Nanowalls, Nanosheets	
Based on dimension	3-D	All dimensions of 3D nanostructures are outside the nanometer range.	Bulk Materials Composed of Individual Blocks Within the Nanometer Scale	
Based on structural configuration	Carbon Based Nanomaterials	These nanomaterials are ellipsoids, tubes hollow sphere, fullerenes, nanotubes.	Ellipsoids, Tubes Hollow Sphere, Fullerenes, Nanotubes	
Based on structural configuration	Dendrimers	These nanostructures are highly branched. They are highly symmetric, monodispersed and spherical compounds.	Highly Branched, Highly Symmetric, Monodispersed and Spherical Compounds	
Based on structural configuration	Composites	They are multiphases solid nanostructures in which at least one of the phases	Multiphases Solid Nanostructures	
Based on structural configuration	Metal Based Material	These nanomaterials are made of metals which include nanogold, metal oxides, nano silver,	Nanogold, Metal Oxides, Nanosilver, Quantum Dots	

2.2.3.3 The elements metal and metal oxide

Among all NPs, metal NPs have the most promise for use in a wide range of fields, including medicine, diagnosis, environmental cleaning, catalysis, antibacterial agents, electrons, cosmetics, biotechnology, packaging, and coating.

Metal NPs:

Copper particles smaller than 100 nanometres are known as copper nanoparticles, or CuNPs. They are beneficial in a range of applications due to their special qualities. CuNPs are added to lubricants, polymers/plastics, metallic coatings, and inks in order to enhance the performance and coulombic efficiency of lithium-ion batteries. They are also included in skin care products, among other things. Additionally, it is applied as a bioactive coating to stop the growth of microorganisms and as an antibacterial, also CuNPs' copper nanoparticles possible utility in medicine, including drug delivery and cancer treatment, is also insecticide (El-Sawy et al., 2022; Masuda et al., 2020; Sharma et al., 2020; Vardhana et al., 2022).

AgNPs, silver nanoparticles, are gaining attention in biomedicine due to their antibacterial, therapeutic, and therapeutic properties. They have applications in catalysis, textile engineering, water purification, and medicine. They are used in dental resin composites, ion exchange fibers, and medical equipment surface coatings to prevent microbial colonization and biofilm formation (Shanmuganathan et al., 2019; Wei et al., 2021). Gold NPs are valuable with its bio-application in the field of labelling, delivery, heating, sensing etc (Nooranian et al., 2022).

Metal oxide NPs:

Metal oxide nanoparticles (NPs) are gaining importance as a novel class of materials in the pharmaceutical industry and other health-related applications. One significant property of these NPs is their biocompatibility, which allows for the immobilization of enzymes and enables selective sensing of biomolecules. Numerous metal oxide NPs, such as CuO, NiO, ZnO, MnO_2, Fe_2O_3, TiO_2, and CeO_2, have been extensively studied for their electrochemical detection capabilities of biomolecules (Chavali & Nikolova, 2019; Nikolova & Chavali, 2020).

2.2.3.4 Synthesis of NPs

Nanoparticles (NPs) are produced through different methods, broadly categorized as bottom-up and top-down approaches (figure 2.4). In the bottom-up method, nanomaterials are constructed by assembling basic building blocks like atoms, molecules, or nanoclusters using biological or chemical techniques. Commonly employed bottom-up methods for NP production include sol-gel, spinning, pyrolysis, chemical vapor deposition (CVD), and biosynthesis.

Top-down method/ destructive method

Nanometric-scale particles can be obtained by reducing bulk materials through physical and chemical methods. This process involves techniques such as nanolithography, thermal decomposition, laser ablation, mechanical milling, sputtering, and pyrolysis, which are widely utilized. However, these methods can result in surface defects, internal stress, and contamination of the nanoparticles (NPs). Additionally, they tend to be costly due to the requirement of high temperature and pressure (Sonanwane et al., 2022).

In contrast to the top-down approach, constructive methods offer advantages in producing high volumes of nanoparticles (NPs) with a more uniform chemical composition and fewer surface imperfections, all at a lower cost (Yun et al., 2020). However, it is important to note that the chemical synthesis of NPs carries potential hazards such as carcinogenicity, genotoxicity, cytotoxicity, and general toxicity. As a result, there is a current emphasis on developing "green" NPs using principles of green chemistry, which prioritize eco-friendliness, energy efficiency, and convenience (Bateni et al., 2022; Pal Singh et al., 2020).

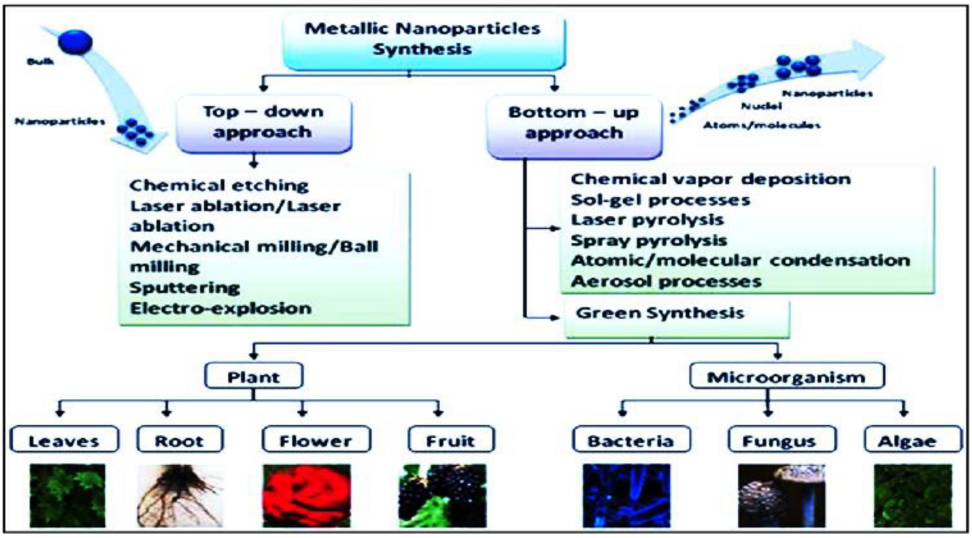


Figure 2-4 diagram of Various approaches for the synthesis NPs. (This figure has been adapted from ref (Ghotekar et al., 2021)

The 12 principles of green chemistry. These principles are guidelines for designing and carrying out chemical processes in a way that minimizes environmental impact (Bodach et al., 2023):1. Waste prevention over clean-up. 2. Maximize the use of all atoms in starting materials. 3. Use and create less hazardous substances. 4. Design safer chemicals. 5. Choose safer solvents and auxiliaries. 6. Design energy-efficient processes. 7. Utilize renewable feedstocks. 8. Minimize unnecessary derivatives. 9. Utilize catalysis for safer reactions. 10. Design products that degrade harmlessly. 11. Employ real-time analysis for pollution prevention. 12. Create inherently safer chemistry processes.

