ASSESSMENT OF ENTOMOPATHOGENIC FUNGI MEDIATED SILVER NANOPARTICLES AGAINST MOSQUITO LARVAE AND ITS TOXICITY IMPACT ON SELECTED AQUA BIOTA

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

ZOOLOGY

By

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Declaration

I, hereby declared that the presented work in the thesis entitled "**Assessment of entomopathogenic fungi mediated silver nanoparticles against mosquito larvae and its toxicity impact on selected aqua biota**" in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of Dr A. Najitha Banu working as Associate Professor, in the **School of Bioengineering and Biosciences,** of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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Certificate

This is to certify that the work reported in the Ph.D. thesis entitled **Assessment of entomopathogenic fungi mediated silver nanoparticles against mosquito larvae and its toxicity impact on selected aqua biota** submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in **Zoology, School of Bioengineering and Biosciences**, is a research work carried out by **Natasha Kudesia, 11916775** is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course..

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ABSTRACT

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Since ancient times, mosquitoes have been acting as a vector for different species of pathogens that act as a source of various ailments affecting humans. Pathogens spread by mosquitoes include those that cause encephalitic viral infections as well as the parasites that cause Malaria and Filariasis. Mosquito-borne diseases have been flourishing for ages. They cause a considerable amount of illness and mortality over the world, especially affecting children and adolescents. It has been estimated that approximately 3000 species of mosquitoes are found all around the world, although only half of them are concerned with disease transmission. The most threatening vectors are mosquitoes from the *Culicidae* family. *Anopheles* (Malaria, Filariasis), *Aedes* (Dengue, Chikungunya), and *Culex* (Japanese encephalitis, Filariasis) are some of the main genera of mosquitoes that transmit parasites that cause human disease. These insects are reported to be distributed worldwide. Approximately 700 million people are infected with these vector-borne diseases every year. Along with this, such infections result in the death of more than a million people. As a result of this 1.13 million people contracted Dengue, and Chikungunya in India. Mosquito-borne diseases are now being recognized as a major epidemic. The growing urbanization of developing nations has contributed to the perfect environment for the spread of vector-borne diseases. Along with this, climate change, temperature shifts, and rainfall patterns are important factors which contribute to increased mosquito longevity. In most instances, the resurgence of the infectious disease is associated with the ecological changes such as construction of dams, deforestation, irrigation etc that have resulted in the increase in the vector population. Increased human movement has also caused the infectious agents to expand, bringing them into regions where they had previously been absent.

Therefore, in this scenario, it becomes highly important to undertake a vector control strategy for the prevention of these diseases. A number of larvicides aim to kill mosquito larvae in their breeding habitat before they may develop into adult mosquitoes and spread. By treating breeding places with larvicide, the number of adult mosquitoes in the surrounding region is reduced. Insecticide-treated bed nets using chemicals such as carbamates, organophosphates, pyrethroids and indoor residual spraying are typically used to target mosquito larvae. However, these chemicals pose adverse effect to public health and ecosystem. Along with this, recent studies have also shown that different life stages of mosquitoes have shown resistance against various classes of chemical insecticides. Environmentally friendly strategies such as various bio-control methods have also been employed for the control of mosquito larvae.

Plant-borne materials have been considered for sustainable vector management. Many different plant species have been shown to exhibit mosquito-repelling properties. A wide variety of plant species have been identified to have mosquitocidal activity. Essential oils obtained from the plants are highly efficient in controlling mosquito larvae. Yet still, research gaps concerning mammalian toxicity of many essential oils

restricts their use as a mosquito larvicide. Several aquatic organisms, like fishes and amphibians, feed upon mosquito larvae and act as the natural enemies of mosquito larvae. *Gambusia* and *Poecilia,* two genera of larvivorous fish, have been extensively used worldwide in mosquito control program. However, larvivorous fish are frequently seen as a threat to the local aquatic biodiversity, which includes amphibians. In a similar way, bacterial insecticides synthesized using *Bacillus thuringiensis* and *Bacillus sphaericus* have been utilized as mosquito larvicides throughout the world, although at this time, resistance has been seen against them.

Nowadays, entomopathogenic fungi (EPF) are acquiring a lot of popularity as potential candidates for controlling mosquito larvae. Unlike any other biological agent, EPF has the capacity to produce a variety of enzymes and proteins which have mosquitocidal activity, making it a highly promising choice. Conidia and spores of EPF play a crucial role in imparting pathogenicity to the larvae. Attachment of conidia or spores to the cuticle of mosquito larvae initiates the release of adhesins that cause infection which ultimately led to death. Studies have confirm the larvicidal impact of EPF metabolites on the mosquito larvae. Currently, to increase the efficacy of the EPF, nanoparticles (NPs) are being synthesized. Nowadays EPF based silver nanoparticles (AgNPs) are rising candidates for mosquito larvae control. To date, only a few EPF have been identified for the synthesis of AgNPs that could control vector larvae population. Along with this, the mechanism behind the toxicity of EPF based AgNPs in mosquito larvae is not yet known. On the contrary a lot of work has been already done to evaluate the detail of morphological and physiological deformities caused by plant-based nanosilver.

Therefore the current study is based on the *Aspergillus fumigatus* synthesized nanosilver along with its larvicidal activity in *Aedes aegypti.* The optical, elemental, morphological, and structural properties of newly synthesized nanoparticles were studied through UV–Vis spectroscopy, Zeta potential, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM) coupled with Energy-dispersive X-ray analysis (EDX). The characterization was confirmed by the surface plasmon resonance band at 434 nm. The results validated the spherical shape and size (28–33 nm) of the nanoparticles. AgNPs have been evaluated for their effectiveness in controlling *A. aegypti* larvae. The larvicidal effect was evident in the experiment when *Aedes* larvae were subjected to five different log concentrations of *A. fumigatus* based-AgNPs ranging from 1ppm to 5ppm. The mortality in the larvae had been observed at various exposure times. Exposure of third instar larvae of *A. aegypti* to biosynthesized silver nanoparticles resulted in lethal concentrations (LC50 and LC90) of 2.624 and 4.728 ppm, respectively. Moreover, the biotoxicity screening against non-target organism *Daphnia magna* and Rohu fingerlings revealed the nontoxic nature of biogenic nanosilver against non-target fauna. Thus the findings indicate that the EPF *A. fumigatus* has the potential to facilitate the swift synthesis of silver nanoparticles, presenting an innovative approach for vector population control, without causing any threat to other aquatic organisms occupying the same ecological niche.

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I dedicate this degree to my family, whose love and support have been my guiding light. My father, though no longer with us, has always been a constant presence in my heart. I envisioned celebrating this achievement with him, but fate had other plans. Yet, not a day goes by without thinking of him and knowing

he is watching over me with pride. My mother, a woman of incredible strength, has always believed in me. She has encouraged me to pursue my dreams, even though it meant being apart. I know the sacrifices she has made will be worth it when I finally earn my doctorate. To my brother, words cannot express my gratitude. He has selflessly sacrificed so much for me, enabling me to complete my studies. He has turned my dreams into reality and has been my unwavering source of strength. I love him dearly.

Natasha Kudesia

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LIST OF ABBREVIATIONS

S/No	Abbreviations	Full meaning
1	EPF	Entomopathogenic fungi
2	NPs	Nanoparticles
3	AgNPs	Silver nanoparticles
4	SEM	Scanning electron microscopy
5	FTIR	Fourier transform infrared spectroscopy
6	ZNPs	Zinc oxide nanoparticle
7	LC	Lethal concentration
8	DDT	Dichlorodiphenyltrichloroethane
9	IPM	Integrated Pest Management
10	XRD	X-ray diffraction
11	*Bs*	*Bacillus sphaericus*
12	*Bti*	*Bacillus thuringiensis*
13	EDX	Energy-dispersive X-ray analysis
14	MNPs	Metal nanoparticles
15	AuNPs	Gold nanoparticle
16	SiNPs	Silica nanoparticle
17	WWTP	Wastewater treatment plant
18	ICP-MS	Inductively coupled plasma mass spectrometry
19	PS	Photosystem
20	OECD	Organization for Economic Co-operation and Development
21	LCQTOFMS-MS	Liquid chromatograph quadrupole-time of fight-mass spectrometry
22	GC-QTOF-MS	Gas chromatograph-quadrupole time of fight mass spectrometry
23	CARS	Coherent anti-stokes Raman scattering
24	nm	nanometre
25	PDA	Potato dextrose agar
26	NCMR	National Centre for Microbial Resource

27	PDB	Potato Dextrose Broth
28	$AgNO_3$	Silver nitrate
29	ICMR-NIV	Indian Council of Medical Research-National Institute of Virology
30	DO	Dissolved oxygen
31	SPR	Surface plasmon resonance
32	UCL	Upper confidential level
33	LCL	Lower confidential level
34	PTTH	Prototho racicotropic hormone
35	(H&E)	hematoxylin and eosin
36	Er-AgNPs	*Euphorbia rothiana* leaf extract-synthesized silver nanoparticles
37	L	Lumen
38	ENS	Endoperithophic space
39	CA	Cardia cells;
40	BM	Basement membrane
41	EC	Epithelial cells
42	FB	Fat body
43	N	Nucleus
44	EXO	Exoskeleton
45	FAs	(Fatty acids)
46	mM	Milli molar
47	F1	Field 1
48	F2	Field 2
49	F3	Field 3
50	C	Cadaver
51	℃	Degree centigrade
52	Hr	Hour
53	gm	gram
54	ml	millilitre

CHAPTER 1

INTRODUCTION

CHAPTER 1- INTRODUCTION

By disseminating viruses and parasites like malaria and dengue, mosquitoes contribute to problems with global health. Unfortunately, resistance and high cost make the current insecticide-based control methods only moderately effective. In order to control mosquito-borne diseases, scalable, long-lasting, and affordable strategies are required. At present entomopathogenic fungi are being considered as a naturally occurring vector control agent. Spores and metabolites of entomopathogenic fungi have been confirmed to cause histopathological toxicity in mosquito at its larval stage. The ability of entomopathogenic fungi to kill mosquito larvae has also made them increasingly desirable in recent years for use in the biological synthesis of silver nanoparticles. At extremely low concentrations, fungi-based nanoparticles are an effective mosquito larvicidal agent.

There are two strategies by which silver nanoparticles can be synthesized are: top-down route in which a bulk counterpart decreases in size forming NPs and bottom-up route in which the self-assemblage of atoms and molecules generating nano-sized particles (Fig 1.1) (Thakkar et al., 2010). There are physical methods like evaporation-condensation, laser ablation, arc discharge method, etc using a top-down approach and chemical methods involving chemical reduction, microemulsion techniques, electrochemical synthetic techniques using bottom-up approach that are employed in the manufacture of NPs (Iravani et al., 2014). However, the physical and chemical methods are highly popular, disadvantages like being highly costly, toxic, complicated, and outdated limit their utilization. A wide variety of biological resources like plants, and microbes such as fungi and bacteria are used in the synthesis of NPs. The biological method is of low cost, provide high yield, is non-toxic, and environment friendly. Biological extracts comprising amines, amides, proteins, flavonoids, terpenes act as a reducing agent, this property makes them highly useful for the synthesis of NPs extracellularly or intracellularly (Asmathunisha & Kathiresan, 2013a; Thakkar et al., 2010).

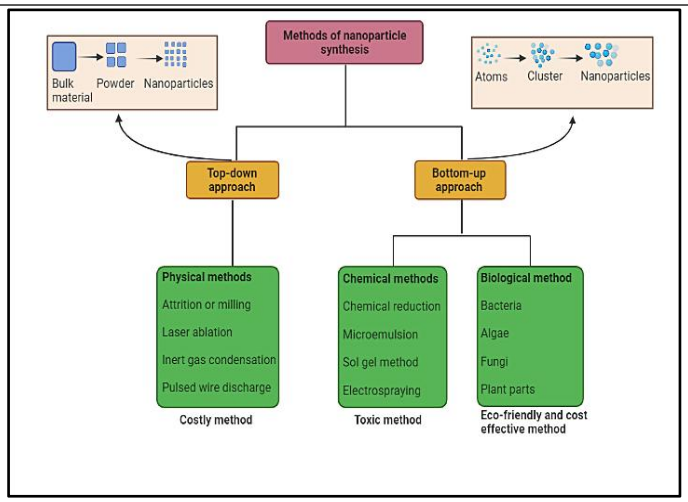


Fig 1.1: Schematic representation of various methods involved for the synthesis of nanoparticles

Insects are the most diversified and successful group of organisms dominating the world for 400 million years ago. There are approximately 5 million species of insects out of which 1 million have been identified (Grimaldi & Engel, 2005). The vast majority of insects are harmless to mankind but a small percentage of them act as pests and disease-transmitting vectors. Although only 1 percent of the known species have a detrimental impact, they too require special attention to reduce their negative impact on agriculture and human health (Kaya & Vega, 2012). Less than 10 percent of the total insect population is categorized as a major pest. It has been estimated that about 10,000 species of them are involved in crop damage (Dhaliwal, et al 2010). Insect pests are the major rival of humans causing great damage to agricultural productivity especially in cash crops and grains (OERKE, 2006). Different species of pests damaging a wide variety of crops have been listed in Table 1.1. The arthropod pests are a nuisance causing an 18-20 percent loss of crop yield globally. These losses are predominant during the harvesting season (Sharma et al., 2017). The loss of crops due to pests is a matter of concern in developed and developing nations. The boosting of the agriculture practices during the early 2000s has substantially increased the crop loss, owing to which India suffers an annual loss of 36 billion US$ (Dhaliwal et al., 2015). Similarly, a majority of insect vector such as belonging to the order *Phthiraptera, Siphonaptera, Heteroptera, and Diptera* are the causative agent of contagious diseases in humans.

Mosquitoes belonging to the family *Culicidae* are the most intimidating vectors. They act as a storehouse for various viruses that are passed on to the host thereby causing deadly diseases like Dengue, Chikunguniya, Yellow fever, etc. The vector ingest the pathogen while sucking blood from the host body. The disease causing pathogen replicates and is passed on to the new host after each subsequent bite. Table 1.2 lists different mosquito species harboring pathogenic micro-organism that acts as a causative agent for infectious diseases in humans (WHO, 2020). In India, vector-borne diseases are emerging as a major health concern, because over 1.13 million people in the country had suffered from Dengue, Malaria, and Chikungunya in the

year 2018 (Shinde et al., 2019). The global incidence of these diseases has dramatically increased in the past few years. Due to this reason more than half of the world`s population is at risk of having the infection (WHO, 2021). Mosquito-borne diseases were highly prevailing in most parts of the world a century ago, several measures were taken which proved to be successful in reducing the distribution of these vectors. Despite all this, in a recent decade, rapid urbanization in developing countries has created ideal conditions for the outbreak of vector-borne disease (Fernandes et al., 2018).

Pest species	**Crops**	**References**
Diabrotica virgifera virgifera	Maize	(Wesseler & Fall, 2010)
Phenacoccus solenops *Sphenaeches cafer* *Henosepilachna vigitioctopunctata and Epilachna dodecastigma*	Brinjal, tomato, okra Bottle gourd Cowpea and bitter gourd	(Rai AB, 2014)
Pyrilla perpusilla, Chilo infuscatellus, Odontotermes obesus	Sugarcane	(Srikanth et al., 2016)
Rhopalosiphun padi	Wheat	(Duan et al., 2017)

Table 1.1 Insect pest species causing damage to a broad range of crops including fruits, vegetables, and cash crops

Causative agent	**Vector organism**	**Vector-borne disease**
Plasmodium parasites	*Anopheles*	Malaria
Dengue virus	*Aedes aegypti* and *Aedes albopictus*	Dengue
Chikungunya virus	*Aedes aegypti* and *Aedes albopictus*	Chikungunya
Japanese encephalitis virus	*Culex tritaeniorhynchus, Culex vishui, Culex paseudovishnui*	Japanese encephalitis
Zika virus	*Aedes aegypti* and *Aedes albopictus*	Zika virus disease
Filarial worms (*Wuchereria bancrofti, Brugia malayi and Brugia timori)*	*Culex quinquifasciatus,* and *Mansonia annuifera/ Mansonia uniformis*	Filariasis
Yellow fever virus (arbovirus of the flavivirus genus)	*Aedes aegypti* and *Haemogogus species*	Yellow fever

Table 1.2 Vector organisms responsible for the transmission of infectious agents that cause several diseases in humans

Several control strategies have been undertaken for the management of pests, like the application of chemical pesticides. Dichlorodiphenyltrichloroethane (DDT) was considered to be an ideal agent against mosquitoes during the 1950s and 1960s. Advantages like being cheap, highly toxic towards pests, and harmless towards humans made it highly popular and demandable. However, in later years due to its excessive application, there was the development of widespread resistance among the pest population (Davies et al., 2007). The first case of resistance against DDT was seen in 1946. About 428 arthropods and 91 plant pathogen species have been reported to develop resistance against pesticides (Georghiou, 2012). Haphazard usage of these kinds of chemical pesticides though helped initially in keeping a check on pests but on contrary also harmed the environment (Rai & Ingle, 2012b). Chemical-based insecticides have also caused detrimental impacts on the environment and human health. Adverse effects on birds and animals have been observed due to the entry of chemicals pesticides in the food chain. Presently, insecticides like neonicotinoids and fipronil are highly efficient in controlling a wide range of pest populations. Till now no resistance has been discovered in any pest. However, they are highly toxic to the environment and can lead to lethal impacts on nontarget organisms (Simon-Delso et al., 2015).

In this context, Integrated Pest Management (IPM) has also been considered as a sustainable program developed to replace chemical pesticides with biological control methods (natural enemies of pests), and biopesticides (obtained from plants, micro-organisms, and entomopathogens). Microbial pesticides obtained from bacteria, fungi, algae, viruses, or protozoans are an alternative for chemical insecticides. In spite of this microbial insecticides are only toxic against a specific group of insects. Factors like heat desiccation or exposure to UV radiation affect their efficiency. Although microbial larvicide such as *Bacillus sphaericus (Bs)* and *Bacillus thuringiensis (Bti)* have been effective but currently mosquito vector has developed a high level of resistance against them (Poopathi et al., 2015). Similarly, disease-causing EPF like *Metarhizium anisopliae, Beauveria bassiana* are able to control the vector population. Despite being successful, they certainly have several limitations like their mass production is needed for the application. Factor like high humidity is required for the spread of infection in insects. Lastly, mycosis fungal pathogens are slow killers that make them inefficient in pest control (Charnley & Collins, 2007).