2.2.3.5 Green Synthesis of NPs

Many different biological entities, including plants, microbes, and proteins, can be used to produce nanoparticles (NPs).

a. Nanoparticle production mediated by plants

Because plants contain a wide variety of phytochemicals and bioactive components, plant extracts, also known as phytochemicals, are still of interest for the manufacture of nanoparticles (NPs). It is a quick and affordable way to get very stable NPs with an efficient processing stage. The benefits of synthesizing nanoparticles through plant mediation include numerous advantages such as readily accessible plant extracts, biocompatibility, minimal or no contamination, the ability for large-scale production, cost-effectiveness, the utilization of phytochemicals as both reducing and stabilizing/capping agents, and the use of aqueous solvents. The approach of synthesizing nanoparticles through plant mediation relies on the plant's capacity to absorb, utilize, store, and recycle various minerals. Recently, there has been considerable interest in the production of nanoparticles by plants due to their environmentally friendly approach. The plant-mediated methods for nanoparticle production involve intracellular, extracellular, and phytochemical-mediated synthesis processes, which are discussed in detail below.

b. Phytochemical mediated synthesis of NPs

A diverse group of molecules, including alkaloids, polyphenols, terpenoids, antioxidants, glutathione, quinone, amino acids, organic acids, alcoholic compounds, sugars, proteins, and polysaccharides, have been occupied in the synthesis of nanoparticles composed of secondary metabolites (Table 2.5). Flavonoids, the maestros of this molecular group, frequently conduct the synthesis of NPs. Compounds such as carboxyl, sesquiterpenes, amide groups, alkynes, and hydroxyl groups of monoterpenoids correspond with plant extracts from species like *Acacia mearnsii* bark, leading to the formation of nanoparticles (Shaikh et al., 2021).

Table 2-5 Lists plants -mediated synthesis of NPs

Botanical Names	NPs	NP size (nm)	Application	Refs
Anacardium occidentale	gold	10–60	Antimicrobial Anticancer	(Sunderam et al., 2019)
Dracocephalum kotschyi	gold	5–21	Anticancer	(Chahardoli et al., 2019)
Euphorbia fischeriana	gold		Antioxidant	(Zhang et al., 2020)
Camellia sinensis	silver	4-50	Antibacterial	(Göl et al., 2020)
Ferula	silver	20-80	Antibacterial	(Ahmed

gumosa, Ferula latisecta				Uttu et al., 2022)
Musa acuminata colla L. flower	silver	Nanoclusters	Antibacterial Antifungal	(Valsalam et al., 2019)
Trianthema portulacastrum Extract	Zinc oxide	25-90	Cytotoxic Antibacterial Antifungal Antioxidant	(Khan et al., 2019)
Punica granatum Extract	Zinc oxide	32.98–81.84	Cytotoxic Antibacterial	(Mohamad Sukri et al., 2019)
Tecoma castanifolia Extract	Zinc oxide	70-75	Antiseptic Antioxidant Antitumor	(Sharmila et al., 2019)
Ocimum tenuiflorum Extract	Copper oxide	20-30	Antibacterial	(Altikatoglu et al., 2017)
Moringa oleifera Extract	Copper oxide	35-95	Antifungal	(Pagar et al., 2020)
Eichhornia crassipes Leaves	Copper oxide	28	Antifungal	(VANATHI et al., 2016)
Gloriosa superba Leaves	Copper oxide	5-10	Antibacterial	(Naika et al., 2015)
Artocarpus heterophyllus Extract	Titanium dioxide	15-20	Cytotoxic antibacterial anticancer	(Ullah et al., 2019, p. 2)
Citrus sinensis fruit	Titanium dioxide	20-50	Antibacterial, Cytotoxic, Anticancer	(Rueda et al., 2020)

c. Microbiology mediated synthesis of NPs.

Numerous microorganisms, including prokaryotes and eukaryotes, including algae, fungus, bacteria, and actinomycetes, have been used to synthesize NPs both intracellularly and extracellularly, as summarized in Tables 2.6, 2.7, and 2.8.

Table 2-6 Various algae used in the synthesis NPs and their applications

Algae	NPs	size (nm)	Application	Refs.
Ulva armoricana	Ag	12.5	Antibacterial	(Massironi et al., 2019)
Chlorella vulgaris	Ag	40-90	Catalyst for the synthesis of quinolines	(Mahajan et al., 2019)
Neodesmus	Au and	5-34	Antioxidant,	(Omomowo et al.,

pupukensis	Ag		antimicrobial	2020)
Gelidiella acerosa	Au	5 -117	antibacterial, antioxidant	(Senthilkumar et al., 2019)
Tetraselmis indica	ZnO	20-40	Fabric, cosmetic, biomedical, food wrapping	(Thirumoorthy et al., 2021)
Padina boryana	Pd	11	Antibacterial and anticancer	(Sonbol et al., 2021)
Macrocystis pyrifera	CuO	2-50	N.M	(Araya-Castro et al., 2021)
Botryococcus braunii	Cu and Ag	10-70 (Cu), and 40-100 (Ag)	Antimicrobial	(Arya et al., 2018)

Table 2-7 Various fungi used in the synthesis NPs and their applications

Fungi	**NPs**	**size (nm)**	**Applications**	**Refs.**
Phanerochaete chrysosporium	ZnO	50	Antimicrobial	(Sharma et al., 2021)
Trichoderma harzianum	Ag	21	Antioxidant, antibacterial	(Konappa et al., 2021)
Rhizopus Oryzae	MgO	20	Germicide, insecticidal, and tanning treatment	(Hassan et al., 2021)
Morchella esculenta	Au	16	Biomedical	(Acay, 2021)
Aspergillus sydowii	Ag	1-24	Fungicidal and antiproliferative action to HeLa cells	(Wang et al., 2021)
Aspergillus flavus	Cu	2-60	Biomedical	(Saitawadekar & KAKDE, 2020)
Ganoderma lucidum	Ag	15-22	Antimicrobial, antiseptic, antimycotic	(Aygün et al., 2020)
Periconium sp	ZnO	16-78	Germicide and antioxidant	(Ganesan et al., 2020)
Fusarium solani	Au	40-45	Antitumor	(Clarance et al., 2020)
Trichoderma Harzianum	Ag, CuO	5-18	biotechnological method	(Consolo et al., 2020)

Table 2-8 Various bacteria used in the synthesis NPs and their applications

Bacteria	**NPs**	**size (nm)**	**Application**	**Refs**

Actinobacter spp	Au	5–500	Antimicrobial, fungicidal, nano compost	(Bharde et al., 2007)
Haloferax volcanii	Au	*10*	*Antiseptic activity,* *Nano biosensors*	(Costa et al., 2020)
Deinococcus radiodurans	Au	*43*	*Antibacterial action*	(Li et al., 2016)
Acintobacter species	Au	*15*	*Antioxidant action*	(Nadhe et al., 2020)
Escherichia coli	Ag	100	plant growth, antibacterial action, and antifungal action	(Kannan et al., 2010)
Bacillus licheniformis	Ag	18–63	Fungicidal effect	(Shanthi et al., 2016)
Pseudomonas deceptionensis	Ag		antibacterial action and bio film suppression	(Jo et al., 2016, p. 5)
Pseudomonas fluorescens	Ag	5-50	Antibacterial activity pesticide	(Syed et al., 2016, p. 417)
Sporosarcina koreensis	Ag	varied	Antibacterial action	(Singh et al., 2016)
Acinetobacter sp.	Ag	10	Fungicidal - biofilm inhibition	(Nadhe et al., 2019)
Pseudomonas rhodesiae	Ag	20-100	Antibacterial action	(Hossain et al., 2019)
Streptomyces capillispiralis *Streptomyces zaomyceticus*	Ag	23-63 11-36	Antibacterial action Antifungal action Larvicidal	(Fouda et al., 2020)
Haloalkaliphilic Streptomyces	Ag	16	Antibacterial action Antifungal action	(Marathe et al., 2021)
Streptomyces griseus	Cu	5-50	fungicides	(Ponmurugan et al., 2016)
Lactobacillus casei	Cu	30–75	Plant fertilizer	(Kouhkan et al., 2020)

2.2.3.6 Mechanism synthesis of NPs by plants:

a. Intracellular synthesis

Plants, with their characteristic abilities for phytoextraction and phytoremediation, have emerged as promising tools for the synthesis of metal nanoparticles (NPs). This process, driven by the phenomenon of bioaccumulation, involves the uptake of metal ions by plants at a rate faster than their removal through metabolic processes. This accumulation of excess metal ions within plant tissues can induce the production of reactive oxygen species (ROS), potentially harming plant cells. To combat this metal-induced toxicity, plants have advanced, elegant detoxification mechanisms. Phytochelatins, metallothionein, cysteine-rich proteins, and oligopeptides play crucial roles in removing metal ions, making them less toxic. Additionally, plants activate enzymatic antioxidant systems and synthesize phenolic compounds and flavonoids to maintain ROS homeostasis (Garg et al., 2020).

b. Extracellular synthesis:

The use of plant extracts for nanoparticle (NP) synthesis has advantages over other methods. It avoids the need for toxic chemicals and the strict aseptic conditions required for microorganism-mediated synthesis. Plant extracts serve as natural capping agents, stabilizing the nanoparticles. The process is simple, efficient, cost-effective, and rapid. Different plant parts, including roots, stems, leaves, flowers, pods, latex, and bark, have been used for NP synthesis. Metallic salts dissociate into anions and cations, with cations forming hydroxyl complexes. Crystallite growth of metals with oxygen species occurs when hydroxyl complexes become supersaturated. Capping agents from plant extracts stop the growth of high-energy atomic planes, and heat provides the necessary energy. Reducing agents donate electrons to convert metal ions into nanoparticles. Stabilizing and reducing agents prevent nanoparticle aggregation and promote smaller nanoparticle production. Polyphenols, amino acids, polysaccharides, organic acids, and proteins in plant extracts suppress the superoxide-driven Fenton reaction, facilitating the formation of metallic nanoparticles (Yuan et al., 2021).

2.2.3.7 Characterization of NPs

Nanoparticles (NPs) have emerged as a revolutionary class of materials with applications ranging from medicine to electronics. However, their unique properties, such as their small size and high surface area, can also pose challenges in terms of characterization. Characterization of NPs typically involves assessing their size, shape, surface area, polydispersity, and stability. Various diagnostic methods have been used to study the produced NPs based on different features such as special functional classes, light scattering and absorption potentials, and so on (Table 2.9). In most cases, UV-visible spectroscopy is utilized to confirm the stability and synthesis of NPs (Teixeira et al., 2022).

Table 2-9 Various methods used in the characteristic NPs

Techniques	Instrument	Size	Agglomerati	Shap	Chemical

	s	Distribution	on State	e	Composition
Spectroscopy Techniques (Vázquez-López et al., 2022)	Visual ultraviolet spectroscopy	✓			✓
	X-ray Diffraction	✓			✓
	FTIR analysis				✓
Microscopy Techniques (Deepak et al., 2018)	(Scanning Electron Microscopy) SEM	✓	✓	✓	
	(Transmission Electron Microscopy) TEM	✓	✓	✓	

The features of NPs, including composition and concentration, surface characteristics, surface functional groups, and atomic organization, are measured using Fourier transform infrared (FTIR) spectroscopy (Jeyaraj et al., 2019). The location, size, and morphology of NPs can be seen by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) (Ingale & Chaudhari, 2013). The crystalline structure is determined via X-ray diffraction (XRD) (Strasser et al., 2010).

2.2.3.8 *Coffee arabica* mediated synthesis of NPs

Coffee extract has been used until today to create nanoparticles at room temperature without the need for a surfactant, capping agent, or template. Saha et al. explored a facile and green synthetic protocol for synthesizing bismuth nanoparticles (BiNPs) in an aqueous solution employing green coffee bean extract. The obtained BiNPs have a spherical shape and a diameter range of 20 to 40 nm. (Saha et al., 2023). Nadagouda et al. reported the green approach to obtain AgNPs in the size range of 20–60 nm and crystallized in face-centered cubic symmetry (Nadagouda et al., 2014).

The AgNPs using roasted dry *Coffea arabica* seed extract based on a green method were observed to have strong antimicrobial activity (Al-hashimi, 2021). Chien et al. showed that the coffee extract could be employed as a green reductant for synthesizing AgNPs on the surface of carbon microspheres (Chien et al., 2019). Abdelmigid et al. successfully synthesized zinc oxide nanoparticles (ZnO NPs) using coffee ground extracts. The NPs showed antibacterial properties and lower cytotoxicity compared to chemically synthesized NPs (Abdelmigid et al., 2022).

In a study, *Coffee arabica* leaf extracts (CAE) were utilized for the eco-friendly synthesis of silver nanoparticles (AgNPs) as biosensors. The CAE-AgNPs showed excellent sensing performance for the amino acid (Haridas et al., 2022). Also, researchers explored the use of spent coffee grounds (SCGs) as a green and sustainable method for synthesizing gold and silver nanoparticles (NPs). The SCG extracts were effectively utilized as reducing agents in the synthesis of gold and silver NPs (Yust et al., 2022). A study successfully developed a green deposition method for zinc oxide nanoparticles (ZnO NPs) using coffee leaf extraction, X-ray diffraction, and SEM analysis, which confirmed the highly crystalline nature of the deposited nanoparticles with a cubic shape structure (Abel et al., 2021).