The above-mentioned limitations of the traditional methods urged for the development of a novel strategy that could be employed in the control of insect pests. Nanotechnology is a promising field that can transform the agriculture sector by providing a solution to various problems like insect pest management, rapid disease detection in plants, better nutrient absorption in plants, increasing the efficiency of pesticides, etc. Nanotechnology is an eco-friendly approach in comparison to the traditional methods that are harmful to the ecosystem (Rai & Ingle, 2012b). NPs find a wide range of applications in biology and medicine due to their small size and peculiar physicochemical properties that help in drug delivery, tissue engineering, etc (Salata, 2004). Nanotechnology has a considerable role in agribusiness. It helps in the effective smart delivery of

fertilizers, pesticides, and also serve as biosensors which provide information on the well-being of crops. Metal nanoparticles (MNPs) such as ZNPs (Zinc oxide nanoparticle), AuNPs (Gold nanoparticle), SiNPs (Silica nanoparticle), and AgNPs (Silver nanoparticles) are considered to be highly effective against a wide array of pests and insects (Kashyap et al., 2013; Malaikozhundan & Vinodhini, 2018; Najitha Banu et al., 2014; Subramaniam et al., 2016).

Out of all the metals, AgNPs are considered to be exceptionally beneficial due to the excellent insecticidal and antimicrobial activity possessed by them. Silver metal has a broad range of applications due to its pathogenic action, nanotechnology has increased the potency of this metal by utilizing it in the form of AgNP. As the small size of these nanosized particles increases the surface area to volume ratio that makes them highly reactive towards insects pest (Alghuthaymi et al., 2015a). AgNPs due to their unique properties has emerged as a highly popular and demanding biopesticide in the agriculture sector. Due to the enhanced usage of AgNPs it becomes extremely necessary to study their interaction with the ecosystem (Mishra & Singh, 2015). Increased consumption in industrial and agriculture fields tends to intensify the release of AgNPs in the aquatic environment (VandeVoort & Arai, 2012). Few studies that have been carried out reveals that biologically synthesized AgNPs used as a larvicidal agent against mosquito vector exhibit the least toxicity to aquatic organisms (Sarkar et al., 2014a). Hence it could be stated that AgNPs are the most suitable candidate to be used for mosquito control due to their less harmful behaviour towards the environment (Najitha Banu et al., 2014).

AgNPs made from plants and microbes are a greener and cost-effective approach in contrast to the other chemical and physical methods of synthesis. Nowadays NPs synthesized using biological entities such as bacteria, algae, fungi, and plant extracts are gaining a lot of prominence in the agriculture sector (Banu et al., 2021). EPF are highly suitable candidates in contrast to any other biological agent, due to the potential of secreting a wide variety of enzymes and proteins that assists in the reduction of metal ions to metal NPs (Alghuthaymi et al., 2015a). EPF mediated AgNP acts as a potent mosquitocidal agent at a very low concentration which makes them significant in vector control strategy (Najitha Banu et al., 2014). Therefore EPF synthesized AgNP can be considered as eco-friendly, cost-effective, highly reactive nano pesticides capable of putting control over the vector population (Vivekanandhan et al., 2018a). Currently, these biologically fabricated NPs have been confirmed as highly stable mosquitocidal (Marimuthu et al., 2011) and pesticide agents (Kamil D, 2017) as they are effective at a very low concentration.

Due to the enhanced usage of AgNPs it becomes extremely necessary to study their interaction with the ecosystem (Mishra & Singh, 2015). Increased consumption in industrial and agriculture fields tends to intensify the release of AgNPs in the aquatic environment (VandeVoort & Arai, 2012). Even though AgNPs exhibit mosquitocidal property, very little information is present regarding the effect of low doses of these nanosized particles on aquatic organisms (Benelli, 2016b). Few studies that have been carried out reveals that biologically synthesized AgNPs used as a larvicidal agent against mosquito vector exhibit the least toxicity to other aquatic organisms (Sarkar et al., 2014a). Hence it could be stated that AgNPs are the most

suitable candidate to be used for mosquito control due to their less harmful behaviour towards the environment (Najitha Banu et al., 2014).

Therefore this work emphasizes the potential of green synthesized AgNPs as a unique and effective measure to combat dengue vector. *Aspergillus fumigatus* has been successfully used as a reducing and stabilizing agent for the synthesis of AgNPs. A comprehensive analysis of the AgNPs was conducted using a combination of techniques, including UV–vis spectroscopy, FTIR (Fourier transform infrared spectroscopy), SEM (Scanning electron microscopy), EDX (Energy-dispersive X-ray analysis), XRD (X-ray diffraction), and Zeta potential. The study assessed the toxicity of mycosynthesized AgNPs against Ae. aegypti. Furthermore, toxicity was also evaluated against *Daphnia magna* to determine the implication of Asp–AgNPs on non-target organisms to validate the associated risk.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2-LITERATURE REVIEW

2.1 Introduction

Nanotechnology has a pronounced perspective in transforming human lives. The progress happening in this field is much ahead of the evaluation of their consequences on the ecosystem (Krysanov et al., 2010). Currently fabricated nanomaterials have gained recognition in cosmetics, biosensors, medications, and therapeutics. The rise in the exposure of nanomaterials makes it highly essential to analyze their physicochemical properties such as size, shape, chemical composition, and surface morphology. These characteristic features play a crucial part in imparting the toxic nature of nanomaterials (Gatoo et al., 2014). Engineered nanoparticles encompass unique chemical, biological, and optical properties not possessed by their bulk forms. These attributes make them highly preferable to many industries. The uniqueness in their property which makes them exceptionally beneficial causes a negative impact on living organisms (Oberdörster et al., 2005). Metal and non-metal oxide-based nanomaterials are highly in demand. Consequently, various life forms have already become highly vulnerable to nanomaterials due to their large-scale productivity. Out of all the metal-based nanoparticles, carbon and silver are widely accepted groups (Walters et al., 2016).

Silver metal has been used since prehistoric times in ornamental, photography, utensils, health, and hygiene. Silver vessels have antimicrobial properties, due to which they played an important role in preservation and storage. Silver has also been used in the treatment of serious burn wounds, ophthalmia neonatorum, conjunctivitis, bladder irritation, cervicovaginitis, and syphilis (Balasubramanian & Banu, 2016). Modern science and technology have transformed metallic silver into ultrafine nanoform, because it is having extraordinary physicochemical properties. Therefore, the nanoparticles important are uplifted in the nanotechnology industries (Chen & Schluesener, 2008). Uniqueness in the properties of silver nanoparticles has made it applicable in more than 250 consumer products such as food packaging, pharmacy, and cosmetology (Anjum et al., 2013). This is due to the antiseptic property being possessed by them. Silver nanoparticles have an extensive range of applications as antimicrobial and biocidal agents, water purification, in medicine, biodiagnostics, drug delivery, and textiles (Austin et al., 2014; Lansdown, 2006; Gajbhiye, 2016). Consequently, the demand for nanosilver is being anticipated to increase by 600 tons by 2021 in global market. Besides this, the worldwide production of nanomaterials is expected to rise by $ 55,016 million by 2022 (Anjum et al., 2013)

The endless applications and high production of silver nanoparticles indirectly affect the terrestrial and aquatic life forms. The majority of synthesized silver nanoparticles end up in the water bodies, causing a threat to the aquatic organisms (Lekamge et al., 2019). Anthropogenic activities, utilization in medicine, healthcare facilities, and industries are the major cause of pollution. Similarly, a major amount of nanosilver

is retained in sewage sludge which is finally released into the aquatic ecosystem (Tiede et al., 2010). Characteristic properties such as size of the particle, surface coating, and the concentration of the silver nanoparticles help in the determination of their fate and toxicity in water. Inside the water body, silver nanoparticles transform through aggregation, oxidation, as well as sulfidation and ultimately interact with the aqua biota (Levard et al., 2012). There have been innumerable studies conducted to show the presence of silver nanoparticles in lower aquatic organisms and fishes (Fabrega et al., 2011; Huang et al., 2019). Currently, chemically reduced nanosilver are receiving a lot of popularity due to their multipurpose application. However, the production of toxic chemical residues during the synthesis limits their utilization. Therefore it can be confirmed that chemical-based silver nanoparticles can be toxic to aquatic organisms. It has been confirmed that chemically synthesized silver nanoparticles are highly toxic to aquatic life. Substantial accumulation of silver that leads to toxic impacts has been observed in aqua microcosms (Ge et al., 2014).

In contrast to this silver nanoparticles which are synthesized biologically are considered a simpler, cheaper and sustainable approach. Biological resources such as different microorganisms and plants are considered ideal for nanoparticles synthesis (Thakkar et al., 2010). Nowadays, biomediated nanosilver is getting a lot of prominence in agriculture, because of its antibacterial, pesticidal, and mosquitocidal nature (Abdel-Raheem et al., 2020; Arvind Bharani & Karthick Raja Namasivayam, 2017; Banu & Balasubramanian, 2014; Franci et al., 2015). Owing to their wide acceptance, the probability of the release of biologically based silver nanoparticles might increase during their manufacture, application, and disposal. Runoff from the terrestrial land is a major source of nanomaterial into the aquatic ecosystem. This would increase the chance of accumulation of silver nanoparticles in the water bodies in future, thereby affecting aquatic life. On account of the increasing demand for biocompatible silver nanoparticles, the concern regarding their toxicity seems to escalate (Tortella et al., 2020). Till now, no severe effect of biosynthesized nanosilver has been reported by any aquatic organism, but few researchers have observed the least toxicity. It is confirmed that bio-based silver nanoparticles exhibit minimum toxicity in comparison with chemically mediated silver nanoparticles (Chandramohan et al., 2016)

2.2 Chemical- based nanoparticles application and impact

2.2.1 Routes to exposure of silver nanoparticles in the aquatic environment

The boost in the nanotechnology field has led to the massive disposal of nanoparticles into the aquatic ecosystem. The aquatic environment acts as a sink for different sources of nanomaterials released from industries (Walters et al., 2016). It has been predicted that nanoparticles can enter the ecosystem during their life cycle in three phases. They can be discharged at the time of their production, product utilization, and during the disposal of nano-based products (Bundschuh et al., 2018). Out of all the metal-based nanoparticles, nanosilver imposes a significant hazard to the environment. On account of the indiscriminate applications of silver nanoparticles, the concern seems to intensify (Khaydarov et al., 2009). Due to such a

broad range of utilization, the likelihood of silver nanoparticles entering into the ecosystem including the food chains might increase manifold times (Anjum et al., 2013). This ubiquitous usage of nanosilver enables them to enter into the ecosystem thereby imposing risk to the organisms (Kahlon et al., 2018).

Figure 2.1 illustrates the possible route of entry of silver nanoparticles in water bodies. The nanosilver discharged from industries and wastewater treatment plants ultimately ends up in waterways. In recent days, nano-based pesticides are getting attention in the agriculture area and it is finally released into the aquatic bodies through the surface run-of. Along with this, the silver nanoparticles employed in food packaging, water purification, fabrics, and healthcare sector such as catheters and dentistry also remain the primary reason for their release

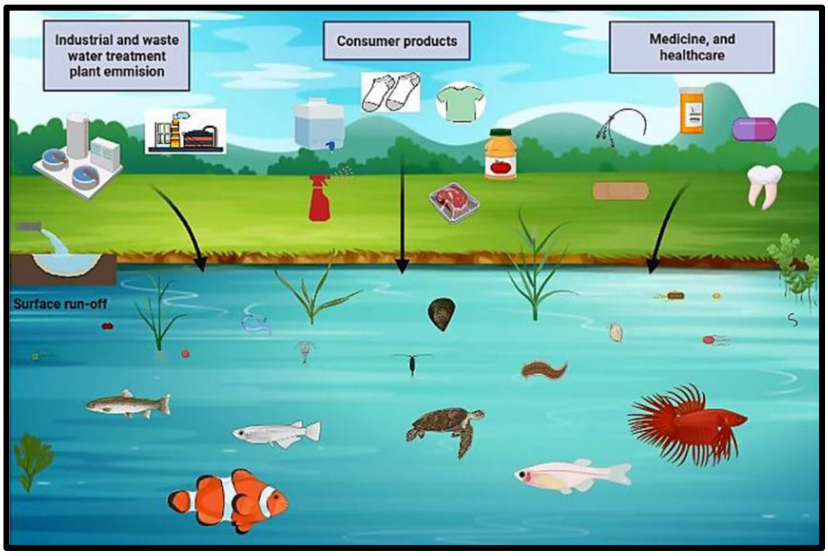


Fig 2.1: Possible pathways of silver nanoparticles from industry, consumer products, agricultural field, and biomedical into the aquatic environment

Water is the fastest medium to spread the contamination caused by these nanoparticles, as they tend to concentrate on the water surface. As a result, silver nanoparticles can easily accumulate and invade the food chains. This can cause a considerable threat to the aquatic organisms occupying different hierarchical orders. It has been concluded that approximately 15 percent of the total silver released into the Rhine River was through the nanosilver incorporated in the textiles and plastic industry (Blaser et al., 2008). As per the study conducted by Benn & Westerhoff (2008) haphazard usage of nanosilver leads to its release into the wastewater treatment plant (WWTP). An obstruction encountered in removing silver nanoparticles from WWTP accelerates their discharge in sewage. This ultimately leads to their entry into other water bodies, thereby creating a potential hazard to the ecosystem. Nanotechnology has increased the potency of silver metal by utilizing it in the form of silver nanoparticles. The smaller size of silver nanoparticles has a larger surface area to-volume ratio which makes them highly reactive toward pests (Alghuthaymi et al., 2015b). The uninterrupted dumping of these nanoparticles takes place at the time of direct application of the nano-pesticides and nanofertilizer on the soil (MacCormack & Goss, 2008). Currently, no safety guidelines exist

for the release of nano-pesticides in an aquatic environment. Agricultural runoff of nano-pesticides acts as a direct source of entry into water bodies. Thus, the silver nanoparticles have become prevalent in the aquatic systems through different sources. For this reason, it becomes essential to assess their potential impacts on aquatic fora and fauna. The toxicity imposed by nanosilver majorly depends upon its fate and transformation in the aquatic environment.

2.2.2 Transformation of silver nanoparticles in water bodies

The response generated by the silver nanoparticles entirely depends upon its properties and that of the nearby surrounding. Upon entering the aquatic ecosystem, the nanoparticles may stay in suspension form or tend to form aggregates because of their high surface area (Yu et al., 2013). Research in past have been done to study the transformation of the nanoparticles in a liquid medium, whether they will undergo aggregation or dissolution (Benn & Westerhoff, 2008; Furtado et al., 2016; Jorge de Souza et al., 2019). Physicochemical properties such as particle size, shape, ionic strength, and pH influence the response and the stability of the silver nanoparticles in water. These characteristics determine the fate of silver nanoparticles in aquatic mediums. It was concluded that the release of silver ions in suspended form is the predominant cause of nanosilver toxicity (Yang et al., 2012; Yu et al., 2013).

Silver nitrate has the highest tendency to liberate Ag^+ ions, whereas bulk silver releases Ag^+ ions to a lesser extent. As the size of bulk silver decreases and reaches the nanometre range, the tendency to release silver ions increases (Nowack et al., 2011). Silver nanoparticles exert different toxic effects depending upon the ionic environment. It has been elucidated that low chloride ion concentration influenced the dissolution of Ag^+ ions from silver nanoparticles that caused serious toxicity in the zebrafish embryo (Lee et al., 2018). The dissolution of silver nanoparticles released from commercial clothing in water has also been investigated. In an experiment, six different brands of socks were immersed in water. Results show that three out of six sock samples released silver nanoparticles. The silver nanoparticles transformed into a substantial amount of colloidal and ionic form in washed water. In each wash, different amounts of silver nanoparticles are released (Benn & Westerhoff, 2008). The stability, dissolution, and toxicity of citrate coated and polyvinylpyrrolidone-coated silver nanoparticles were investigated in an aquatic medium. It was observed that the citrate-coated silver nanoparticles had a faster dissolution rate in freshwater as well as seawater media, while the polyvinylpyrrolidone-coated silver nanoparticles remained stable. It was also seen that citrate-coated nanosilver was more toxic toward *Ceriodaphnia dubia* due to the rapid release of silver ions (Angel et al., 2013).

The state of silver nanoparticles in an aqueous environment is different from the change in pH. It was observed that at the pH range of 3 to 6.5, silver nanoparticles formed agglomerates, whereas in lower pH value (0.5), they completely suspended into Ag^+ ions. These suspended silver nanoparticles and dissolved silver have caused fatal implications in aquatic organisms (Elzey & Grassian, 2010) (Fig 2.2). The release of Ag^+ ions also depends on the particle size. It was confirmed that the smallest-sized silver nanoparticles are

completely dissolved. Rather, larger particles were able to persist for a long time to release silver ions (Dobias & Bernier-Latmani, 2013). The dissolution of citrate stabilized nanosilver to release Ag^{+} ion depends upon the presence of oxygen. The oxidation process generates the reactive oxygen species that are responsible for the toxicity mechanism (Liu & Hurt, 2010). The long-term transformation was investigated after 18 months of dosing silver nanoparticles in the terrestrial soil and water column. The silver nanoparticles were sulfdized in both compartments. The silver nanoparticles in the terrestrial soil were transformed into Ag_2S (silver sulfide), whereas silver nanoparticles in the aquatic sediment were present as Ag_2S and Ag–sulfhydryl compounds. It was also confirmed that surface runoff and erosion are the potential pathway for silver nanoparticles to enter the water bodies (Lowry et al., 2012). Finally, it can be stated that the behaviour of the silver nanoparticles is not the same in all aquatic environments. The reason behind this is the higher dilution in freshwater and seawater. The nature of silver nanoparticles is highly problematic to investigate in the aquatic ecosystem (Liu & Hurt, 2010).

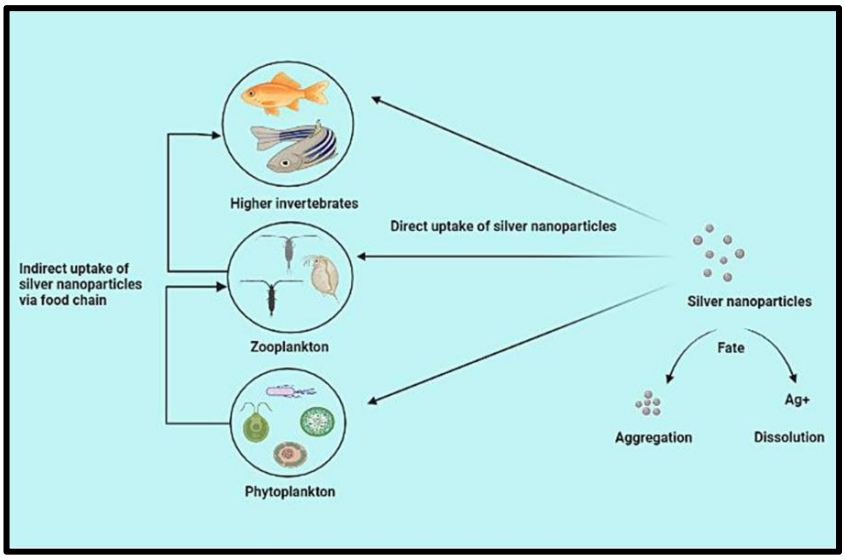


Fig 2.2: Mode of entry of silver nanoparticles in aquatic micro and macrocosms, and the fate of silver nanoparticles in water bodies

2.2.3 Uptake and bioaccumulation of silver nanoparticles by aquatic organisms

Bioaccumulation is the increase in the level of contaminants in the body of an organism. Waterborne and food-borne uptakes are two ways by which any pollutant enters the body (Wang et al., 2016a). Particles may accumulate in different organisms via the direct entry from water to gills, skin, or by the consumption of contaminated food. The intake of nanoparticles by the living organism can have two effects, either it will be stored in the body or it will be excreted in benign form (Uddin et al., 2020). Bioaccumulation is highly significant in evaluating the risk assessment posed by silver nanoparticles. The process explains whether the nanoparticles would penetrate the cuticle of the organism or would be retained on the surface. Bacteria and fungi have semi-permeable cell walls that prevent the entry of any large molecules to reach the plasma membrane (Fabrega et al., 2011). The prokaryotes are protected against nanoparticles due to the lack of a

transport mechanism, although the higher organisms exhibit specialized processes, such as endocytosis and phagocytosis for the internalization of nanoparticles (Moore, 2006). Various factors that influence the rate of accumulation of silver nanoparticles include the concentration, nature, route of exposure, and the biology of the organism (Luoma & Rainbow, 2005).

The silver nanoparticles in the aquatic environment may aggregate to form a large cluster or they dissolve to release Ag^+ ions. The silver ions are the primary reason for the toxicity induced by silver nanoparticles. These nanoparticles can enter either directly or via contaminated food sources into the body of various aquatic organisms occupying different trophic levels (Fig. 2.2). Studies have reported the uptake of silver nanoparticles in various aquatic organisms. Waterborne exposure is the usual route of uptake of silver nanoparticles by aquatic biota. Either the nanoparticles induce toxicity by adhering to the cell surface (Turner et al., 2012) or they accumulate inside the body of organisms (McTeer et al., 2014). Prolonged exposure to nanosilver leads to inhibition of photosynthesis in phytoplankton. The reproduction capacity is hampered in zooplankton, and organ deformities are observed in higher level organisms (Dewez et al., 2018; Kannan et al., 2011). The entry of silver nanoparticles was studied in wild-type and cell-wall-free mutants of green algae *Chlamydomonas reinhardtii*. The result shows that the cell wall containing green algae acted as a barrier and limits the uptake of silver. On the contrary, the cell-free wall had a higher accumulation rate of silver nanoparticles (Piccapietra et al., 2012). Adsorption (or attachment) of silver nanoparticles to zooplankton is believed to involve exoskeleton, appendages, respiratory epithelium, and digestive tract. More than 70 percent of silver nanoparticles were ingested by *Daphnia magna* through dietary uptake. Food ingestion in *Daphnia* occurred due to the consumption of silver nanoparticles-exposed algae (Nam et al., 2014). Internalization of nanosilver particles was studied in *Caenorhabditis elegans*. Different sizes of polyvinylpyrrolidone silver nanoparticles and citrate-coated silver nanoparticles were able to enter the cell membrane (Meyer et al., 2010). A comparative study of the interaction of silver nanoparticles with algal cells and fish gill cells was done by Yue et al., (2017). It was concluded that silver nanoparticles were adsorbed by the algal cells on their surface, whereas the fish cells followed the endocytic pathway for the uptake.

Waterborne exposure of citrate and polyvinylpyrrolidone coated silver nanoparticles was conducted in rainbow trout. Inductively coupled plasma mass spectrometry (ICP-MS) confirmed the presence of citrate-coated silver nanoparticles in the gills. Owing to their small size, citrate silver nanoparticles had a much faster transport rate within 48 h of exposure (Farkas et al., 2011). The toxic Ag^+ ions interact with the skin surface and get transported by gills to reach the brain and liver (Kwok et al., 2012). The transport of silver nanoparticles in developing zebrafish embryos was studied by Lee et al. (2007). The embryo was incubated with silver nanoparticles. Optical microscopy reveals that chorion pore channels were the site for the uptake and transport of nanosilver. The larger size of the pore channel permitted the passive diffusion of silver nanoparticles in the chorionic space of the embryo. From the above studies, it can be elucidated that silver nanoparticles are readily uptaken by aquatic organisms through various routes. The risk assessment requires

consideration of the uptake and accumulation of nanosilver in an aquatic ecosystem. Both these factors are crucial predictors of toxicity in different aqua biota ranging from lower to higher organisms.

2.3 Accumulation and toxicological effects of nanosilver in phytoplankton

Phytoplankton is the primary source of energy, forming the basis of the food chain in the aquatic ecosystem. Alteration in the aquatic ecosystem will have a direct implication on these microorganisms (Winder & Sommer, 2012). Therefore, it becomes important to understand the danger posed by any chemical pollutant released into the aquatic ecosystem. There has been an increased consumption and release of nanosilver in water bodies. So, it is important to know the impact caused by them on lower-level organisms. Algae are autotrophic unicellular or multicellular eukaryotes that are primarily affected when silver nanoparticles are present in water (Boxall et al., 2007). Similarly, aquatic plants or macrophytes function as the primary producers. They also provide habitat to zooplankton and lower-level invertebrates. Macrophytes have an important role in the regulation of biogeochemical cycles (Bornette and Puijalon, 2009). According to US EPA (1980) there is a high probability of retention and uptake of silver by the aquatic plants. It has been estimated that plants can accumulate approximately 2 to 58 mg Ag/kg DW. The nanoparticles are likely to accumulate in the different parts of the plant through osmotic pressure or via pores of the cell wall (Madigan et al. 2003).

The toxicity of chemically reduced nanosilver was observed on the growth of aquatic plants *Lemna minor* and *Eichhornia crassipes*. It was seen that the growth was inhibited in plants after exposure to nanosilver for 14 days. At a higher exposure concentration, silver nanoparticles were accumulated in tissues and roots that ultimately led to necrosis. This was followed by the decrease in the growth of the plant due to the interference in the metabolic activities. Thus, it can be concluded by this study that the toxicity of silver nanoparticles increased with time (Gubbins et al., 2011; Rani et al., 2016). Silver nanoparticles leds to the development of oxidative stress in the aquatic plant *Spirodela polyrhiza*. It was observed that there was an increase in the level of reactive oxygen species after treatment with 6-nm silver nanoparticles. In addition to this, chloroplasts had silver nanoparticles-accumulated starch grains, along with the reduction in the thylakoids. These results indicate that silver nanoparticles could penetrate the plant cells and can alter various physiological functions (Jiang et al., 2014). Phytotoxicity caused by silver nanoparticles was observed in the aquatic plants *Egeria densa* and *Juncus efusus*. Both the plants on exposure showed enzymatic defense to tolerate silver nanoparticles. Still, there were changes in the physiological characteristics. Accumulation of silver content was seen in all the tissues of *E. densa*. In contrast to this, silver accumulated only in the roots of *J. efusus*. Membrane damage was seen in *E. densa*, confirming that silver nanoparticles exerted more stress in submerged plants (Yuan et al., 2018). Seagrass *Cymodoce anodosa* exposed to silver nanoparticles concentration ranges (0.0002–0.2 mg L^{-1}) for 8 days was studied by Mylona et al. (2020). It was seen that the concentration of 0.0002 mg L^{-1} caused an increase in the level of oxidative stress. The cytoskeleton, photosystem II (PS II), leaf rhizome, and cell organelles were severely

damaged after 8 days at a concentration of 0.02 mg L^{-1}. The aggregation effect of silver nanoparticles was observed in cell compartments on the eighth day.

The comparative toxicity induced by silver nanoparticles and $AgNO_3$ on freshwater *algae C. reinhardtii* was evaluated for a short time by (Navarro et al., 2008). The higher toxicity of silver nanoparticles is due to the increase in the Ag^+ ions released as compared to that of silver nitrate. The silver nanoparticles led to a reduction in the algal photosynthetic yield. Miao et al. (2010) tested the impact of silver nanoparticles on freshwater alga *Ochromonas danica*. They found that a noticeable amount of silver nanoparticles was present in the vacuoles of *O. danica*. The presence of silver nanoparticles was confirmed by an X-ray spectrometer. The silver nanoparticles exerted direct implications on the algal growth. Growth inhibition was caused due to the dissolving of silver nanoparticles in the bulk media, with the release of silver ions to the algal surface. This can also occur through the direct entry of nanosilver into the cell, finally liberating metal ions and thereby imposing toxic effect. The implication of chemical silver nanoparticles on green algae *Pithophora oedogonia* and *Chara vulgaris* was done by Dash et al. (2012). After 10 days of exposure to silver nanoparticles, the nuclear cytology was studied. The scanning electron microscopy reveals cytological abnormalities along with the rupture of the cell wall. Accumulation of silver nanoparticles aggregates was observed on the surface of the algal filaments. The present study has also been interpreted for the control of algal weeds in the municipal water supply.

The uptake of silver nanoparticles by the algal cells depends upon the semipermeable cell wall that constitutes polysaccharides, cellulose, and glycoproteins. Nanoparticles can easily pass through the cell wall since its pore size is bigger than that of the particles.The pellicle of the green alga acts as a barrier to the entry of silver nanoparticles. In this study, nanoparticles were not internalized; rather, they are absorbed by the pellicle. Within 1–2 h of exposure, the silver nanoparticles were able to change the morphology of the cell (Li et al., 2015). Very few studies have been carried out stating the internalization of nanoparticles by algae. One of these studies was conducted by Wang et al., (2016), in which cellular internalization and intracellular biotransformation of silver nanoparticles were studied in *C. reinhardtii*. After 48 h of exposure, bioaccumulation of silver was observed in algae. The Ag^+ ion released from the silver nanoparticles was sequestered into subcellular compartments after entering the cytoplasm. Silver was also found to coexist with sulfur in the cytoplasm, on account of sulfidation. Despite this, to date, the uptake and accumulation of silver nanoparticles in algae remain unclear (Zhang et al., 2020). It can be concluded from the above mentioned studies that silver nanoparticles exert adverse effects on the phytoplankton. Concern increases when the transfer of these nanoparticles takes place from phytoplankton to higher organisms.

2.4 Bioaccumulation and toxicological effects of nanosilver in zooplankton

Zooplankton regulates the mechanism through which pollutants and energy are transferred to the higher trophic level. The zooplankton community comprises organisms of different body sizes, taxonomy, and ecological role (Havens, 2002). Zooplankton is highly sensitive species that acts as the main food constituent

for organisms of higher trophic level. These organisms are important bioindicators for the assessment of the accumulation and transfer of contaminants in the food chain (Battuello et al., 2016).

Daphnia, a highly sensitive zooplankton commonly known as water flea, is a filter feeder, found in abundance in water bodies. Being an essential part of the diet for aquatic predators, *Daphnia* occupies an important position in the food web. They have a short life span and high sensitivity toward various chemicals and heavy metals. *Daphnia* is widely used as a model organism for toxicity testing by Organization for Economic Co-operation and Development (OECD) (Tatarazako & Oda, 2007). According to the OECD guidelines 202 (OECD, 2004), acute toxicity tests are conducted for a period of 48 h on neonates of less than 24 h. Similarly, a reproductive toxicity test is conducted according to the OECD protocol 211 (Ocde, 2012), to find out the impact of toxins on the reproductive output of *Daphnia.*

Countable reports have confirmed the accumulation of silver nanoparticles on *Daphnia* (Asghari et al., 2012; Khan et al., 2015). *D. magna* was subjected to a study by Zhao & Wang (2011a) to evaluate the chronic and acute toxicity when exposed to silver nanoparticles. Cysteine was used as a chelator to decrease the toxic effect of silver ions released from silver nanoparticles. No significant toxicity was seen after the 48-h. However, accumulation of silver nanoparticles was observed in the gut lines at a higher concentration. In contrast to this, *Daphnia* was also exposed to waterborne and foodborne silver nanoparticles for 21-day chronic test. The growth inhibition occurred at the lowest dose of 5 mg/L. The underlying reason behind the growth inhibition was stated as low quality of food (having silver nanoparticles), low ingestion, and depuration rate. Silver nanoparticles lead to reproductive toxicity in *D. magna* after 21 days of exposure. The individuals suffered from survival, growth, and reproductive abnormalities at the concentration above 10 µg Ag/L (Mackevica et al., 2015). The chronic physiological effect of low doses of silver nanoparticles (0.02 and 1 ppb) was evaluated on *D. similis* for 21 days. Techniques such as liquid chromatograph quadrupole-time of fight-mass spectrometry (LCQTOFMS-MS) and gas chromatograph-quadrupole time of fight mass spectrometry (GC-QTOF-MS) were used in the study. Results were based on these techniques, which elucidated the down regulation of fatty acid contents that caused an inhibition of reproduction. Thus, this study provides details of chronic toxicity by the low doses of silver nanoparticles (Wang et al., 2018). The effect of surface coatings of silver nanoparticles on the feeding behaviour and bioaccumulation was investigated in *Daphnia*. T- (tyrosine-reduced) silver nanoparticles, C- (curcumin) silver nanoparticles, and E- (epigallocatechin gallate) silver nanoparticles were used in the study. Silver nanoparticles were transmitted from algae to Daphnids, confirming the trophic transfer. For silver nanoparticles with different coatings, there were differences in the bioaccumulation of the particles in algae and the diet-borne bioaccumulation of the particles in Daphnids. In algae, C-silver nanoparticle bioaccumulation was 1.5 times greater than T-silver nanoparticle bioaccumulation. Nonetheless, there was a 2.6-fold increase in T-silver nanoparticle accumulation in Daphnids through trophic transfer (Lekamge et al., 2019). In the recent study done by Yan et al. (2020), maternal transfer of bioaccumulated silver nanoparticles in *D. magna* was studied for the first time. The present study confirms the maternal transfer of silver nanoparticles which were present

within the lipid droplets in the embryo. The reproduction capability of F0 and F1 generations was inhibited, justifying the need to synthesize silver nanoparticles with a less toxic nature. Silver nanoparticles leds to the disruption of the food chain was studied by Yan & Wang (2021). In their study, citrate-coated silver nanoparticles and aggregation-induced emission fluorogen silver nanoparticles were internalized by the algae which were fed to *Daphnia*. Trophic transfer of nanosilver from algae to *Daphnia* was confirmed in the study. It was observed that more than 95 percent of accumulated silver nanoparticles were eliminated in the form of Ag^{+} ions by *Daphnia*. Yet, retention of aggregation-induced emission fluorogen silver nanoparticles was seen due to their low dissolution rate.

Artemia salina (brine shrimp) is another highly significant model organism used for ecotoxicology study in both field and laboratory conditions. Factors such as small body size, short life span, ability to adapt to adverse conditions, and high fecundity make *Artemia* relevant in ecotoxicity testing (Nunes et al., 2006). *A. nauplii* were exposed to spherical-shaped silver nanoparticles for 24 and 48 h. It was observed that due to its nonselective filter-feeding behaviour, *Artemia* ingested a large amount of silver nanoparticles that led to mortality, DNA damage, and inhibition of the hatching behaviour. A phase-contrast microscopy study reveals the accumulation of nanosilver particles in the mouth and gut region (Arulvasu et al., 2014). The magnetic silver nanoparticles that are recommended as a water disinfectant may impose risk to the aquatic ecosystem. The effect of magnetic silver nanoparticles was studied on *A. salina* to investigate the mortality rate and the effect on enzymatic activity. Although no mortality was seen in the experiment, a slight increase in oxidative stress was observed. An imbalance in the antioxidant defense mechanism was observed. The silver nanoparticles were found in the gut and the outer compartment of the body (Demarchi et al., 2020). The wide occurrence of the zooplankton makes them more vulnerable to nanosilver contaminants. They interact strongly with their ambient environment, but the uptake and accumulation of silver nanoparticles lead to the disruption of this normal function.

2.5 Bioaccumulation and toxicological effects of nanosilver in higher aquatic organisms

In an aquatic ecosystem, fish are the top most consumers in the food chain. They depend upon zooplankton and phytoplankton as a source of nutrition. Species diversity is disturbed in the food chain due to chemical contaminants. This creates havoc on the whole of the aquatic ecosystem. In this connection, the toxic substances can also reach the birds and humans when the contaminated fishes are consumed (Sudha & Baskar, 2017). Edible fishes can be considered as the primary route for the exposure and accumulation of nanoparticles in humans. Direct aqueous exposure, dietary uptake, or transfer via the food chain is some of the routes by which nanoparticles can get accumulated. Their transfer via the food chain is a recent topic getting a lot of prominences (Liu et al., 2016). Studies have been carried out stating the bioaccumulation of silver nanoparticles in fishes (Abarghoei et al., 2016; Clark et al., 2019; Jang et al., 2014; Kakakhel et al., 2021; Joo et al., 2013).

Zebrafish (*Danio rerio*) is a famous model organism for toxicity studies (Dai et al., 2014). Several works have evaluated the implications of nanoparticles on zebrafish embryos (Asharani et al., 2011; Lee et al., 2013). Transport of the silver nanoparticles into the embryo can take place due to the larger pore size of the chorion (Lee et al., 2007). Silver nanoparticles ranging from size 10 to 72 nm were easily able to diffuse into the embryo via chorionic pores, stating size-dependent nanotoxicity (Chen et al., 2020; Lee et al., 2013). The silver nanoparticles of size 10 nm and 35 nm induced dose dependent lethality. Coherent anti-stokes Raman scattering (CARS) microscopy confirmed the presence of silver nanoparticles on the outer edge of the embryo. Various morphological defects such as damage to the yolk sac membrane were also observed. Embryos exposed to a higher concentration of silver nanoparticles developed abnormalities such as reduced yolk size and bend tails (Osborne et al., 2013).

The adult *D. rerio* was exposed to 20-nm and 110-nm citrate-coated silver nanoparticles to investigate the target organ for Ag+ toxicity. Results obtained show that the 20-nm silver nanoparticles severely disrupted the Na/K ion channels in the gills. After subjecting the organism to 7 days of depuration period, silver nanoparticles of both sizes were still retained in the intestines (Osborne et al., 2015). The effect of silver nanoparticles on the gut microbiota was studied for 35 days in different sexes of zebrafish. It was observed that the accumulation of silver nanoparticles took place in the digestive tract. Male zebrafish suffered from a significant change in the microbial community residing in their intestine, whereas no such effect was observed in females. The underlying reason could be the difference in the gut microbiota in both sexes (Ma et al., 2018). The recent study of Seyedi et al. (2021) describes silver nanoparticles toxicity in the brain, oocyte, liver, and muscle tissues in *D. rerio*. Results illustrate that unsaturated fatty acyl chains present in these tissues are highly susceptible to degeneration. This can lead to an ill effect on the maternal and offspring population dynamics in future.