A study focuses on the green synthesis of copper oxide nanoparticles (CuO NPs) using coffee extracts as reducing agents. The synthesized CuO NPs are characterized using various techniques and found to be effective catalysts for the photocatalytic degradation of methylene blue dye (Mandrekar & D'Souza, 2023). A paper presents a fast and environmentally friendly method for synthesizing iron oxide nanoparticles (Fe_2O_3-NPs) using coffee seed aqueous extract as a bio-reducing agent. The synthesized Fe_2O_3-NPs were characterized using X-ray diffraction (XRD) and ultraviolet-visible (UV-Vis) spectrophotometry, revealing a highly crystalline cubic structure with particle sizes ranging from 23.2 nm to 37.5 nm (Teoh et al., 2021).

A study focuses on the green synthesis of gold (Au), silver (Ag), and selenium (Se) nanoparticles (NPs) using coffee bean extract under hydrothermal conditions. The synthesized Au, Se, and Ag NPs showed low antioxidant activity. Morphological and antibacterial assessments demonstrated that the synthesized NPs had a spherical shape and exhibited high bactericidal activity against *E. coli* and *S. aureus* (Abbasian & Jafarizadeh, 2020).

Alumina nanoparticles were synthesized using coffee extracts via a rapid and efficient microwave-assisted method. The nanoparticles were spherical and had an average size of 50–200 nm (Sutradhar et al., 2013). Coffee waste residues were successfully used to synthesize iron nanoparticles (FeNPs) in multiple extraction rounds. The FeNPs were found to be effective in degrading chlorinated volatile organic compounds (Rónavári et al., 2023). From coffee, a novel cafestol-chitosan-ZnO NP system with antibacterial properties was synthesized. The synthesized NPs effectively inhibited the bacterial growth of both Gram-positive and Gram-negative strains (Ballıca et al., 2020).

Green synthesized metallic iron nanoparticles (n-Fe) using coffee bean extract as a natural oxidant were used for the removal of Orange II (Lazaar et al., 2022). The synthesis of catalyst materials on coffee ground-derived carbon was successful and produced materials with desirable physical and electrochemical properties (Simson et al., 2023).

2.2.3.9 Copper Nanoparticles

Copper nanoparticles (CuNPs) have gained significant attention due to their unique properties and versatile applications in fields such as catalysis, electronics, energy storage, sensing, and biomedical applications (Bhagat et al., 2021). The synthesis of CuNPs can be achieved through various methods, including chemical reduction, electrochemical deposition, and green synthesis (Tyagi et al., 2023).

CuNPs exhibit excellent physical, chemical, and optical properties, such as high electrical conductivity, catalytic activity, antimicrobial properties, and adjustable optical characteristics (Mali et al., 2020). These properties make them suitable for a range of applications, including catalysis, electronics, energy storage, sensing, and biomedical applications. However, challenges such as stability, toxicity, and scalability need to be addressed for their successful implementation. Additional research and development are required to optimize synthesis methods, understand their potential impacts, and explore novel applications. With continued advancements, CuNPs hold great potential to revolutionize various industries and contribute to technological advancements in the future.

Green synthesis copper nanoparticles

A general representation of the process of producing nanoparticles can be found in Figure 2.5. Copper precursor salts made of metallic copper are combined with extracts of plants during this process. There are a number of compounds that may be discovered in the plant extracts that are accountable for the reduction of copper ions to neutral atoms (OH function group), which finally leads to the formation of copper nanoparticles.

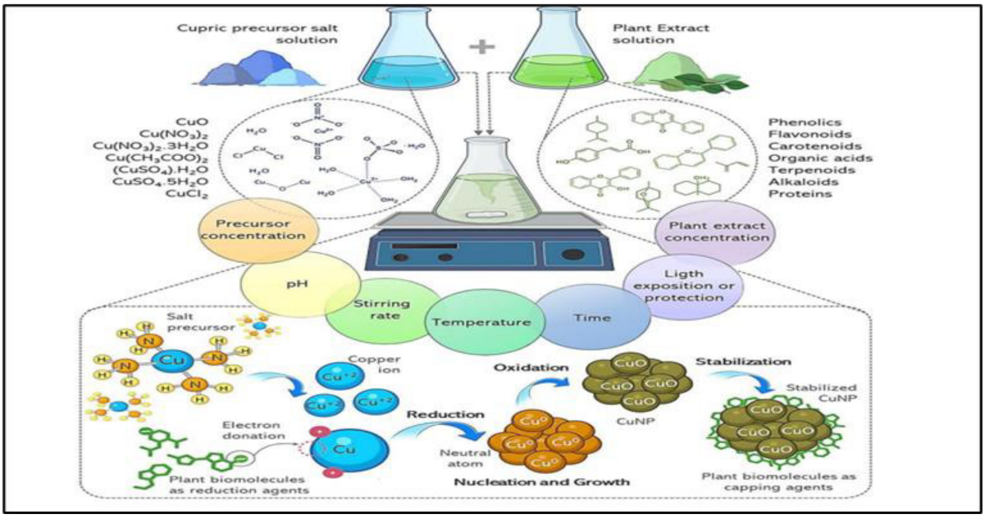


Figure 2-5 Green synthesis copper nanoparticles: steps and mechanisms (Antonio-Pérez et al., 2023).

The stabilization of the nanoparticles is accomplished by the interactions that take place between the nanoparticles and other plant components (Antonio-Pérez et al., 2023). Furthermore, several studies have specifically examined the synthesis of CuNPs using various plant extracts. Table 2.10 presents an example of plants that are utilized for the production of copper nanoparticles.

Table 2-10 CuNPs synthesized using some plant extracts

Plant	Precursor	NPs Characteristics	Applications	Ref.
Azadirachta indica Hibiscus rosa-sinensis, Murraya koenigii, Moringaoleifera, and Tamarindus indica	CuO	Particles are spherical and range in size from 9.8 to 10.77 nm.	antioxidant activity and cytotoxicity	(Rehana et al., 2017)
Kigelia africana	Cu $(CH_3COO)_2$	The study does not report the morphology or size of NPs, it focuses only on antimicrobial activity.	Antimicrobial activity	(Alao et al., 2022)
Centella asiatica	$CuCl_2 \cdot 2H_2O$ MnO_2	Cu/MnO2 nanocomposites, size range 10-30 nm.	High catalytic activity for the reduction of inorganic and organic dyes	(Nasrollahzadeh et al., 2018)
Camelia sinensis	$CuCl_2$.	Agglomerated form with an average size of 60 ± 6 nm.	Dye degradation against Antibacterial activity	(Ahmed et al., 2019)
Ageratum houstonianum	CuCl	Size around 80 nm, agglomerate, and not specific shape. NPs behave as a semiconductor.	Antiviral action	(Chandraker et al., 2020)
Ehretia acuminata	CuCl $2H_2O$	NPs of 500 nm. The. shape was not reported. Green NPs and	Antiviral	(Tahir et al., 2022)