Labeo rohita has an excellent ability to acclimatize in laboratory conditions due to which it has been incorporated in various studies. *L. rohita* was subjected to short-term exposure (7 days) of chemically reduced synthesized silver nanoparticles. Single-cell electrophoresis reveals DNA damage at the concentration of 400 and 800 μg L^{-1}. The accumulation of nanosilver in the tissues was found to be significantly high. Histopathological analysis reveals several abnormalities in the liver like degeneration of hepatocytes (Sharma et al., 2016). Similarly, the effect of chemically stabilized spherical nanoparticles in *L. rohita* was determined for 28 days. It was found that histological alterations such as necrosis of the lamellar cells and hyperplasia were developed in the gills. The liver suffered from a reduction in the size of hepatocytes and the production of the apoptotic body. However, maximum accumulation of silver was found in gills. The study illustrates that gills and the liver are two highly susceptible organs for the absorption of silver nanoparticles (Khan et al., 2018).

An acute toxicity study was done by Khosravi-Katuli et al. (2018) in juvenile carp (*Cyprinus carpio*) according to the OECD guidelines 203 (Ocde, 2012). The median lethal concentration LC50 (96 h) value of 0.4 and 0.8 mg L^{-1} was obtained for silver nanoparticles. In the sublethal exposure test, fish was exposed to

the LC50 values of silver nanoparticles for 21 days. Results show that the highest amount of silver was accumulated in the intestine. There was an increase in the expression of antioxidant enzymes that can lead to tumor formation. The effect of sublethal concentration of silver nanoparticles in freshwater carp after 96 h had severely affected the gill membrane. Oxidization of the long-chain omega-3 unsaturated FAs (Fatty acids) resulted in a decrease in membrane fluidity. This led to the destruction of the normal physiological function of the gill membrane. Further histopathological details reveal damage in the lamellae, thinning of the basement membrane, and inflammation (Xiang et al., 2020). The waterborne sublethal effect of silver nanoparticles on *C. carpio* after 21 days of exposure led to the accumulation of 25 to 50 percent of Ag^{+} content. There was an elevation in the number of WBCs and neutrophils, whereas there was a decline of globulin and albumin content. Thus, it could be concluded that sublethal exposure of nanosilver to common carp led to the deterioration of its health (Vali et al., 2020).

Several studies have been carried out to describe the effect of silver nanoparticles on rainbow trout (*Oncorhynchus mykiss*) (Farkas et al., 2011; Salari Joo et al., 2013). The early life stages of rainbow trout are highly sensitive toward the silver nanoparticles. When juveniles and larvae were exposed at the least concentration of 3.2 mg/L and 1 mg/L, 100 percent mortality was observed (Johari et al., 2015). The silver nanoparticles present in municipal effluents cause immunosuppression and inflammation in *O. mykiss* (Bruneau et al., 2016). Ostaszewska et al. (2018) investigated the cytotoxicity caused by silver nanoparticles in the hepatocyte cells of *O. mykiss*. After 28 days of exposure at a concentration of 0.15 mg L^{-1}, histological analysis reveals shrunken hepatocytes, necrosis in the nucleus, and mitochondrial edema. Silver nanoparticles were visible as black dense spots in the hepatocyte cytoplasm and mitochondria. The latest study of Shabrangharehdasht et al. (2020) states that the blood cell morphology was severely hampered when *O. mykiss* was subjected to 8.9 mg/L of silver nanoparticles. Side by side, there was an increase in the expression of the p53 gene and Hsp70. This could mediate the production of reactive oxygen species and ultimately lead to a change in the cell signaling pathway. Regardless of these studies, the long-term exposure and uptake of silver nanoparticles in fishes are still missing (Dasgupta & Ramalingam, 2016; Lacave et al., 2018).

2.6 Biologically mediated nanoparticles application and impact

Different physical and chemical methods have been used extensively in the previous years to synthesize nanoparticles. However, disadvantages such as generation of hazardous by-products limit their utilization. Therefore, since the last decade, numerous efforts have been undertaken to develop an innovative approach for the production of nanosilver particles. This could prevent the toxic consequences of the traditional methods (Castro et al., 2014). The biological method of synthesis is a novel method with a futuristic approach, for the formation of nanomaterials. It is considered an eco-friendly and cost-effective way for the synthesis of nanoparticles. The plants and microbes of lower taxonomic groups have been considered ideal candidates for green synthesis (Das et al., 2017). The biological extracts helps in the reduction of metal ions

thereby forming nanoparticles. Microbial enzymes such as nitrate reductase and plant extracts consist of phytochemicals such as flavones and terpenes. They act as a reducing agent for the conversion of metal ions into metal nanoparticles (Asmathunisha & Kathiresan, 2013b).

Nowadays, to meet the increasing requirement of silver nanoparticles, biomediated procedure has been considered as an easy and quick strategy that assists in producing a high yield of nanoparticles. Biogenic nanosilver is used in therapeutics, agricultural engineering, and dye degradation. The biosynthesized silver nanoparticles are also emerging as a novel pragmatic way for developing nano-fertilizers and nano-pesticides (Rafique et al., 2017). Different resources employed for the synthesis and applications of biofabricated silver nanoparticles are discussed in Fig 2.3. The enzymes and proteins present in the biological extracts help in the reduction of silver ions and formation silver nanoparticles. These biologically reduced silver nanoparticles due to their insecticidal property are being extensively used in pest control.

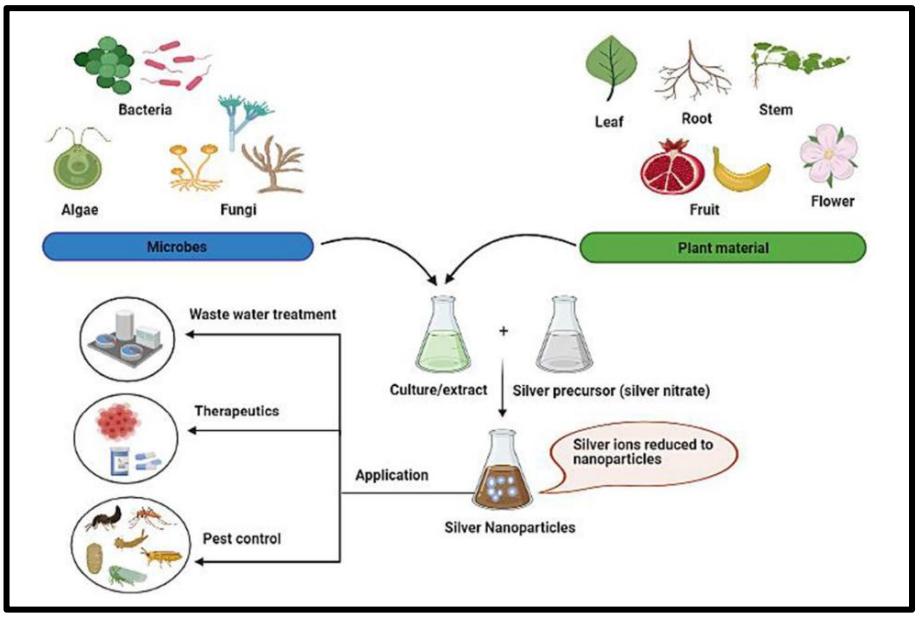


Fig 2.3: Biological extracts obtained from microbes, plants, and their products are utilized for the synthesis of silver nanoparticles. Biofabricated nanosilver is used in pest control, waste water treatment, and therapeutics

2.6.1 Phytofabricated silver nanoparticles and their application

Medicinal plants are considered to be highly effective in the synthesis of silver nanoparticles. The medicinally active biomolecules present in the extract can increase the antibacterial activity of the newly formed nanosilver. *Morinda tinctoria* or Indian mulberry leaf extract comprises carboxylic groups, alcohols, and phenol groups that act as a reducing agent for silver ions. *M. tinctoria*-based silver nanoparticles were found to exhibit excellent photocatalytic activity against dye molecules. This property makes them applicable in water purification systems and dye effluent treatment (Vanaja et al., 2014). Suresh et al. (2015) reported the use of silver nanoparticles biosynthesized from leaf extract of *Phyllanthus niruri* as an effective

mosquitocidal agent. The leaf extract was highly efficient in producing silver nanoparticles that were found to exhibit potential in controlling the *A. aegypti* larvae. Singh et al. (2015) synthesized a herbal plant Korean red ginseng based silver nanoparticles. It was observed that nanoparticles were produced within a short time of 10 min. The synthesized silver nanoparticles were effective against pathogenic organisms such as Staphylococcus aureus and *Bacillus cereus*. *Cleome viscosa* plant extracts-synthesized silver nanoparticles have a potential application in the biomedical field. Fruit extract was employed in the production of silver nanoparticles, which showed a considerable anticancer effect on lung and ovarian cell lines. In the study, silver nitrate was taken as the metal precursor, mixed with the fruit extract, and incubated for 24 h in dark. The fruit extract significantly reduced the silver ions, whereas the secondary metabolites acted as a natural capping agent for the silver nanoparticles (Lakshmanan et al., 2018). Capping or stabilizing agents have an important role in preventing the overgrowth of nanoparticles. They also prevent the agglomeration of newly synthesized nanoparticles. These agents help in increasing the functionality by maintaining the stability of the nanoparticles (Javed et al., 2020).

2.6.2 Microbes- based silver nanoparticles and their application

Likewise, microorganism such as bacteria acts as a renewable alternative for the synthesis of nanoparticles. Bacterial strains of *Listeria monocytogenes, B. subtilius,* and *Streptomyces anulatus* have been employed for silver nanoparticles formation. The synthesized nanoparticles were of varied shapes and size ranges, and they exhibited antifungal as well as mosquitocidal activity (Soni & Prakash, 2015). The pesticidal and antibacterial properties of biosynthesized silver nanoparticles have been evaluated against various pests and diseases in different studies. An entomopathogenic bacterium, *B. thuringiensis*, was highly effective in producing silver nanoparticles which appeared to have brownish-yellow color. The nanoparticles were studied for their characterization properties. It was stated that the size of the particles was 85 nm. In this study, *B. thuringiensis*-mediated silver nanoparticles showed pesticidal activity against cabbage looper, *Trichoplusia ni*, and black cutworm, *Agrotis ipsilon* and dengue vector (Najitha Banu et al., 2014; Sayed et al., 2018). Likewise, silver nanoparticles of particles size 25–50 nm synthesized using endophytic bacterium *B. siamensis* were efficient in protecting plants from bacterial infections. The silver nanoparticles synthesized in this study were used to control rice pathogens *Xanthomonas oryzae* and *Acidovorax oryzae* (Ibrahim et al., 2019). In the latest study of (Ameen et al., 2020) silver nanoparticles were synthesized using urban soil bacterium *Cuprividus sp.* Amides present in the bacterial culture were considered as a key component which helped for the silver nanoparticles synthesis. Spherical crystalline structures were obtained that caused a significant antibacterial activity against human clinical pathogenic bacteria.

Marine algae are considered to be ideal candidates for the synthesis of silver nanoparticles that have varied applications (Jeevitha & Rajeshkumar, 2019; Kathiraven et al., 2015). Aqueous extract of *P. oedogonia*, a freshwater alga, was used in the synthesis of silver nanoparticles. Algal biomass mixed with $AgNO_3$ was incubated, to give a brownish-yellow-colored solution. A colored solution is an indicator for the reduction of

silver ions. *P. oedogonia* extract comprises of phytochemicals such as carbohydrates, saponins, steroids, and terpenoids which acted as the capping agents. The biosynthesized silver nanoparticles exhibited strong antibacterial activity. This property makes them significant in pharmaceuticals against multidrug-resistant bacteria (Sinha et al., 2015). Silver nanoparticles synthesized from seaweed *Hypnea musciformis* are an eco-friendly tool against mosquito vectors and agricultural pests. Lower doses of *H. musciformis*-based silver nanoparticles showed toxicity against *A. aegypti* and the cabbage pest *Plutella xylostella* (Roni et al., 2015). In the same way, green alga *Caulerpa serrulata*-synthesized silver nanoparticles had a promising application in wastewater treatment (Aboelfetoh et al., 2017). *Amphiroa anceps*-based silver nanoparticles have been considered as eco-friendly nanobiofertilizer. The synthesized silver nanoparticles accelerated the seed germination by 70% for *Abelmoschus esculentus* and 80% for *Raphanus sativus*. In contrast to this, the germination rate was only 20–40% in seeds treated with water, seaweed extract, and liquid biofertilizer (Roy & P, 2018).

Similarly, EPF are a highly suitable candidate in contrast to any other biological agent. This is due to the potential of secreting a wide variety of enzymes and proteins that assists in the reduction of metal ions to metal nanoparticles (Bhainsa & D'Souza, 2006). EPF of genus *Metarhizium* and *Beauveria* are commonly isolated from soil. They have advantages such as high enzymatic activity, production of secondary metabolites, and insecticidal properties. Hence, EPF is considered an ideal alternative to chemical and physical synthesis (Litwin et al., 2020). Many studies have documented the potential of biomediated nanosilver in mosquito vector control strategy. A potent mosquitocidal agent was produced by Banu & Balasubramanian (2014). In their study, *Beauveria bassiana* was employed to produce nanoparticles. *B. bassiana* was isolated from an infected coffee berry borer, *Hypothenemus hampei*. The mycelia extract-synthesized silver nanoparticles exhibited mosquitocidal activity against dengue vector *A. aegypti*. Amerasan et al. (2016) produced *Metarhizium anisopliae* based silver nanoparticles of rod shape with 28–38 nm size. The study confirms the presence of biomolecules such as proteins that functioned as the stabilizing agent. The silver nanoparticles produced in this study were highly efficient in controlling the Malaria vector *Anopheles culicifacies*. Vivekanandhan et al. (2018) synthesized silver nanoparticles using *Fusarium oxysporum*. The nanosilver synthesized was an effective mosquito larvicidal agent at a very low concentration. According to the latest studies carried by Abdel-Raheem et al. (2020) EPF-mediated silver nanoparticles acted as an effective pesticidal agent against *Rhynchophorus ferrugineus*. *R. ferrugineus* is a serious date palm pest in Asia. Silver nanoparticles were synthesized from *M. anisopliae, B. bassiana, Verticillium lecani*, and *Trichoderma harzianum*. The study confirmed the highest efficacy of *M. anisopliae*-based nanosilver against *R. ferrugineus*, thereby making it useful in pest management. Thus, it can be seen that biomediated silver nanoparticles are being highly welcomed in the agricultural and healthcare sectors. They have countless advantages and applications on account of which there has been a rapid increase in their production.

2.7 Impact of biomediated silver nanoparticles on aquatic microcosms

At present, biologically synthesized silver nanoparticles are widely accepted in various applications. Despite this, only moderate information is available concerning their toxicity in the ecosystem (Guo et al., 2019; Rani et al., 2016). At most, till now many studies discuss the comparative toxicity of chemically and biologically synthesized silver nanoparticles. This helps in evaluating the eco-friendly nature of biomediated nanosilver and to verify their safe level in various applications (Jadoun et al., 2021). To date, studies concerning the biologically synthesized nanosilver reveal their least toxic behaviour toward aqua biota (Govindarajan & Benelli, 2016b). The chemical-based nanosilver is more toxic and leads to the highest mortality in contrast to biomediated silver nanoparticles. The less toxic nature of biobased nanosilver is due to the presence of antioxidants in the biological extracts that helped in the formation of silver nanoparticles. However, it has been observed that on increasing the concentration, both kinds of silver nanoparticles show toxicity, in which chemical-based silver nanoparticles are most toxic and bio-based silver nanoparticles are least toxic. An acute toxicity test was conducted on zebrafish using silver nanoparticles-synthesized sodium borohydride and guava (*Psidium guajava*) leaf extract. LD50 for chemically and biologically synthesized nanosilver was 80 and 400 $\mu g\ l^{-1}$. Chemically synthesized nanosilver caused follicular dystrophy and atresia in the ovary. On contrary, guava-based silver nanoparticles showed mild symptoms due to the presence of antioxidants in the guava leaf extract (Sarkar et al., 2014). Girilal et al. (2015) determined the toxicity caused by chemically reduced and *T. atroviride*-based silver nanoparticles in *Oreochromis niloticus*. The fish was exposed to three different concentrations of chemically synthesized and biologically synthesized silver nanoparticles (50, 100, and 200 µg/mL). After one week of exposure, stress developed in fish tissues on exposure to both chemically and biomediated silver nanoparticles, although stress protein analysis reveals that chemical silver nanoparticles caused a higher expression of Hsp70. Swelling and breakdown of muscles were also noticed in *O. niloticus*. At a concentration above 100 µg/mL, 50 percent mortality was observed for chemical-based nanosilver. At the same time, no mortality was observed for *T. atroviride*-synthesized silver nanoparticles up to the concentration of 50 and 100 µg/ mL. The acute toxicity caused by *Turbinaria ornata* (brown algae) (L- silver nanoparticles) and commercially purchased silver nanoparticles (C- silver nanoparticles) on zebrafish was investigated. Within 48 h of exposure, no abnormal behaviour was observed in L- silver nanoparticles-exposed organisms up till the concentration of 50 mg L^{-1}. Histological analyses revealed that no damage was induced by L- silver nanoparticles. Although minor uptake of silver by gills, brain and body tissues were observed in L- silver nanoparticles-exposed zebrafish (Renuka et al., 2020).

The above evidence confirms the least toxic nature of biogenic nanosilver than chemically synthesized. Although in recent years, studies have also been carried out to evaluate the toxicity of biologically synthesized silver nanoparticles against non-target organisms (Pandiarajan & Krishnan, 2017). Biosynthesized silver nanoparticles are emerging nanofertilizers and nano-pesticides agents. Therefore, it becomes highly essential to evaluate the toxicity of these products after their application. The major concern lies when the nanofertilizers are discharged from the agricultural fields to the water bodies (Castillo-

Henríquez et al., 2020). According to the review paper of (Benelli, 2018; Pavela & Govindarajan, 2017) maximum research work has been conducted on the plant-based silver nanoparticles to evaluate their acute toxicity in non-target aquatic organisms. However, those studies have confirmed that biomediated nanosilver does not have any harmful impact on aquatic organisms till now. Exposure to sublethal concentrations of bio-silver nanoparticles did not have any significant effect on nontarget organisms such as *Gambusia affnis* (mosquitofish) and *Poecilia reticulata*. Biogenic silver nanoparticles due to their toxicity toward mosquito larvae are also coming to light (Benelli, 2016). Subarani et al. (2013) synthesized mosquitocidal silver nanoparticles using *Vinca rosea* leaf extract. The nanosilver toxicity was tested against a healthy guppy fish (*P. reticulata*). After 72 h of exposure at a concentration of 10 mg/mL nanosilver, no toxicity was observed in fish. Subramaniam et al. (2015) used *Mimusops elengi* leaf extract for the synthesis of silver nanoparticles, which had an excellent impact on the larvae and pupa of *A. stephensi* and *A. albopictus*. The impact of exposing one third of LC50 (4 ppm) doses for mosquito larvae was studied on the predation efficacy of mosquitofish. Results illustrate that the predation rate increased in *G. affnis* after exposure, without having any post-treatment toxicity.