		phytochemicals were coated on a cotton textile surface.		
Aloe vera	Cu $(NO_3)2$-$3H_2O$	Monoclinic phase, average particle size of 20 nm	Bactericidal against fish pathogens	(Kumar et al., 2015)
Galeopsis herba	Cu $(NO_3)2$-$3H_2O$	Size of 5-10 nm with spherical shape	High antioxidant activity and catalytic activity	(Dobrucka, 2018)
Hagenia abyssinica	Cu $(NO3)_2$.3H2O	Spherical, hexagonal, triangular, cylindrical, and prismatic shapes. Size range of 10-50 nm	Antibacterial	(Murthy et al., 2020)
Cinnamomum zelanicum	Cu $(NO_3)2$-$3H_2O$	Spherical morphology with size of 19.55 to 69.70 nm	Cardioprotective against isoproterenol-	(Liu et al., 2021)
Berberis vulgaris	Cu $(NO_3)2$-$3H_2O$		Positive effect on various physiological and biochemical characteristics of Solanum lycopersicum seedlings	(Tu et al., 2023)
Carum carvi	Cu $(NO_3)2$-$3H_2O$	Regular and homogenous spherical distribution with 12.4 nm size	Antimicrobial activity	(Oraibi et al., 2023)
Syzygium alternifolium	$CuSO_4$-$5H_2O$	Spherical, 2-69 nm	Antiviral	(Yugandhar et al., 2018)
Falcaria vulgaris	$CuSO_4$-$5H_2O$	Spherical, 20 nm	Nematicidal, antibacterial	(Zangeneh et al., 2019)
Orobanche aegyptiaca	$CuSO_4$-$5H_2O$	Spherical, <50 nm	Antioxidant, antifungal, antibacterial, wound healing	(Akhter et al., 2020)
Gnidia glauca, Plumbago	CuSO4-$5H_2O$	Variable size, 5-93 nm	Antidiabetic	(Jamdade et al., 2019)

zeylanica				
Eucalyptus camaldulensis	$CuSO_4$-$5H_2O$	Spherical, 41-65 nm	Antibacterial	(Asghar & Asghar, 2020)
Prunus nepalensis	$CuSO_4$-$5H_2O$	Spherical, 35-50 nm	Anticancer	(Biresaw & Taneja, 2022)
Nigella sativa	$CuSO_4$-$5H_2O$	Size 98.23 nm	Antiobesity	(Kumar et al., 2023)]
Zingiber officinale	$CuSO_4$-$5H_2O$	Crystalline, 60 nm	Antibacterial	(Abbas & Fairouz, 2022)
Haplophyllum tuberculatum	Cu $(NO_3)2$ $3H_2O$	Amorphous particles, 85 nm	Nematicide	(M. M. Soliman et al., 2022)
Krameria sp.	$(CuSO_4)$ $5H_2O$	Spherical NP's, 6.16 nm	Antioxidant, antimicrobial	(Alshammari et al., 2023)

Coffee arabica mediated synthesis of copper NPs

The synthesis of CuO nanoparticles was achieved by utilizing coffee powder extract and copper sulfate pentahydrate. Analysis using XRD, UV-Vis, TEM, and SEM techniques indicated the presence of spherical CuO nanoparticles with sizes ranging from 15 to 30 nm. The produced CuO nanoparticles demonstrated a suppressive impact on testosterone levels in both male and female serum, indicating their potential function in controlling hormone levels (Jassim & Salman, 2021).

A study focused on the green synthesis of copper oxide nanoparticles using plant extracts as reducing agents. Coffee extracts were used to reduce copper sulfate solution, forming copper oxide nanoparticles. These biosynthesized nanoparticles showed promising photocatalytic activity in degrading methylene blue dye (Mandrekar & D’Souza, 2023).

Coffee bean extracts were used to synthesize nanoparticles (NPs) in a cost-effective manner. While the NPs had reduced antioxidant potency compared to air-oxidized NPs, they still exhibited free-radical scavenging activity and were biocompatible with human dermal fibroblasts. Additionally, the NPs showed antiproliferative effects on MCF7 breast cancer cells, particularly the copper sulfate-oxidized NPs (Sunoqrot et al., 2021).

In a study, cupric oxide nanoparticles were successfully synthesized using coffee powder extracts through the sol-gel method at various calcination temperatures. The synthesized nanoparticles underwent characterization using X-ray diffraction, scanning electron microscopy, ultraviolet-visible spectroscopy, and Fourier transform infrared spectroscopy. Analysis of the powder X-ray diffraction data confirmed the formation of single-phase copper oxide nanoparticles with a monoclinic crystal structure (Taghavi Fardood & Ramazani, 2016).

In another study, copper nanoparticles (CuNPs) were successfully synthesized using a single-step, facile, and green method, employing a solution of green coffee bean extract as both a reducing and stabilizing agent. The synthesized CuNPs demonstrated excellent stability, with average particle sizes ranging from 5 to 8 nm. These methodologies served as efficient and easily recyclable catalysts for the rapid and efficient reduction of methylene blue (Wang et al., 2021).

Mosquitocidal green nanoparticles

The World Health Organization (WHO) has established guidelines for conducting tests on larvicides and pupicides, which involve the process of rearing mosquito larvae and evaluating their susceptibility to these substances. To rear the larvae, eggs are collected and hatched in containers filled with dechlorinated tap water or distilled water, along with larval food. The containers are maintained at a temperature of 25 ± 1 °C. Once the larvae hatch, they are fed at regular intervals and closely monitored for their development. When they reach the desired growth stage, they are ready for testing. The time it takes for the eggs to hatch and the larvae to reach the desired stage can vary depending on the species of mosquito. For example, *Ae. aegypti* larvae generally hatch within approximately 12 hours, while *Culex spp*. larvae may take 1–2 days to hatch. Alternatively, some researchers choose to collect larvae directly from their natural habitats and select the specific growth stage needed for conducting larvicidal tests (Onen et al., 2023).

Table 2.11 provides a summary of various green sources and the larvicidal and pupicidal activities of nanoparticles that have been developed. Analyzing the data presented in the table, it becomes evident that a significant portion of the studies focused on the potential of metal nanoparticles for controlling mosquito vectors, specifically in terms of their larvicidal and pupicidal effects. It is worth noting that half of the studies specifically investigated the use of silver nanoparticles. The mosquito species that were predominantly tested included Ae. aegypti, followed by *Cx. quiquefasciatus* and *An. stephensi*. Interestingly, the metal nanoparticles demonstrated LC_{50} values ranging from 5 to 500 ppm. Additionally, it is important to mention that the tested mosquito species were primarily derived from laboratory strains rather than wild populations.