The toxicity of *Clerodendrum chinense* leaf extract-based silver nanoparticles was studied in *Diplonychus indicus, Anisops bouvieri*, and *G. affnis*. The nanosilver formed exhibited high toxicity toward mosquito vectors. When LC50 doses toxic to mosquito larvae were exposed to non-target organisms, no behaviour changes were observed (Govindarajan & Benelli, 2016b). Murugan et al. (2016) synthesized silver nanoparticles using *Moringa oleifera* seed extract, which was highly effective against *Culex quinquefasciatus* larvae. After exposing the low doses of biologically synthesized silver nanoparticles, an increase in the predation efficiency of water bugs was observed. The underlying reason behind the increase in predation was stated that low doses of silver nanoparticles made mosquito larvae less motile, thereby making them highly susceptible to predation by the bug. Overall, this study proved that a single low dosage of *M. oleifera* silver nanoparticles did not cause any toxicity in water bugs. Alyahya et al. (2018) synthesized silver nanoparticles using leaf extract of *Holostemma ada-kodien*. Silver nanoparticles were highly effective against *A. stephensi, A. aegypti,* and *Cx. quinquefasciatus.* The impact of biomediated silver nanoparticles was also evaluated against the water bug *Diplonychus indicus. D. indicus* was exposed to different concentrations of *Holostemma ada-kodien*-based silver nanoparticles (300, 600, 900, 1200, and 1500 µg/mL). After 48 h, LC50 value was observed at a higher concentration of 623.48 µg/mL in *D. indicus.* Therefore, it can be concluded that mortality is directly proportional to the dose of synthesized silver nanoparticles.

Many studies confirm the bio-compatible property of biosynthesized silver nanoparticles (Govindarajan & Benelli, 2016; Ottoni et al., 2020). Although few studies have also stated that biomediated silver nanoparticles might pose threat to aquatic organisms. In the study of Krishnaraj et al. (2016) leaves extract of *Malva crispa* was used for the synthesis of silver nanoparticles. Adult zebrafish were exposed to the synthesized silver nanoparticles for 96 h and 14 days. The LC50 concentration of silver nanoparticles was

observed at 142.2 µg/L. Half of the value of LC50 (71.1 µg/L) was exposed for 14 days. Results illustrate that no mortality and difference in fish behaviour were observed. However, oxidative stress and localization of the silver nanoparticles were observed in the tissues. Similar results were observed by Ramachandran et al. (2018). The effect of *Acalypha indica* leaves extracts-mediated silver nanoparticles led to nuclear abnormality along with the generation of reactive oxygen species in zebrafish. Jenifer et al., (2020) concluded in their comparative study that *Solanum nigrum*-based silver nanoparticles were very less toxic than silver nitrate. In their study, dose-dependent toxicity was observed. Hundred percent mortality was observed for ionic silver at 30–40 µg mL^{-1} in *Ceriodaphnia cornuta* and *P. reticulata*. In *P. reticulate*, LC50 values for silver nanoparticles after 48 h and 96 h were found to be 38.3 and 34.5 µg mL^{-1}, whereas the LC50 for *C. cornuta* was 23.5 µg mL^{-1}. These values were very less as compared to the values obtained for ionic silver. The study confirmed that a lower concentration of silver nanoparticles did not cause any toxic implications. On increasing the dose of silver nanoparticles, toxicity and accumulation could occur in both organisms. Nowadays, biofabricated silver nanoparticles are emerging as a potent candidate in various sectors such as agriculture, pest, and vector control. Still, no reports concerning their worse implications have been recorded yet. So far, the bioaccumulation of biogenic silver nanoparticles has not been reported in aquatic organisms. Therefore, in-depth studies are required to evaluate their toxicological implications. Further studies are needed to know the toxicity of these silver nanoparticles in the aquatic ecosystem on non-target organisms as negligible information is present on this topic.

Conclusion

Nanotechnology has its offshoot in several sectors. Nanoparticles on account of having unusual properties have been deployed in several industries. Metal nanoparticles have been in demand for a long time. Out of all the metal-based nanoparticles, silver nanoparticles have been consistently favoured due to their antimicrobial, pesticidal, and mosquitocidal nature. Silver has been used since prehistoric times as a preservative and antiseptic. Currently, silver nanoparticles are actively engaged in textiles, electronics, medicine, and health care. The innumerable applications and continuous production of nanosilver have increased the rate of its exposure to the environment, especially in the aquatic ecosystem. Water bodies are the ultimate sink for the runoff containing silver nanoparticles coming from different industries and other sources of applications. The review highlights the bio accumulation and toxicity of silver nanoparticles in various aquatic organisms of different trophic levels. From the studies, it has been concluded that chemical-based nanosilver induces severe effects on aquatic life forms. In recent years, biologically synthesized silver nanoparticles are gaining a lot of prominence in various applications. Microbial and plant-based silver nanoparticles are tremendously desired in the agriculture and biomedical sector. The present article has also spotlighted the consequences of biogenic silver nanoparticles on aquatic fora and fauna. Up to now, studies conclude that a lower concentration of silver nanoparticles does not induce any toxicity and behavioral change in aqua biota. Although the growing demands for biofabricated nanosilver might increase the

likelihood of their accumulation in aquatic microcosms in future, currently, the long-term impact of these silver nanoparticles is not yet known. These findings suggest that further research is required to evaluate the toxicity of bio-silver nanoparticles on aquatic organisms. Along with this, the biodegradable nature of bio-silver nanoparticles in the aquatic environment should be focused to investigate their toxicity. This would help in framing safety guidelines and policy-making for the field application of biologically synthesized silver nanoparticles.

CHAPTER 3

HYPOTHESIS

CHAPTER 3-HYPOTHESIS

Mosquitoes are a great threat to mankind, vectoring diseases like Malaria, Dengue, Chikungunya, Zika virus, etc. According to a study, in India, around 40 million people contract mosquito-borne diseases annually. The major burden of these diseases is borne by the most backward, poor, and remote parts of the country. There are no specific medications available for these diseases, hence only some preventive measures are followed which too have limitations. Therefore, it is necessary for researchers to identify biological agents to control mosquito vectors. Owing to their enormous significance, AgNPs have been the subject of substantial research. The one-step, low-cost, and environmentally benign green synthesis approach has recently attracted considerable interest. Previous studies have claimed that biologically produced AgNPs have antibacterial and larvicidal characteristics. The AgNPs produced are very stable and significantly effective in controlling vector larvae

Hence this study will investigate the larvicidal efficacy of mycosynthesized AgNPs against Dengue vector. Fungal colony will be isolated from the soil samples and insect cadaver. The samples will be collected from the Palampur tea garden, Himachal Pradesh. The fungal extract and metal precursor $AgNO_3$ will be subjected to reduction for the synthesis of AgNPs. The synthesized nanosilver will be used further to evaluate its efficiency against *Aedes aegypti* larvae. Due to the increasing demand for biofabricated AgNPs, concerns regarding their toxicity to non-target organisms seem to have escalated. Hence it is important to determine the effect of these nanoparticles on other aqua biota. Furthermore, toxicity is also evaluated against *Daphnia magna* and *Labeo rohita* fingerlings to determine the implication of biosynthesized AgNPs on non-target organisms to validate the associated risk.

AIM OF THE STUDY

This study aims to achieve a dual objective: first, to synthesize and comprehensively characterize silver nanoparticles (AgNPs) utilizing a sustainable, mycosynthetic approach employing the entomopathogenic fungus *Aspergillus fumigatus*. This characterization will involve a multi-modal analysis of their optical (UV-Vis spectroscopy), surface charge (Zeta potential), chemical composition (FTIR, EDX), crystalline structure (XRD), and morphology (SEM) to establish a thorough understanding of their physicochemical attributes. Second, the study seeks to rigorously evaluate the larvicidal potential of these *A. fumigatus*-derived AgNPs against the third instar larvae of the significant vector *Aedes aegypti*, determining key toxicological parameters such as LC50 and LC90 values. Crucially, a parallel investigation will be conducted to assess the toxicity of these biogenic AgNPs towards representative non-target aquatic organisms (*Daphnia magna* and Rohu fingerlings) to ascertain the environmental compatibility of this novel vector control strategy.

CHAPTER 4

OBJECTIVES

CHAPTER 4-OBJECTIVES

Objectives for the proposed work

1. **Isolation and identification of the entomopathogenic fungi from soil and insect cadavers.**

2. **Larvicidal efficacy of entomopathogenic fungi synthesized AgNPs against mosquito larvae.**

3. **Toxicity impact of AgNPs on aquatic organism *Daphnia* and *Labeo rohita* (fingerlings)**.

CHAPTER 5

MATERIAL AND METHODS

CHAPTER 5-MATERIAL AND METHODS

5.1 Methodology for Objective 1:

5.1.1 Collection of soil samples and insect cadaver from Palampur tea garden (Himachal Pradesh)

Palampur a popular town in Himachal Pradesh's Kangra region, is surrounded by beautiful deodar trees and various tea gardens (Fig 5.1). The city is located at an altitude of 1260 m and have a sub humid sub temperate type climate (Sood, 2016; Verma et al., 2016)

Fig 5.1: Map of the tea plantation garden Palampur (Google map)

As per the literature survey the microbes are highly prominent in cultivable land such as tea plantations which have high organic matter and acidity (Bihal et al., 2023; Quesada-Moraga et al., 2006). Soil samples were collected according to the method of Sayed et al. (2018) with minor modification. The 50 gm of soil were collected randomly from three different tea garden namely F1, F2, and F3 (Field1, Field2, Field3) respectively. Samples were collected in triplicates (Fig 5.2). Sampling was done by collecting the topsoil down to 15 cm depth, with a help of a scoop like tool made up of stainless steel. All samples were collected in sterile zipper polyethylene bags, which were correctly marked, brought to the laboratory of Lovely Professional University (56 block), and were refrigerated until further use.

Likewise, an insect cadaver (grasshopper) was also collected from F1 field.

Fig 5.2: Site of the Tea gardens (a); F1, F2, F3 field (b) Collection of soil samples in zipper bags

5.1.2 Isolation and identification of fungi from soil samples and insect cadaver

The fungi were isolated from soil samples and insect cadavers according to the method of Haraprasad et al. (2001). Total of 27 soil samples were collected from field F1,F2 ad F3. The soil samples were subjected to a serial dilution procedure followed by the spread plate technique as mentioned by Aziz & Zainol (2018) with minor modifications. Total of 9 samples collected from every field was mixed to make a single sample. 1 gm of soil sample was taken in a test tube containing 10 ml of distilled water. The test tube was vortexed for 2 mins. 1 ml of solution was drawn from the test tube with the help of a pipette, and was added to the next tube having 9 ml distilled water to make the total volume of 10ml. The same process was then repeated for the

remaining tubes, taking 1 ml from the previous tube and adding it to the next 9 ml diluent. Now 100µl solution from each dilution tube of 10^{-4} to 10^{-8} was plated on PDA (Potato dextrose agar) media plates supplemented with streptomycin by spread plate technique. With the help of a micro pipette samples were transferred to the plates, which were incubated at 28 ℃. After 5 days the plate was subjected to sub-culturing for isolation of pure colonies.

This experiment also investigated the presence of EPF on an insect cadaver collected from a tea plantation. Given the diverse microbial communities found in tea plantations, these environment are hypothesized to harbor a rich variety of EPFs, acting as natural biocontrol agents for insect pests. Therefore, the cadaver was presumed to have a high probability of harboring fungal spores. Two isolation methods were employed. Spores were gently dislodged from the cadaver using a sterile brush and dusted onto a PDA plate (spore dusting). Alternatively, the cadaver was directly mounted onto another PDA plate (direct cadaver mounting). Both plates were incubated at 28°C for five days. Following incubation, distinct fungal colonies were observed on the dusted plate, while fungal growth was directly observed on the cadaver in the second method. Sub-culturing from these sources onto fresh PDA plates was performed using a sterile inoculating loop to obtain pure isolates. Notably, the cadaver mounted on the PDA plate was also transferred to a new plate for further observation. It's crucial to transfer the fungus growing on the insect body to a new PDA plate before significant cadaver degradation occurs. Any mixed cultures obtained during the isolation process were further sub-cultured to achieve pure colonies to further identification and characterization.

5.1.3 Microscopic identification of fungi (Slide culture method)

The isolated pure fungal culture was stained with lactophenol cotton blue, and observed under a light microscope (Riddell, 1950). This method is considered to be superior to the other methods for the microscopic identification of fungi. 20 ml of the chosen agar medium was liquefied and carefully dispensed into a sterile Petri dish (Fig 5.3). Upon solidification a layer of approximately 2 mm in depth was formed. After the medium had completely set, a sterile dissecting needle and glass rod were used to draw a grid of 1 cm squares on the agar surface. To minimize the risk of contamination, the lid was removed carefully. Utilizing sterile forceps, a sterilization microscope slide was positioned within the lid of the Petri dish. A coverslip, likewise sterilized by flame, was then placed upon the slide with one extremity overlapping the other. Finally, a square section of agar was meticulously excised from the Petri dish and expeditiously transferred to mitigate the introduction of contaminants**.** Following the creation of the agar block, a sterile needle was used to inoculate each of the four edges in the centre with a spore suspension, ensuring penetration throughout the 2 mm depth**.** Using sterile forceps, a coverslip was placed centrally on the agar square. The entire slide was then transferred to a moist chamber for fungal growth**.** To minimize disruption during microscopic observation, the coverslip remained undisturbed during the entire growth period. Growth was terminated before excessive sporulation occurred, typically within 1-3 mm from the coverslip edge**.** To prepare the slide for observation, the coverslip was lifted vertically from the agar block. Using a sterile lifter,

the agar and most of the fungal growth were removed, leaving behind four isolated growth zones connected by thin mycelial lines on both the slide and the coverslip**.** A drop of 95% ethanol was applied to the centre of each growth zone, allowed to spread, and then followed by a drop of lactophenol blue stain applied just before complete evaporation of the alcohol**.** Finally, a clean coverslip was gently placed on the slide to spread the stain over the fungal growth. The slide was then used for microscopic examination**.** The stained culture was observed under the microscope with the fungal identification manual of (Humber, 2005).

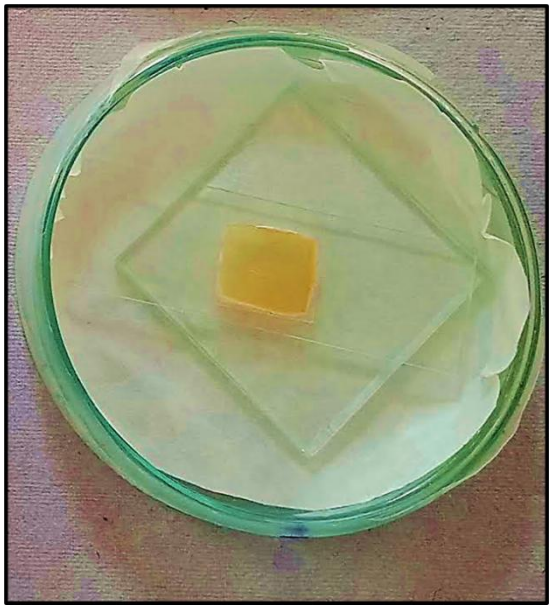


Fig 5.3: Slide culture technique

5.1.4 Molecular identification of fungi (18s rRNA sequencing)

The molecular identification of the pure culture was done through 18s rRNA sequencing at the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune, following the standard protocol. The phenol/chloroform extraction technique, a widely used method, was used to extract genomic DNA at the facility (Malke, 1990). The ITS regions were amplified using PCR with the universal primers ITS1 and ITS4. The amplified ITS PCR product was purified using PEG-NaCl precipitation and then promptly sequenced using an ABI® 3730XL automated DNA sequencer from Applied Biosystems, Inc. (Foster City, CA). To ensure accuracy, each location was sequenced from both ends, resulting in at least two reads per location. The Lasergene software was used to assemble the sequences, which were then compared to sequences from the type material using NCBI BLAST for preliminary identification (Boratyn et al., 2013)

5.2 Methodology for Objective 2:

5.2.1 Silver nanoparticles synthesis using mycelial extract

The isolated pure colony of fungi was maintained on PDA plates. To prepare the mycelial extract the fungus was grown on Potato Dextrose Broth (PDB) (Hi-Media). Following a 72-hour incubation period in PDB the fungal biomass was collected by filtration using Whatman No. 1 filter paper. To eliminate any residual media components, the biomass underwent washing procedure using double-distilled water. The fresh biomass of 20g was added to 200 ml of Milli-Q water in an Erlenmeyer flask. The flask was then incubated at 25°C for 72 hours while being agitated. The fungal extract was obtained after passing it through the Whatman No. 1 filter paper. To synthesize extracellular AgNPs, 50ml of fungal cell-free extract was added to 1mM $AgNO_3$ (Silver nitrate) (LOBA CHEMIE PVT.LTD) (0.017 g/100 ml) in 1:1 ratio in a 250-ml Erlenmeyer flask and incubated under dark conditions at 25℃ for 72 hours together with the control flask (Banu & Balasubramanian, 2014).

5.2.2 Characterization of the synthesized silver nanoparticles

For morphological, topographical and functional analysis of silver nanoparticles were carried out in the Central instrumentation Laboratory in Lovely Professional University.

UV-visible spectroscopy

UV-vis spectroscopy analysis was performed on Shimadzu UV-1601 (Tokyo, Japan) at wavelength of 300 to 700 nm. The liquid sample measured in standard quartz cuvette of 3.5ml was used for the analysis. The ultraviolet-visible spectra of prepared silver nanoparticles were recorded along with the positive and negative control.

FTIR analysis

FTIR (Perkin-Elmer, USA) was used to identify the biomolecules that assist in reducing and stabilizing the NPs. The liquid sample was transfer in Petri plates and dried in the hot air oven at 50°C for overnight. The dried sample was collected by scrapping. The measurement was done with a 500–4000 cm^{-1} range for the relevant spectrum scans.