Table 2-11 Lists mosquitocidal green nanoparticles

Plant	Metal Precursors	Shape and Size	Activity and Species	LC_{50}	Reference
Syzgium cumini	Zn $(CH_3COO)_2$, 1 mM	Spherical, 50-60 nm	Larvicidal and Ovicidal (*Ae. aegypti*)	51.94	(Roopan et al., 2019)
Ficus racemosa	$AgNO_3$, 1 mM	Cylindrical, 250.06 nm	Larvicidal (*Cx. quinquefasciatus* and *Cx. gelidus*)	12.00	(Velayutham et al., 2013)
Piper longum	$AgNO_3$, 1 mM	Spherical, 25-32 nm	Larvicidal (*Ae. aegypti*, *An.*	8.97	(Yadav et al., 2019)

			stephensi and *Cx quinquefasciatus*)		
Holarrhena antidysenterica	$AgNO_3$, 1 mM	Spherical, 32 nm	Larvicidal (*Ae. aegypti* L. and *Cx quinquefasciatus*)	93	(Kumar et al., 2018)
Ammannia baccifera	$AgNO_3$, 1 mM	Triangular and hexagonal, 10-30 nm	Larvicidal (An. subpictus and *Cx. quinquefasciatus)*	29.54	(Suman et al., 2013)
Tridax procumbens	$CuSO_4$, 1 mM	Spherical, 16 nm	Larvicidal (*Ae. aegypti*)	4.21	(Muthamil Selvan et al., 2018)
Grewia asiatica	$CuSO_4.5H_2O$, 1 mM	Spherical, 60-80 nm	Larvicidal (*Ae. aegypti)*	100	(Tahir et al., 2022)
Annona squamosa	$CuSO_4$, 1 mM	Spherical, 5.99-24.48 nm	Larvicidal (*An. stephensi*)	170	(Vivekanandhan et al., 2021)
Mangifera indica	$TIO(OH)_2$, 5 mM	Spherical, 30±5 nm	Larvicidal *An. Subpictus, Cx. quinquefasciatus*	5.84	(Rajakumar et al., 2015)
Momordica charantia leaves	TICl, 5 mM	Irregular	Larvicidal and pupicidal (*An. stephensi*)	3.43	(Rajiv Gandhi et al., 2018)
Morinda citrifolia roots	$TIO(OH)_2$, 5 mM	Spherical, oval, and triangular	Larvicidal (*An. stephensi*, *Ae. Aegypti* and *Cx. quinquefasciatus*)	05.03	(Suman et al., 2015)]
Vitex negundo, leaves	$TiCl_4$, 5 mM	Spherical	Larvicidal (*An. subpictus* and *Cx. quinquefasciatus*)	7.52	(Gandhi et al., 2016)
Clausena dentate, leaves	H_2SeO, 1 mM	Spherical,	Larvicidal (*An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*)	240.71	(Sowndarya et al., 2017)
Ceropegia bulbosa, tuber	H_2SeO, 40 mM	Spherical	Larvicidal (*Ae. albopictus*)	250	(Cittrarasu et al., 2021)

Nigella sativa, seed	H_2SeO_3, 0,01 mM	Clusters,	Larvicidal (*Cx. pipiens*)	17.39	(Farag et al., 2020)
Nilgirianthus ciliates leaves	H_2SeO 30 mM	Spherical	Larvicidal (*Ae. aegypti*)	0.92	(Meenambigai et al., 2022)
Opuncia ficus-indica peel	Na_2SeO_3, 2 mM	Spherical	Larvicidal (*Cx. pipiens*)	75.41	(Hashem et al., 2022)
Ulva lactuta seaweed	Zn $(CH_3COO)_2 . 2H_2O$	Sponge-like	Larvicidal (*Ae. aegypti*)	38	(Ishwarya et al., 2018)
Myristica fragrans fruit	Zn $(NO_3)2.6H_2O$	Semispherical, hexagonal	Larvicidal (*Ae. aegypti*)	5	(Faisal et al., 2021)
Cocos nucifera fruits	Pd $(OAc)_2$ 1 mM	Spherical, 323 nm (TEM)	Larvicidal and ovicidal (*Ae. aegypti*)	288.88	(Elango et al., 2016)
Citrus limon leaves	PdCl, 1 mM	Spherical, 1.5-18.5 nm (TEM)	Larvicidal (*An. stephensi*)	10.83	(Minal & Prakash, 2020)
Nephrolepis exaltata whole plant	$FeCl_3.6H_2O$ 0.01 M	Spherical, 30-70 nm (TEM)	Larvicidal (*An. stephensi*)	25	(Nadeem et al., 2022)
Aegle marmelos, leaves	$NiCl_2$ 1 mM	Triangular, 80-100 nm (SEM)	Larvicidal (*An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*)	534.83	(Angajala et al., 2014)
Cocos nucifera, fruits	Ni $(OAc)_2$ 1 mM	Cubical, 47 nm (TEM)	Larvicidal (*Ae. aegypti*)	259.24	(Elango, Roopan, Dhamodaran, et al., 2016)[
Artemisia vulgaris, leaves	HAuC, 1 mM	Spherical, triangular, and hexagonal	Larvicidal (*Ae. aegypti*)	74.42	(Sundararajan & Ranjitha Kumari, 2017)
Moringa oleifera, leaves	$HAuC_4.3H_2O$ 1 mM	Spherical, oval, triangular, and pentagonal	Larvicidal (*Cx. quinquefasciatus)*	8.24	(Mondal et al., 2022)

Copper NPs larvicidal *Aedes aegypti*

The recent study utilized *Pistia stratiotes* leaf extract to synthesize copper oxide nanoparticles (CuONPs). The synthesized CuONPs were tested for their larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* mosquito vectors. Larvicidal activity testing revealed that a concentration of 15 ppm resulted in 100% mortality of *Culex quinquefasciatus* larvae. The calculated LC_{50} and LC_{90} values for *Culex quinquefasciatus* were 4.23 ppm and 13.67 ppm, respectively. Opposite 93% mortality of *Aedes aegypti* larvae, the LC_{50} and LC_{90} were 4.41 and 15 ppm, respectively (Ansari et al., 2023).

In another study, researchers focused on synthesizing biocompatible copper oxide nanoparticles (CuONPs) using extracts from *Rubia cordifolia* bark. The CuONPs had a spherical shape and an average particle size of 50.72 nm. Notably, they exhibited significant larvicidal activity against various mosquito larvae, including *Aedes aegypti* (65 ± 8.66%), *Anopheles stephensi* (80 ± 13.69%), and *Culex quinquefasciatus* (72 ± 13.04%) (Vinothkanna et al., 2023).

Another study centered on utilizing the *Strobilanthes cordifolia* plant for the eco-friendly production of FeNPs and CuNPs. UV analysis demonstrated absorption peaks at 462 nm and 438 nm for FeNPs and CuNPs, respectively. FTIR and XRD techniques confirmed the existence of functional groups and the crystalline structure of the nanoparticles. Notably, *Aedes aegypti* mosquitoes exposed to CuNPs showed a 65% larval mortality rate; the LC_{50} and LC_{90} values were 6.32 µg/mL and 51.30 µg/mL, respectively (Kirubakaran et al., 2023).

2.2.4 Pathological affects

Regarding larvicide and morphological change, a study focused on the use of copper sulfide nanoparticles (CuSNPs) synthesized through sonochemical irradiation to assess their larvicidal potential against *Aedes aegypti* mosquitoes. The CuSNPs exhibited significant larvicidal effects, with 100% mortality observed within 24 hours at a concentration of 7 ppm. Morphological changes and damage to the larvae's epithelium layer were observed in the treated group (Sandhu et al., 2022).