SEM-EDX

SEM measurement was done using JEOL (Tokyo, Japan), to study the morphology of the synthesized AgNPs. Images of highest resolution uptill 100,000X were obtained. The EDX technique is used when coupled with SEM. This technique analyzes the X-rays released from the powdered sample when excited by a beam of electron. The EDX analysis determines the elemental composition the bio-synthesized AgNPs.

Zeta Potential

Zeta potential evaluated the surface charge of mycosynthesized AgNPs by using a Zetasizer Nano ZS90 (UK). It was used to confer the stability of the colloidal suspensions. Measuring the zeta potential of particles provides useful information on particle stability in solution as well as the potential for particle aggregation. Zeta potential analyzer uses the light scattering technique for calculating the Zeta potential of liquid sample (Fig 5.4a).

XRD

XRD measurements helped in the identification of the elemental composition of the nanoparticles. XRD was conducted employing a Bruker D8 Advance diffractometer (Germany) using the powdered sample of silver nanoparticles (Fig 5.4b).

a b

Fig 5.4: (a) Liquid sample of AgNPs; (b) Powdered sample of AgNPs

5.2.3 Laboratory evaluation of mycological synthesized AgNPs against *Aedes aegyptii*

A. aegypti eggs were procured from ICMR-NIV (Indian Council of Medical Research-National Institute of Virology) Pune (Fig 5.5). The eggs were transferred in a tray containing 2L of water. They were supplemented with a nutrient medium consisting of dog biscuits and yeast extract in a ratio of 3:1. The standard method of IVM MANUAL (NVBDCP, 2015) was followed for the rearing of the larvae. After 24 hrs of rearing larvae were obtained (Fig 5.6).

The mosquito larvae were exposed to various test concentrations and a control to determine the activity range of the compounds under test. To determine LC50 and LC90 values, a narrow range of 4-5 concentrations yielding 10% to 95% death in 48hrs was utilized after confirming larvae mortality throughout a wider range.

5.2.4 Bioassay

The larvicidal efficacy of AgNPs, Fungal extract, and $AgNO_3$ in *A. aegypti* was evaluated by following the standardized protocol (WHO, 2005). The bioassay was conducted in which 25 larvae per concentration per replication (5 replicates maintained) in 250 ml glass beakers. The temperature was maintained at 27° C. Under the same conditions, a control group of larvae was placed in dechlorinated water. The effect on larvae was observed using a stereo-microscope. Even after a gentle touch with a glass rod, there is no indication of any movement, such dead larvae were counted according to the instructions of the WHO technique report series. After 48 hours, the mortality percentages were determined using the equation below:

$$\text{Mortality percentage (\%)} = \frac{\text{Number of dead individuals}}{\text{Number of treated individuals}} \times 100$$

Fig 5.5: *Aedes aegyptii* eggs procured from ICMR (NIV)

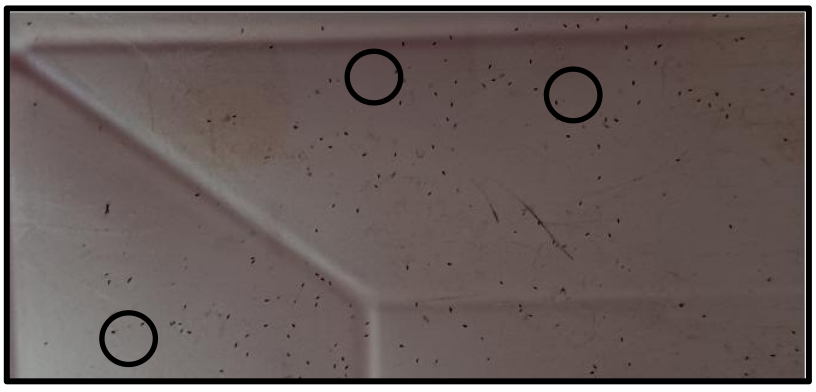


Fig 5.6: Rearing of *Aedes* eggs

5.2.5 Histopathology of *Aedes* larvae

The histopathological observation post exposure to the AgNPs, $AgNO_3$, fungal extract in *A. aegypti* larvae was conducted following the standard protocol (Sundararajan & Ranjitha Kumari, 2017). Larvae of the control setup (dechlorinated water) was also examined for comparative study. Briefly, the larvae were fixed in a 10% buffered formaldehyde solution for 24 hours. Following fixation, they were dehydrated through a series of increasing ethanol concentrations and cleared with xylene. The dehydrated and cleared larvae were then embedded in paraffin wax blocks. Thin sections (5µm) were obtained from these blocks using a rotary microtome (Labomed International). Finally, the sections were stained with Harris's Hematoxylin and Eosin (H and E) for microscopic examination. Light microscopy with an Olympus CX31 microscope was used to evaluate the sections for any abnormalities. Images with 10X and 40X magnification were taken for histopathology study.

5.3 Methodology for objective 3:

5.3.1 Bio-efficacy of AgNPs against non-target organisms

5.3.1.1 Collection and maintenance of Daphnia culture

Daphnia was collected from a pond near near Burger King, LPU campus, Phagwara, Punjab (Fig 5.7). It was identified in the laboratory through compound microscope at 100X magnification. *Daphnia* were cultured in laboratory condition according to the OECD Protocol 202 (Organization for economic cooperation and development) (OECD 2004). Culture was maintained in a loosely covered glass beakers containing tap water. The water was renewed three times a week. *Daphnia* were fed with yeast powder (0.5g per 100ml water) that act as a highly suitable nutrient source. The culture were kept at 20˚C and 16:8 hr light-dark cycle.

Fig 5.7: Site of collection of *Daphnia magna* from a pond Burger King, LPU campus, Phagwara, Punjab.

5.3.1.2 Acute toxicity test

A 48hrs acute toxicity test was performed on less than 24 hours old neonates of *D. magna* selected from the third brood progeny. The toxicity test on *Daphnia* was performed according to the guidelines of OECD (2004). 10 neonates were placed in 250ml glass beakers having 100ml test concentration of AgNPs, fungal extract and $AgNO_3$ ranging from 1ppm to 5ppm. Five replicates of each concentration were also maintained simultaneously along with the control. Test vessels were maintained at an optimum temperature of 20±2°C in 16:8 light and dark photo-period. Percent survivability was assessed after 48 hours, along with the change in the swimming behaviour. The organism was considered to be dead if no movement was observed in the appendages and lower abdomen even after 15 seconds of agitation in the test vessels.

5.3.1.3 Collection and acclimatization of Labeo rohita fingerlings

Labeo rohita fingerlings were brought to the laboratory from the fish farm (Khanna, Punjab) (Fig 5.8). They were acclimatized in recirculating tanks containing fresh, dechlorinated water which were continuously aerated using fish tank aerators. Before transferring fingerlings were treated with potassium permanganate to eradicate any external infection. All the fingerlings were acclimatized for 15 days before experiments. During this period about 20% of the water in each recirculating tank was replaced daily. All of them were provided with rice bran and groundnut oil cake mixture daily in a ratio of 1:3. The fingerlings were not fed 24 hrs prior before experiment. They were maintained at approximately 28° C with a 14 hrs:10 hrs light-dark cycle. The pH of water was maintained between 6.0-8.5.

During the acclimatization stage, mortality was reported using the following criteria:

If more than 10% of the population dies within seven days, the whole batch was rejected. For mortality between 5 and 10% of the population, acclimatization is extended for seven days. If more than 5% of the population dies during the second seven-day period, the batch was rejected. For mortality of less than 5% in seven days, the batch was accepted.

After the acclimatization period the fingerlings were replaced in treatment tank.

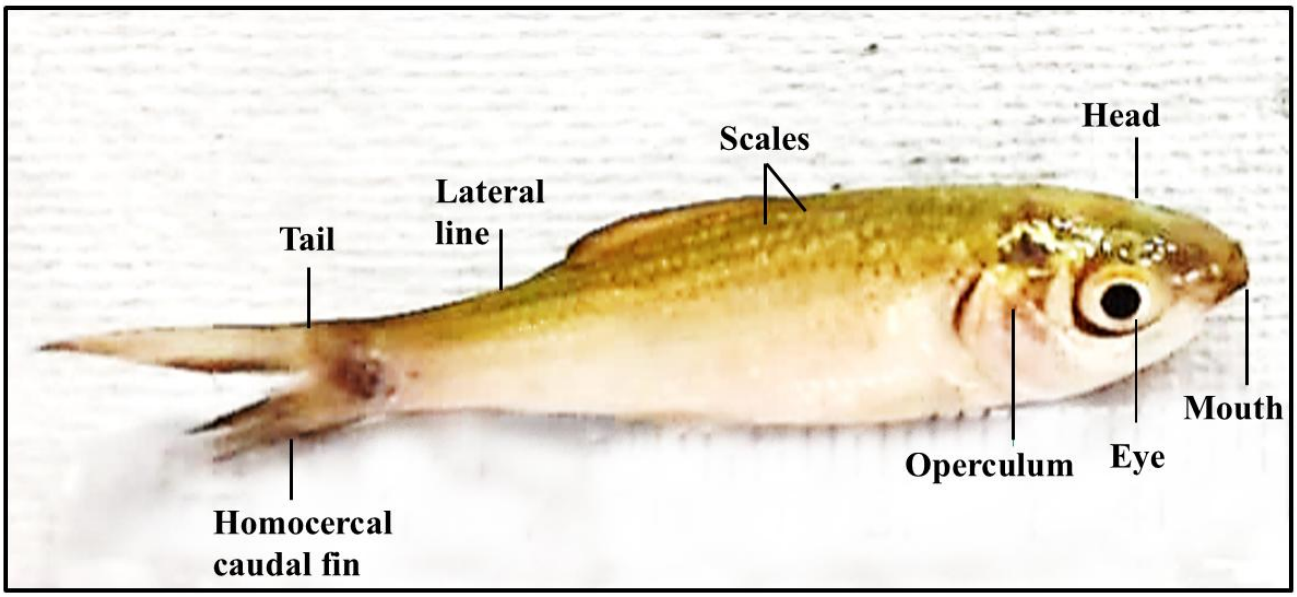


Fig 5.8: *Labeo rohita* fingerling collected from fish farm (Khanna, Punjab)

5.3.1.4Acute toxicity test

Acute toxicity was determined following the OECD (1992) guidelines with minor modification. All the treatment tank were cleaned and filled with dechlorinated water. To each of the treatment tank, 200, 400, 600, 800 and 1000ppm concentrations of AgNPs, fungal extract and $AgNO_3$ suspensions were added, that has been used for mosquito larvae causing LC50 (50 times the LC50 dose used for mosquito larvae). The fingerlings were categorized into 6 groups, each group having 10 organisms (the last group acted as a control). The water temperature and pH was maintained at 28 ± 1℃ and 6.8–7.3. Besides, the dissolved oxygen content (DO) of the water at 5.4 mg/L was maintained using DO meter. Triplicates were maintained under same conditions for each treatment. No feed was provided during the experiment.

All the treatment tank containing fingerlings were inspected twice each day (ideally early morning and afternoon). The study monitored visible abnormalities such as change in appearance, and swimming behaviour. Mortality was recorded at every 24 hrs of intervals for a period of 96hrs and dead fingerling were removed from the treatment tank to avoid contamination. The fingerlings were also monitored for 21 days to assess the post treatment survival and swimming behaviour. At the end of the experiment, the histopathological changes were observed. The fingerlings were dissected, and observed for any changes.

Statistical analysis

The mortality was recorded and analyzed using Probit analytic software (Finney, 1971) and SPSS 22.0 version. The LC50 and LC90 values were used to generate regression equations. Results were considered statistically significant if value of $p<0.05$. Percent survivability was calculated using Excel.

CHAPTER 6

RESULTS AND DISCUSSION

Chapter 6-Results and discussion

6.1 Objective 1:Isolation and identification of the entomopathogenic fungi from soil and insect cadaver.

6.1.1 Collection of soil samples and insect cadaver from Palampur tea garden (Himachal Pradesh)

A well-designed tea agro-ecosystem fosters a microclimate characterized by specific ranges of humidity, temperature, and light penetration. These conditions selectively favor the proliferation of microbial communities. Observations suggest a rich diversity of these beneficial microbes within the tea agro-ecosystem. Within agricultural systems, the resident microbial community plays a multifaceted role, including the regulation of insect pest population naturally (Kumar et al., 2020).

Samples were collected from three different tea estate F1, F2, F3 in the month of August, (2021). On an average more than 80 per cent humidity is observed in the month of August. The sampling site is located in district Kangara, in Palampur, which has an altitude of 700-1500 m above sea level. Three soil samples were collected in triplicates from three different sites F1, F2 and F3 respectively. A total of 9 soil samples were collected from each tea estate (Fig 6.1a). Likewise an insect cadaver was also collected from F1 site. The insect cadaver belongs to the order Orthoptera (grasshopper) (Fig 6.1b).

Sampling was done by collecting the topsoil down to 15 cm depth as because most of the microbial communities resides within the rhizosphere, the zone of influence around plant roots, within tea agroecosystems. Soil samples have been collected from the rhizosphere of tea plantations in the Dooars and Darjeeling regions of India. These samples, meticulously collected in pre-sterilized zip bags and kept with ice packs, were then subjected to a dilution method for the isolation of fungi, specifically focusing on nematophagous fungi, known for their ability to parasitize nematodes, a common group of plant-parasitic pests (Deka et al., 2021). The current research aligns with findings of Berhanu et al. (2022) who investigated the presence of nematophagous fungi in the soil of Ethiopia. Their study employed a randomized sampling approach, collecting 27 soil samples from each of three distinct agroecological zones. Within each zone, triplicate samples were obtained from diverse soil microhabitats – decomposing animal dung, agricultural land, and forested areas highlighting the potential influence of various environmental factors on the distribution and diversity of these beneficial microbes.

The cadaver collected was presumed to have a high probability of harboring fungal spores. The underlying reason can be the diverse microbial communities found in tea plantations. These environments are hypothesized to harbor a rich variety of EPFs, acting as natural biocontrol agents for insect pests. During the active phase, EPFs primarily target soil-dwelling insects in their active life stages. Spore germination and penetration in the insect cuticle occur, with rates influenced by environmental factors and specific EPF species. Once established within the host hemocoel (blood cavity), the fungus undergoes rapid proliferation,

often releasing toxins that contribute to insect mortality. This active infection phase is typically restricted to a brief period within the insect's life cycle. Following host death, the EPF continues colonization. The fungal vegetative network, the mycelium, pervades and eventually consumes the insect cadaver. Notably, some EPF species can enter a dormant state within the soil surrounding the deceased insect. This dormancy serves as a survival strategy, allowing the fungus to persist in the environment until conditions favor spore production. Dead insect cadavers on the soil surface then become crucial reservoirs for EPFs. As the fungus progresses within the cadaver, it produces conidia, specialized airborne spores well-suited for dispersal. The conidia posses a relatively long lifespan, enabling them to remain viable in the soil environment for extended periods. Certain EPF spores on insect cadaver can grow in lab conditions, suggesting they might be useful for pest control (Meyling, 2007).

(a)

(b)

Fig 6.1: (a) Soil samples collected from Tea estate from field F1,F2,F3 (b) Insect cadaver collected from field F1

6.1.2 Isolation of fungi

A total of 27 soil samples were collected from three distinct locations within the tea garden, designated as F1, F2, and F3. These samples were subjected to serial dilution and spread plate technique to isolate fungal colony. Subsequent sub-culturing of resulting colonies led to the isolation of 20 fungal strains from 3 fields. Additionally, a single fungal isolate was obtained through direct plating of an insect cadaver on PDA. The distribution of isolates across the sampling sites was as follows: 7 isolates from field F1, 7 isolates from field F2, 6 isolates from field F3 and 1 isolate from insect cadaver. All isolated fungal strains were subsequently cultured on PDA for further identification.

6.1.3 Morphological and microscopic examination of isolated fungi

All the isolated fungal colonies were morphologically (color, shape, edges of the colony) identified under laboratory condition using standard key of Humber, (2005) and Mycology online website (Fig 6.2).

The isolates primarily belonged to the genera *Mucor, Rhizopus,* and *Aspergillus.* The genus *Mucor* belongs to the zygomycotic order Mucorales. Colonies grew rapidly, reaching several centimeters in height and taking on a fluffy or cotton candy-like appearance. Initially white or beige, the colonies matured to a greyish or brownish color and had loose texture. The initial stage characterized by a white, cottony growth, commonly referred to as white mold. The sporangia exhibit a color transition from grayish-beige to black upon full development. The morphological characters of *Mucor* was similar to the *M. moelleri,* and *M. heterogamus* isolated from the sediment samples. The strains demonstrated optimal growth on PDA at 20°C, reaching a colony diameter of 67-70mm within a five-day incubation period. Initial colony morphology was characterized by a white, aerial mycelium, subsequently undergoing melanization to a brown pigmentation (Nguyen et al., 2020). Figure 6.3 depicts *Mucor* colonies isolated from different sites of Tea garden.

Rhizopus species were characterized by rapidly expanding colonies that form a dense, cotton-like mat on agar surfaces. Initially, these colonies appear white, but as sporulation commences, they gradually transition to a gray or yellowish-brown color. *Rhizopus* is characterized by sporangiophores that are typically solitary or grouped together and lack branching. These structures are generally brown in color. The spherical sporangia vary in color from gray to brown and often have a shiny appearance (Frye & Reinhardt, 1993). *Rhizopus* is easily distinguished from *Mucor* by the presence of stolons that can be several centimeters long, and clusters of rhizoids (root-like hyphae) that emerge from the points where the stolons contact the growth medium. *Rhizopus* also exhibits more vigorous growth compared to *Mucor* (Sarbhoy & Kulshreshtha, 1999). Figure 6.4 shows *Rhizopus* colonies isolated from different sites of Tea garden.

Aspergillus species exhibited a visually diverse group. Their colonies grew rapidly, and displayed a filamentous texture that was fluffy, powdery, showing concentric rings. Colors varied widely, encompassing yellow and green depending on the specific species. Different *Aspergillus* strains were differentiated based

on morphological characteristics. *Aspergillus flavus* exhibited a characteristic colony morphology with a yellowish-green obverse and a hyaline to yellowish reverse. Conversely, *Aspergillus tamarii* displayed colonies characterized by profuse sporulation and a dark green-bronze coloration. A consistent feature of all *A. tamarii* strains was a dark brown reverse colony pigmentation (Makhlouf et al., 2019). Figure 6.5 shows *Aspergillus* colonies isolated from different sites of Tea garden and insect cadaver.