Concerning larvicide and the histopathological effect of copper nanoparticles, I have not yet found any studies, but I have found a lot of studies of other metals such as silver, selenium, zinc, and nickel. A study focused on the green synthesis of silver nanoparticles using two bacterial isolates, *Pantoea stewartii* and *Priestia aryabhattai*. The synthesized nanoparticles were characterized using various techniques, revealing circular-shaped particles with average sizes of 4 nm and 3.6 nm for *Pantoea stewartii* and *Priestia aryabhattai*, respectively. The silver nanoparticles demonstrated strong larvicidal activity against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* mosquito larvae, causing damage to various internal structures. The histopathological analysis of the mosquito larvae revealed evident damage to various structures, including epithelial cells, the food bolus, the basement membrane, muscles, and midgut segments (Wilson et al., 2023).

A study focused on synthesizing selenium nanoparticles (SeNPs) using *Nilgirianthus ciliatus* leaf extracts and evaluating their properties. The SeNPs were characterized using various analytical techniques. The SeNPs also demonstrated strong insecticidal activity *against Aedes aegypti* mosquito larvae, with a median lethal concentration (LC_{50}) of 0.92 mg/L. Histopathological analysis revealed damage to the midgut and caeca regions of the treated larvae, including the epithelial layer and peritrophic membrane (Meenambigai et al., 2022).

In another study, zinc oxide (ZnO) nanoparticles with a spherical-like structure were synthesized using *Indigofera tinctoria* leaf extracts and tested as a mosquito control agent. The efficacy of the green-synthesized ZnO nanoparticles was evaluated against different larval and pupal stages of *A. aegypti*. The nanoparticles demonstrated significant larvicidal activity, with LC_{50} values of 4.030 ppm in first instar larvae and 7.213 ppm in pupae. Histological studies confirmed destructive changes in the larval body tissues, particularly in the fat cells and midgut (Chithiga & Manimegalai, 2023).

In a recent study, researchers aimed to create nickel metal organic frameworks (Ni-MOFs) with strong larvicidal properties. The Ni-MOFs demonstrated remarkable effectiveness in killing *Aedes aegypti* mosquitoes at the larval stage. Histopathological analysis of the treated larvae showed notable alterations in their structure. When exposed to lower concentrations, the larvae experienced tissue fragmentation and damage to epithelial cells. However, higher doses caused more severe consequences, such as the displacement of midgut epithelial cells and complete organ collapse (Raju et al., 2020).

2.2.5 Biochemical effects

Concerning the larvicide and biochemical outcomes of copper nanoparticles and other metal nanoparticles, A study investigating the larvicidal and biochemical effects of copper nanoparticles utilized extracts from *Annona muricata, Azadirachta indica*, and *Pongamia glabra*. The findings of the study revealed a reduction in the total protein level associated with the mortality of *Aedes aegypti* and *Aedes albopictus* larvae (Parthiban et al., 2020).

a study by (Durairaj et al., 2014a) involving *Aedes aegypti* mosquitoes treated with toxic agents derived from fungal-synthesized titanium dioxide nanoparticles. The study findings revealed a notable rise in the glucose levels within the treated insects. According to other researchers, when the larvae of the *Phenacoccus solenopsis* insect were treated with silver nanoliquids, a notable reduction in the total body glucose levels was observed (Madasamy et al., 2023).

In another study, it was observed that alkaline phosphatase enzyme levels decreased in *Culex pipiens* larvae treated with silica nanoparticles (Baz et al., 2022). However, contrasting results were reported in a study by El Gohary et al. (2021) on *Culex pipiens* mosquitoes treated with *Syzygium aromaticum* chitosan nanoparticles, where there was an increase in alkaline phosphatase enzyme activity (El Gohary et al., 2021).

Several studies have suggested that the raised toxicity of insecticides and nanoparticles results in an increase in the aspartate aminotransferase (AST) enzyme levels in mosquito larvae. Halawa and colleagues (2021) conducted a study to assess the efficacy of Chlorpyrifos and *Bacillus thuringiensis israelensis* against *Culex pipiens*. The researchers discovered a rise in the AST enzyme levels within the tissue extracts of the treated insects (Halawa, 2021). According to Sugeçti and Büyükgüzel (2018), the application of a 1.5% concentration of the oxfendazole compound induced oxidative stress in *G. mellonella* insect larvae. This oxidative stress was indicated by an elevated level of the AST enzyme in the haemolymph of the treated insect (Sugeçti & Büyükgüzel, 2018). Durairaj et al. (2018) and their research team conducted a study and observed that the LDH enzyme levels in *Culex* and *Anopheles* larvae decreased as the concentration of chitosan-based silver nanoparticles increased (Durairaj et al., 2018). Conversely, another study discovered raised LDH levels in *Galleria mellonella* insects that were treated with copper oxide nanoparticles (Tunçsoy et al., 2021).

In summary, nanoparticles can enter cells by penetrating the exoskeleton and interacting with sulfur-containing proteins or phosphorus-containing DNA within the cell's interior. This interaction can rapidly lead to the denaturation of organelles and enzymes, which are essential cellular components. These processes represent the primary mechanisms by which nanoparticles apply their effects to cells. Consequently, the distraction of the cell membrane and the proton motive force, which is crucial for cellular energy production, can result in a loss of cellular function and finally end in cell death (Tunçsoy, 2018).

2.2.6 Molecular effects

DNA, a crucial component of cells, is highly sensitive to oxidative damage. Nanomaterials possess random genotoxic characteristics, with various mechanisms influencing their ability to induce DNA damage. It is understood that the excessive generation of free radicals can lead to DNA damage and activate apoptosis. The primary genotoxic impact of nanoparticles arises from the generation of reactive oxygen species (ROS). These ROS can be produced by the nanoparticles themselves, generated by cellular responses or target cell stimulation, or as a result of the presence of metallic contaminants or inflammatory processes induced by the particles. Transition metal ions, including copper, iron, cadmium, nickel, zinc, and titanium, can be released from nanoparticles and act as catalysts for the Fenton and Haber-Weiss reactions. These reactions generate reactive oxygen species (ROS), particularly hydroxyl radicals ($OH^\bullet$), which are highly reactive and can damage DNA (Kessler et al., 2022).

There is a scarcity of genotoxicity data regarding nanoparticles in insects, with more importance placed on their effects on humans and aquatic invertebrates. Gogne et al. (2008) conducted a study and found that high concentrations of Cd telluride quantum dots aggregated and accumulated in the tissues of *Elliptio complanate*, leading to DNA damage in the gills and digestive gland (Gagné et al., 2008). Exposure of Japanese medaka (*Oryzias latipes*) to Ag nanoparticles resulted in the expression of various stress-related genes, indicating cellular and DNA damage (Chae et al., 2009). An investigation conducted with Zebrafish revealed that treatments involving Ag nanoparticles were associated with DNA damage, including the activation of the p53 gene and the occurrence of double-strand breaks (Choi et al., 2010). Subsequently, Kadar et al. (2011) provided evidence that higher concentrations of zero-valent nano iron induced DNA damage in the sperm of *Mytilus galloprovincialis* (Kadar et al., 2011). Furthermore, Gomes et al. demonstrated that both CuO nanoparticles and Ag nanoparticles induced DNA damage in hemolymph cells of *M. galloprovincialis*. The study also revealed a time-dependent response, as the extent of DNA damage increased over time when compared to unexposed mussels (Gomes et al., 2013). According to Mao et al., the presence of Ag nanoparticles (Ag NPs) in the tissues of *Drosophila melanogaster* resulted in the accumulation of reactive oxygen species (ROS). This ROS accumulation subsequently led to apoptosis, DNA damage, and activation of the Nrf2-dependent antioxidant pathway (Mao et al., 2018).

2.2.7 Ecotoxicity of nanoparticles

Artemia sp., commonly known as brine shrimp, are branchiopod crustaceans that exhibit tolerance to a wide range of salinity levels, from 4 to 250 g/L. Due to their sensitivity to chemicals and other toxic substances in aquatic environments, they are easy to use and efficient, and they can be employed for both short-term and long-term studies to evaluate the toxicity of chemicals and pollutants in water. Examples of substances that can be tested using *Artemia nauplii* include plant-produced toxins, manufactured chemicals, and nanoparticles. *A. salina*, as an important component of aquatic ecosystems, is considered a biomarker for evaluating environmental toxicity.

A recent study investigated the effectiveness of a green copper nanopesticide derived from *M. robertsii* against aquatic organisms, specifically *A. nauplii* and *A. salina*. The copper nanoparticles (CuNPs) demonstrated reduced toxicity towards both species compared to mosquito larvae and stored grain pests. The LC_{50} and LC_{90} values for *A. nauplii* ranged from 166.731 to 450.981 μg/mL, while for *A. salina*, they ranged from 293.901 to 980.153 μg/mL. These findings indicate that CuNPs hold promise as a safe and effective pesticide for aquatic environments (Vivekanandhan et al., 2021).

A study aimed to produce copper oxide nanoparticles in an aqueous medium using *Spinacia oleracea* leaf extract and revealed that copper oxide nanoparticles have an impact on both cysts and live organisms of *Artemia salina*. The investigation's findings suggest that the cytotoxic effect increases as the concentrations of copper oxide nanoparticles increase (250, 500, 1000, 2000, 6000, and 10,000 g/mL) (Thandapani et al., 2022).

2.3 Materials and Method

1. Literature Search: A systematic search was conducted using online databases (e.g., PubMed, Scopus, Web of Science) to identify relevant studies published up to [2023]. Keywords such as "coffee plant extracts,"

"green nanoparticles," "mosquito control," "efficacy," and "environmental impact" were used to retrieve relevant articles.

2. Selection Criteria: Articles were screened based on predefined inclusion and exclusion criteria. Inclusion criteria included studies that assessed the efficacy and/or environmental impact of coffee plant extracts and green nanoparticles for mosquito control. Articles focusing on other biocontrol agents or unrelated topics were excluded.

3. Data Extraction: Relevant data from selected articles were extracted, including study design, experimental methods, mosquito species targeted, concentrations or formulations of coffee plant extracts or green nanoparticles used, efficacy measures, environmental impact assessments, and any adverse effects reported.

4. Efficacy Assessment: The efficacy of coffee plant extracts and green nanoparticles in mosquito control was evaluated based on larvicidal, ovicidal, adulticidal, repellent, or other relevant measures reported in the included studies. The effectiveness of these biocontrol agents against different mosquito species and life stages was analyzed.

5. Environmental Impact Evaluation: The environmental impact of coffee plant extracts and green nanoparticles was assessed by examining their potential effects on non-target organisms, such as beneficial insects or aquatic organisms. Studies reporting on the persistence, degradation, and potential accumulation of these agents in the environment were analyzed.

6. Data Synthesis and Analysis: The findings from the included studies were synthesized and analyzed to provide an overview of the efficacy and environmental impact of coffee plant extracts and green nanoparticles for mosquito control. Any limitations or gaps in the existing literature were identified

2.4 Conclusion

In conclusion, our comprehensive review highlights the potential of coffee plant extracts and green nanoparticles as effective and environmentally friendly biocontrol agents for mosquito control. Mosquitoes are a significant public health threat, and the use of chemical insecticides has limitations and environmental concerns. Coffee plant extracts, with their larvicidal, repellent, and adulticidal properties, offer promising alternatives. Additionally, the integration of nanotechnology, specifically metal and metal oxide nanoparticles, shows potential for targeted control. However, further research is needed to evaluate the safety and efficacy of these approaches and consider their environmental implications. By combining diverse strategies and minimizing reliance on conventional insecticides, we can develop sustainable mosquito control methods that protect public health while preserving the environment.

2.5 Research Gap Identification

Numerous research groups have assumed investigations into the production of Cu nanoparticles. However, there is a lack of research literature concerning the environmentally friendly synthesis of Cu nanoparticles. Moreover, there is insufficient documentation regarding the effectiveness of Cu nanoparticles in fighting mosquitoes. Furthermore, there has been limited exploration of a simple, available technique for synthesizing nanoparticles from *Coffee arabica* beans. The main objective of the current study is to examine the green synthesis of Cu nanoparticles using *Coffee arabica* extract and assess their ability to control *Aedes aegypti* mosquitoes.

2.6 Hypothesis

Based on previous studies, this research aims to investigate the insecticidal toxicity potential of CuNPs synthesized using *Coffee arabica* plant extract on mosquito *Aedes aegypti*. The hypothesis of this study is that the polyphenol-rich aqueous extracts obtained from Yemeni *Coffee arabica* beans can generate nanoparticles through oxidative coupling. To test this hypothesis, aqueous extracts have been prepared from coffee beans using the oxidizing agent $Cu5H_2OSO_4$. The insecticidal assays have been conducted to confirm the bioactivity of the nanoparticles and provide evidence on the capacity of coffee polyphenols to support the formation of nanoparticles.

1. *Coffee arabica* seed extract acts as a bioreductant and stabilizer, facilitating the synthesis of copper nanoparticles with specific physicochemical properties.

2. The synthesized copper nanoparticles exhibit concentration-dependent larvicidal activity against Aedes mosquito larvae.

3. The synthesized copper nanoparticles effect on *Aedes* mosquito larvae by causing morphological and histological changes, genotoxicity, and interference with biochemical markers.

4. The coffee-synthesized copper nanoparticles have minimal negative impact on non-target organisms.

5. The phytochemical profiles and larvicidal activity of green and roasted *Coffee arabica* extracts are different from each other.

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