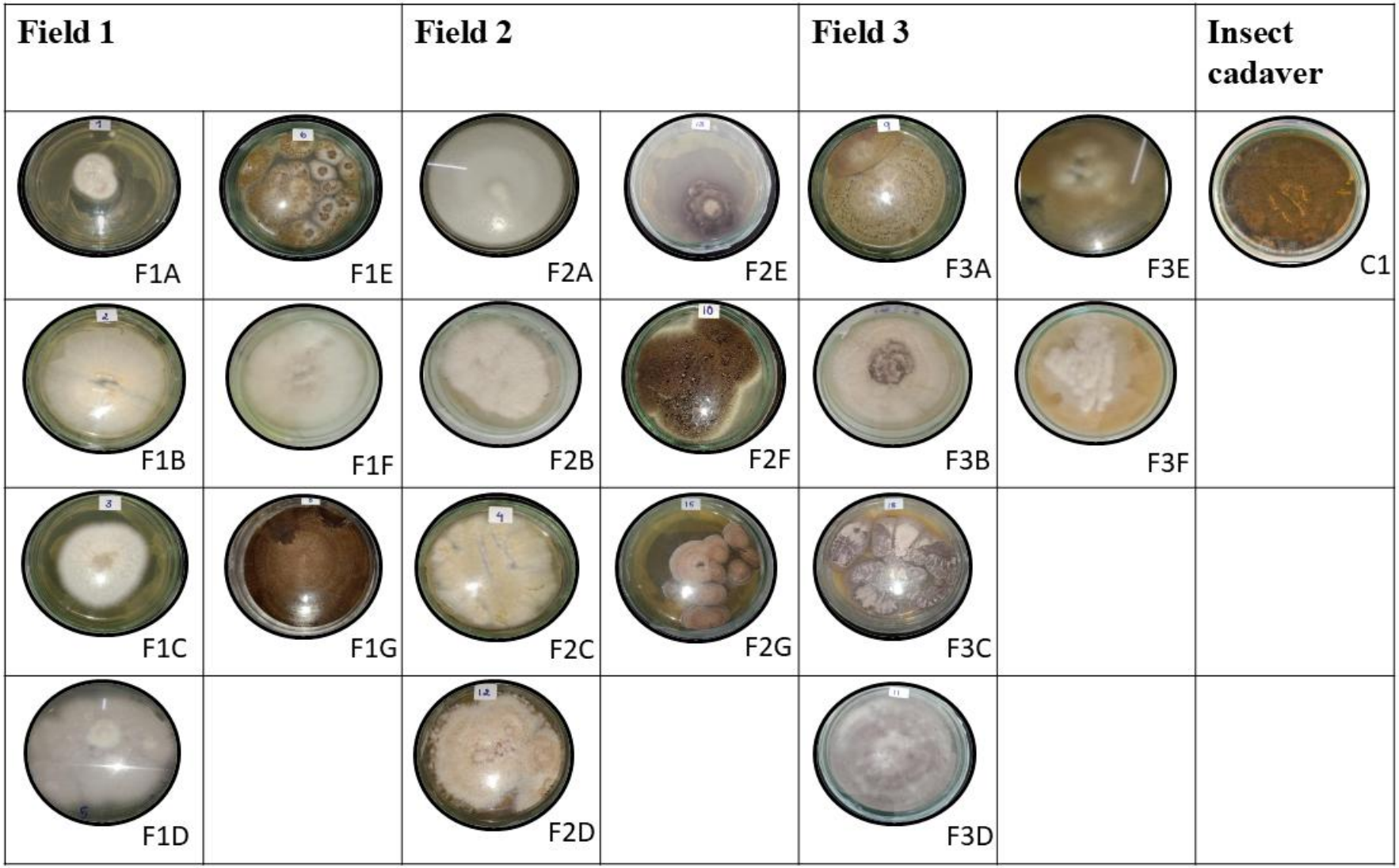


Fig 6.2: **Pure cultures of different fungus isolated from fields (F1, F2 and F3) and insect cadaver**

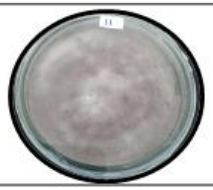
Mucor				
Colony	**Site**	**Colony**	**Site**	**Morphological characteristics**
	F3		F2	The genus Mucor belongs to the zygomycotic order Mucorales. Colonies grew rapidly, reaching and taking on a fluffy or cotton candy-like appearance. Initially white or beige, they matured to a greyish or brownish hue as spores developed.
	F3		F1	
	F1		F2	
	F3			

Fig 6.3: *Mucor* colonies isolated from different sites of Tea garden along with their morphological features

Rhizopus				
Colony	**Site**	**Colony**	**Site**	**Morphological characteristics**
	F1		F1	*Rhizopus* species are characterized by rapidly expanding colonies that form a dense, cotton-like mat on agar surfaces. Initially, these colonies appear white, but as sporulation commences, they gradually transition to a gray, black and yellowish-brown color
	F3		F2	
	F1		F3	
	F2		F2	

Fig 6.4: *Rhizopus* colonies isolated from different sites of Tea garden along with their morphological features

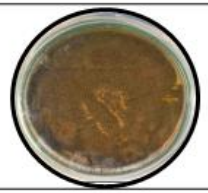
Aspergillus				
Colony	**Site**	**Colony**	**Site**	**Morphological characteristics**
	C1		F2	*Aspergillus* species exhibited a visually diverse group. Their colonies grew rapidly, and displayed a filamentous texture that was fluffy, powdery, showing concentric rings. Colors varied widely, encompassing yellow black and green depending on the specific species.
	F3		F2	
	F1			
	F1			

Fig 6.5: *Aspergillus* colonies isolated from different sites of Tea garden and insect cadaver

6.2 Objective 2: Larvicidal efficacy of entomopathogenic fungi synthesized AgNPs against mosquito larvae

6.2.1 Silver nanoparticles synthesis using mycelial extract

The fungal colonies isolated and identified morphologically in the previous objective were employed for the synthesis of silver nanoparticles. The spore free mycelial extracts were grown in PDB. The filtrate was used for the synthesis of AgNPs. For synthesis of silver nanoparticles, 1mM $AgNO_3$ and mycelial extracts were mixed with 1:1 ratio. The flasks containing the solution were incubated on a shaker for 72 hours, during which a color change was observed (Fig 6.6-Fig 6.9). Total 5 colonies namely F1E, F2F, F2G, F3A and C1 that were used for the synthesis of AgNPs were able to show color change. The resulting solution exhibited a characteristic crystal-clear, pale yellowish-brown hue, indicative of AgNPs formation. The intensity of the color deepened during an increase in incubation period, without any signs of deposition. It indicated that the synthesized AgNPs don't have any impurities. However, the control setup (without silver ions) showed no color change even after 72 hours of incubation. The color change is mainly due to the surface plasmon resonance (SPR), a phenomenon that results from the interaction of an electric field component of the incident light with electrons on the surface of metal particles, which is what causes the color changes to appear (Chutrakulwong et al., 2020). A comparable color change was noted in a study where silver nanoparticles were synthesized extracellularly using *Aspergillus* extract (Bhainsa & D'Souza, 2006).

Extracellular synthesis refers to the formation of the NPs outside the plant and microbial cell because of the presence of biomolecules, proteins, amino acids, enzymes in the cytoplasm. Intracellular synthesis involves the transportation of metal ions in the cell because of the electrostatic interaction between the positively charged metal ions and the negatively charged cell wall. Enzymes present within the cell wall reduces the metal ions to metal NPs (Hulkoti & Taranath, 2014). Fungi utilize enzymes like nitrate reductase to convert silver ions into silver nanoparticles. This eco-friendly process results in the formation of stable silver nanoparticles (Bhainsa & D'Souza, 2006). *Beauveria bassiana* and *Metarhizium anisopliae* have also been employed in the extracellular biosynthesis of silver nanoparticles. Fungal biomass, cultured in a defined liquid medium, was harvested, washed, and suspended in deionized water. Silver nitrate was added to the fungal filtrate, and the mixture was incubated. The subsequent addition of silver nitrate resulted in the formation of silver nanoparticles, visually indicated by a color change to brownish-yellow (Banu & Balasubramanian, 2014; Amerasan et al., 2016).

Colony	Mycelial extract	$AgNO_3$	AgNPs	Colony	Mycelial extract	$AgNO_3$	AgNPs
 F1A			No color change	 F1E			
 F1B			No color change	 F1F			No color change
 F1C			No color change	 F1G			No color change
 F1D			No color change				

Fig 6.6: Mycosynthesized silver nanoparticles using mycelial extract of colonies isolated from field 1

Colony	Mycelial extract	AgNO3	AgNPs	Colony	Mycelial extract	AgNO3	AgNPs
 F2A			No color change	 F2E			No color change
 F2B			No color change	 F2F			
 F2C			No color change	 F2G			
 F2D			No color change				

Fig 6.7: Mycosynthesized silver nanoparticles using mycelial extract of colonies isolated from field 2

Colony	Mycelial extract	AgNO3	AgNPs	Colony	Mycelial extract	AgNO3	AgNPs
 F3A				 F3E			No color change
 F3B			No color change	 F3G			No color change
 F3C			No color change				
 F3D			No color change				

Fig 6.8: Mycosynthesized silver nanoparticles using mycelial extract of colonies isolated from field 3

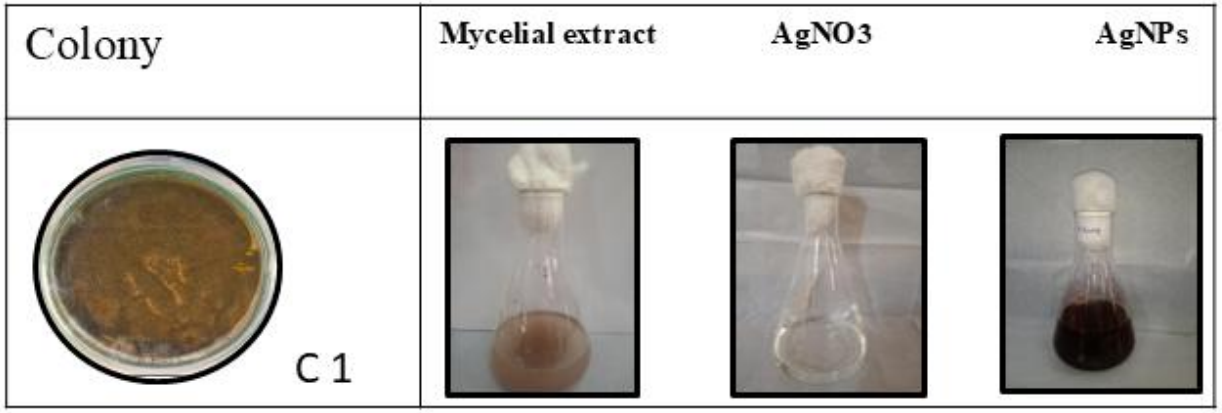


Fig 6.9: Mycosynthesized silver nanoparticles using mycelial extract of colony isolated from insect cadaver

Total 5 colonies namely F1E, F2F, F2G, F3A and C1 that were used for the synthesis of AgNPs were able to show color change. These colonies were identified based on their microscopic appearance using a slide culture technique to confirm their identity. Five fungal isolates, obtained from field sites F1, F2, and F3 as well as from an insect cadaver, were confirmed as members of the Ascomycota. A small portion of mycelium was mounted on the slide, stained with lactophenol cotton blue and was observed under the microscope. Phyllum Ascomycota consists of 476 species of entomopathogenic fungi. Out of all different species such as *Beauveria, Aspergillus, Paecilomyces, Metarhizium,* and *Lecanicillium* demonstrate high effectiveness in killing insects. *Aspergillus* exhibit potent entomopathogenic properties attributed to the production of the diarrheagenic toxin emodin. This metabolite has demonstrated efficacy in limiting pests populations, including mosquito larvae (Bihal et al., 2023).

The microscopic features were studied using the standard key of Humber (2005). The microscopic features revealed that the colonies were biseriate, with philiades having subglobose or globose vesicles of varying sizes. The conidia possessed a globose shape with thin walls and rough texture. The conidiophores had a rough texture and thick walls that were not coloured and unbranched. Whereas the microscopy of colony C1 revealed uniseriate conidiophores bearing columnar conidial heads. Flask-shaped vesicles were partially to fully covered with phialides, producing finely rough, globose conidia of a distinct green hue. The microscopic features of the colonies have been described in Figure 6.10, 6.11 and 6.12. Light microscopy images confirmed that colony F3A has a noticeably smaller vesicle. Despite this limited vesicle size, the colony still possessed well-developed phialides, structures readily visible under light microscopy. *Aspergillus* sps reveals distinct characteristics. The conidial head is uniseriate, meaning it forms a single row of spores. Additionally, the conidial head is columnar, having a cylindrical shape. The conidia themselves can be found in chains, but may also be detached and dispersed. Interestingly, single or paired conidia can sometimes resemble yeast cells in appearance (McClenny, 2005; Suleiman, 2023).

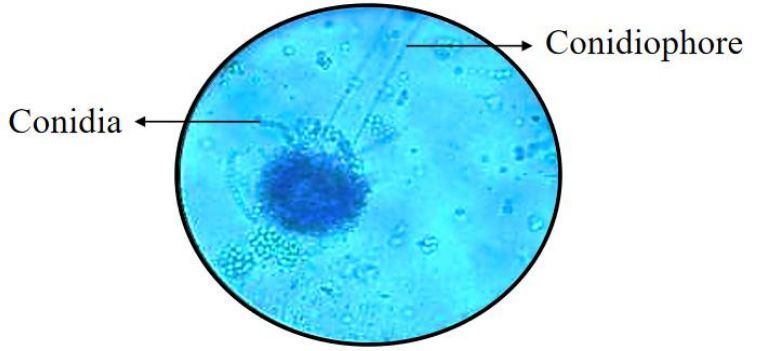
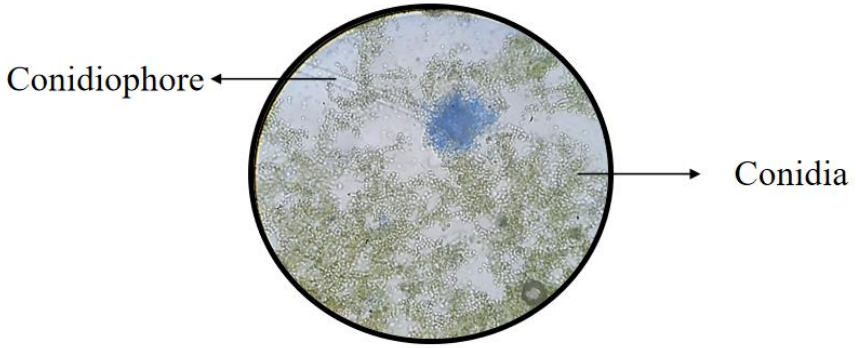
Name	Colony	Microscopic features
F1E		
F2F		

Fig 6.10: Microscopy of the fungus colonies (F1E and F2F)

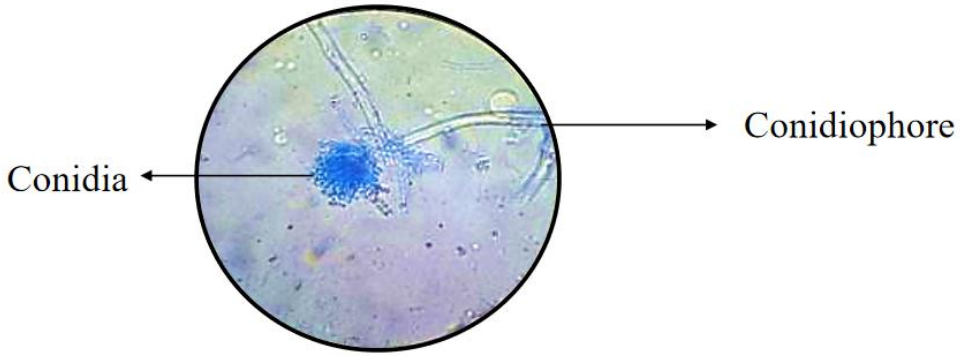
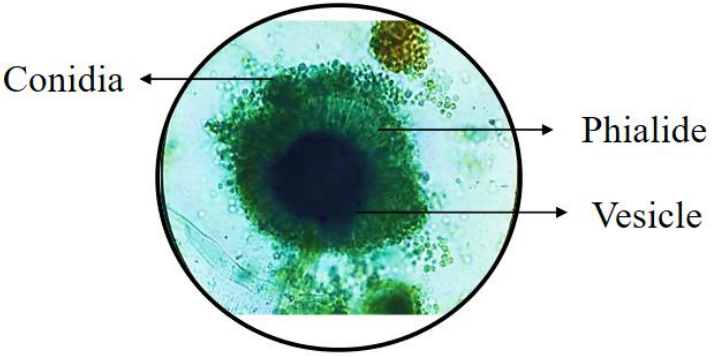
Name	Colony	Microscopic features
F2G		
F3A		

Fig 6.11: Microscopy of the fungus colonies (F2G and F3A)

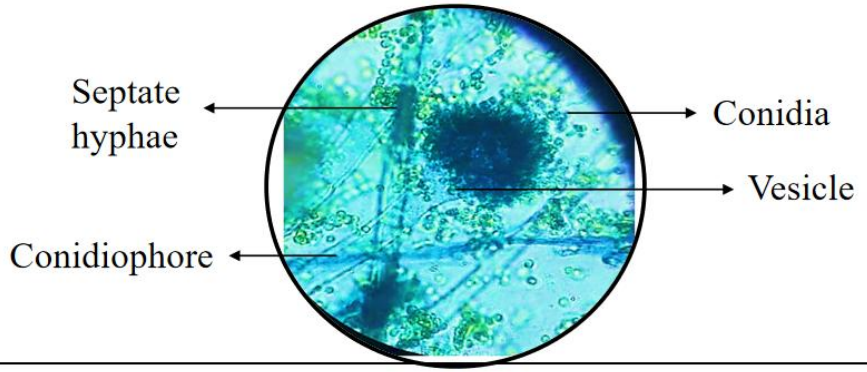
Name	Colony	Microscopic features
C1		

Fig 6.12: Microscopy of the fungus colonies (C1)

6.2.2 Characterization of synthesized silver nanoparticles

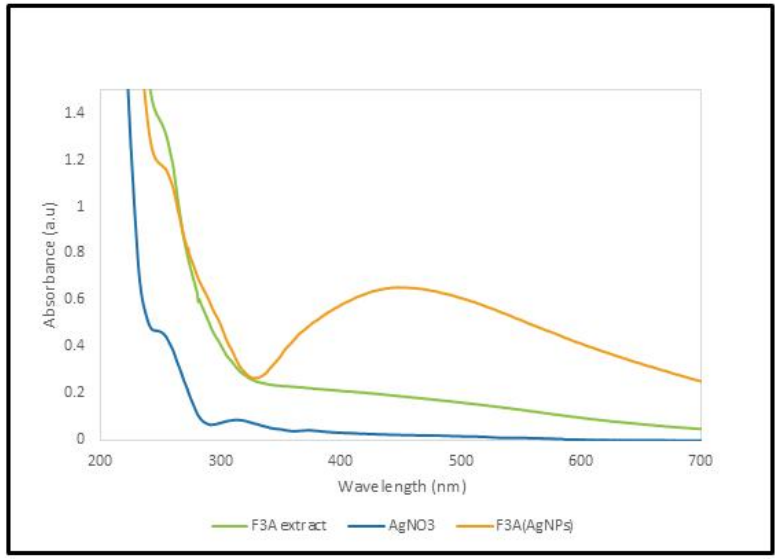
The solutions which were able to show color change after 72 hours underwent characterization techniques to assess their morphological, topographical and functional analysis.

UV-Vis spectroscopy

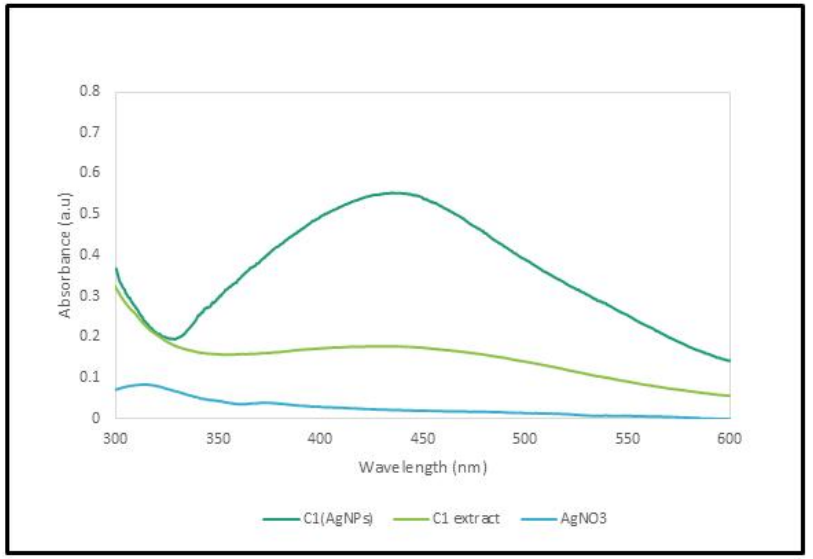
Solutions that were synthesized using F1E, F2F, F2G, F3A and C1 colonies were subjected to UV-vis spectroscopy. The plasma resonance beaks were only observed at 420, 434 nm with respect to C1(AgNPs) and F3A(AgNPs). Rest of the aqueous extracts did not shown any noticeable response to the UV-Vis spectra. Figure 6.13 depicts the UV-Vis absorption spectra recorded for the reaction medium, along with the positive and negative control. Only 2 solutions C1(AgNPs) and F3A(AgNPs) that were synthesized by C1 and F3A colony showed the absorption spectra indicated the peaks from 420 - 440 nm. A similar color inference was observed in the study of Bhainsa & D'Souza (2006) which investigated the silver nanoparticle synthesis using *Aspergillus* extract. After 72 hours of incubation, it was confirmed that this color change is associated with the development of a peak at 420nm in the UV-Vis spectrum.

FTIR analysis

The silver nanoparticles solution C1(AgNPs) and F3A(AgNPs) that showed absorption spectra ranging between 500cm^{-1} - 4000cm^{-1} was used in further characterization techniques. FTIR analysis suggests a potential role of enzymes present in the fungi for the biological synthesis. These enzymes could act as capping agents, helping to reduce metal ions and stabilize the nanoparticles. FTIR spectroscopic analysis of C1(AgNPs) exhibited characteristic absorption bands at 3285 cm^{-1}, 2922cm^{-1}, 1634 cm^{-1}, 1318 cm^{-1} and 1030 cm^{-1} suggesting the presence of specific functional groups like amides, methylene group, aromatic and aliphatic amines that acted as a reducing and stabilizing agents for nanoparticles. Likewise FTIR spectrum of F3A(AgNPs) demonstrated distinct peaks at 3281 cm^{-1}, 2881 cm^{-1}, 1592 cm^{-1}, 1403 cm^{-1} and 1028 cm^{-1} confirming the presence of proteins, alkanes, and amines (Fig 6.14). These distinctive bands of light absorption were identified as a result of the vibrational motions of chemical bonds linked to functional groups derived from fungi. The findings was in concurrence with other studies that confirmed functional groups present in the fungal filtrate helped in the bio-reduction and stabilization of AgNPs (Chandankere et al., 2020; Elshafei et al., 2021).

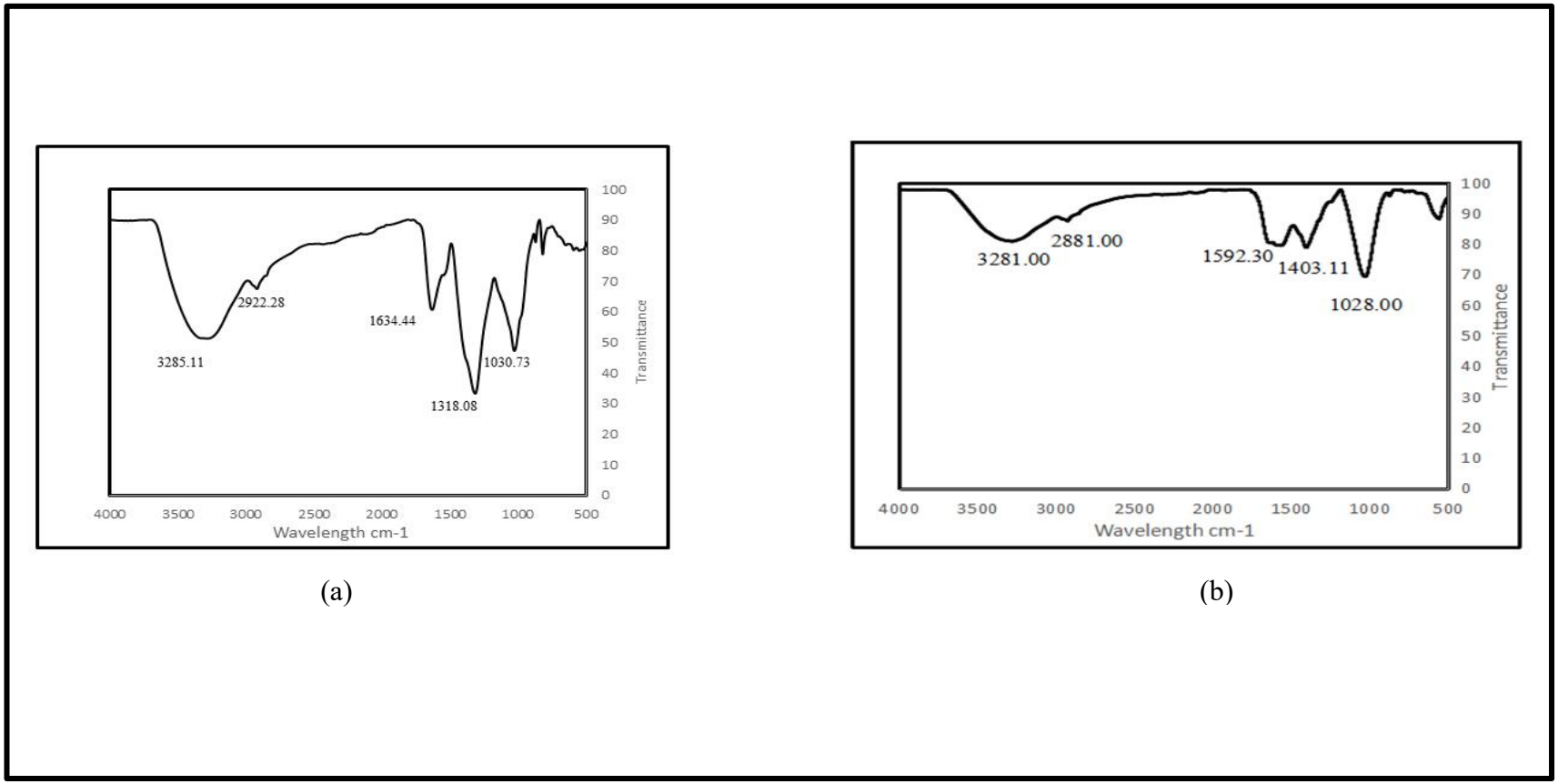


(a)



(b)

Fig 6.13: (a) UV-Vis spectra of C1 extract, C1 *(*AgNPs)and $AgNO_3$ (b) UV-Vis spectra of F3A extract, F3A *(*AgNPs) and $AgNO_3$



(a) (b)

Fig 6.14: (a) FTIR spectra of F3A(AgNPs) nanoparticles solution (b) FTIR spectra of C1(AgNPs) nanoparticles solution

SEM-EDS analysis

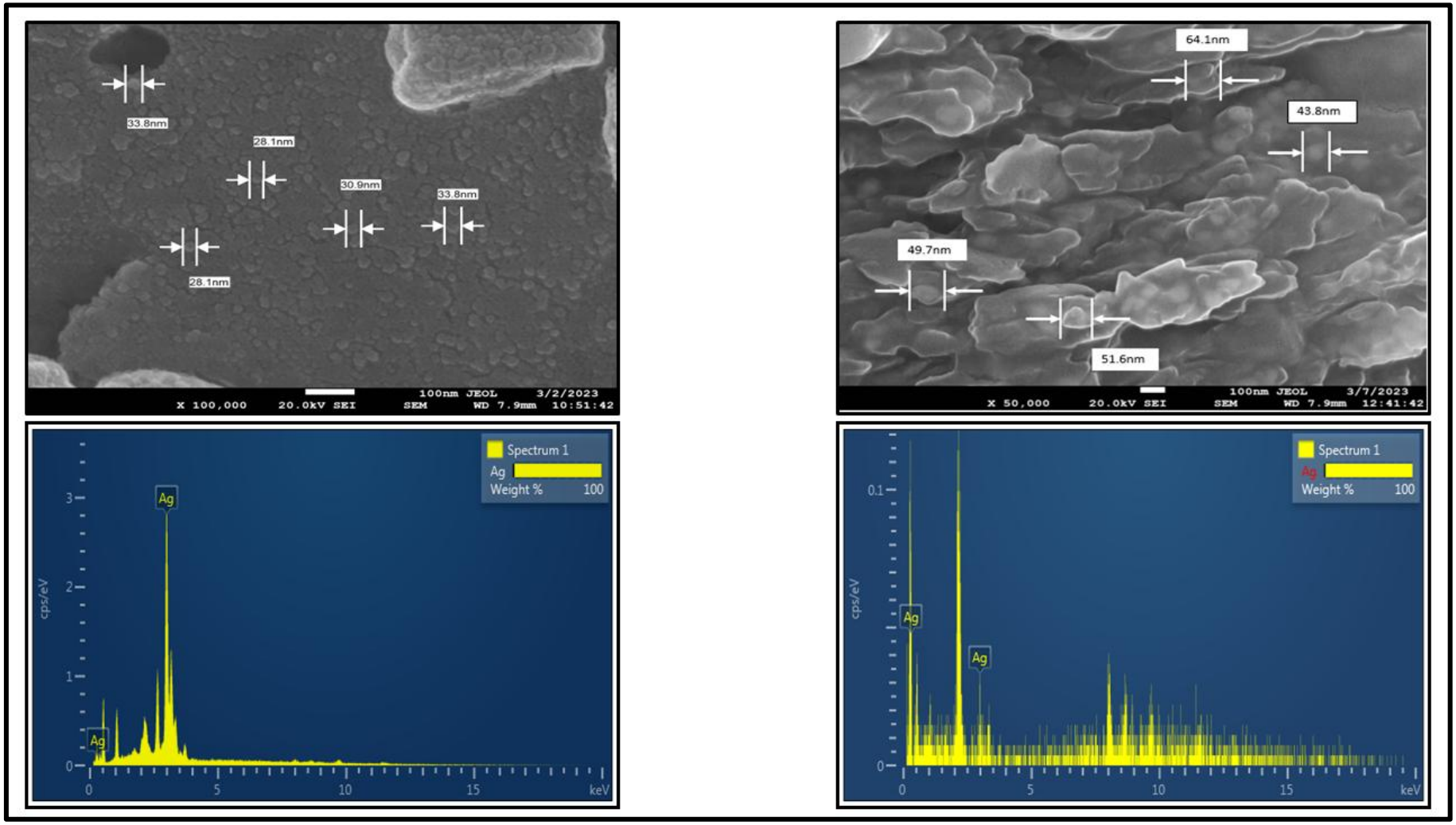
In order to investigate the morphology of the synthesized AgNPs, SEM images were recorded for both the samples. Figure 6.15 shows an enlarged view of the synthesized silver nanoparticles. The average particle size of silver nanoparticles produced by C1 were smaller (30nm) in size in comparision with those produced by F3A (64nm). Zomorodian et al. (2016) conducted a study investigating the extracellular biosynthesis of silver nanoparticles utilizing four diverse *Aspergillus* species*: A. fumigatus, A. niger, A. flavus,* and *A. clavatus*. It was confirmed that the particle sizes of nanoparticles synthesized by the examined fungus differed significantly. *A. fumigatus* produced smaller sliver nanoparticles (mean = 49nm) with stronger mono dispersity. The EDS analysis of synthesized silver nanoparticles validated the presence of pure silver at 3 KeV (Fig 6.13). Similar EDS pattern has been obtained in the study of Tomah et al., (2020) which involved the synthesis of AgNPs through different isolates of *Trichoderma*.

XRD analysis

Figure 6.16 shows the XRD pattern of C1(AgNPs) and F3A(AgNPs). The XRD analysis revealed the diffraction peaks at 2θ angles of 20° to 80°. The XRD analysis of biosynthesized AgNPs showed the diffraction peaks at 2θ angles of 38.2°, 44.4°, 64.6°, 77.8°. The obtained result confirmed that the nature of synthesized silver nanoparticles was crystalline. Similar results were observed in the research work of Ninganagouda et al. (2014), in which *A. niger* synthesized silver nanoparticles showed the presence of sharp diffraction peaks at 111, 200, 220 and 310 planes at 2 theta angle of 20° to 80° thereby confirming their crystalline property.

Zeta Potential

The Zeta potential of C1(AgNPs) and F3A(AgNPs) was found to be -18.3mV and -14.6mV (Fig 6.17). The stability of colloidal dispersions can be correlated with the value of the zeta potential, which measures the strength of attraction between neighboring, similarly charged particles in a dispersion. The inherent poly-dispersity of AgNPs is attributed to their strong negative zeta potential, which generates electrostatic repulsion between the particles, preventing aggregation and enhancing their long-term stability (Elamawi et al., 2018; Phanjom & Ahmed, 2015).



(a) (b)

Fig 6.15: (a) SEM-EDS analysis of F3A(AgNPs) (b) SEM-EDS analysis of C1(AgNPs)

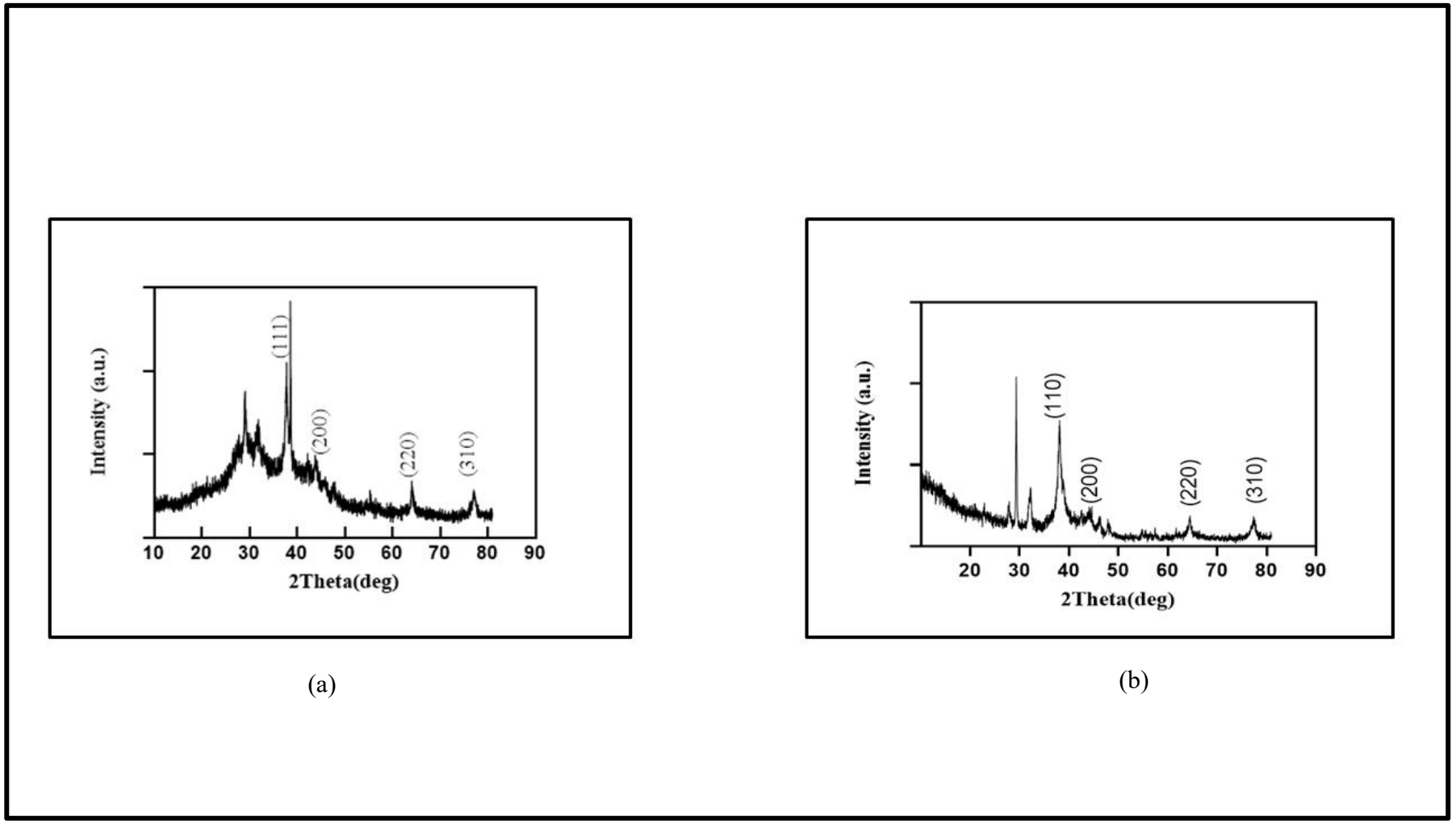


Fig 6.16: (a) XRD pattern spectra of F3A(AgNPs) (b) XRD pattern spectra of C1(AgNPs)

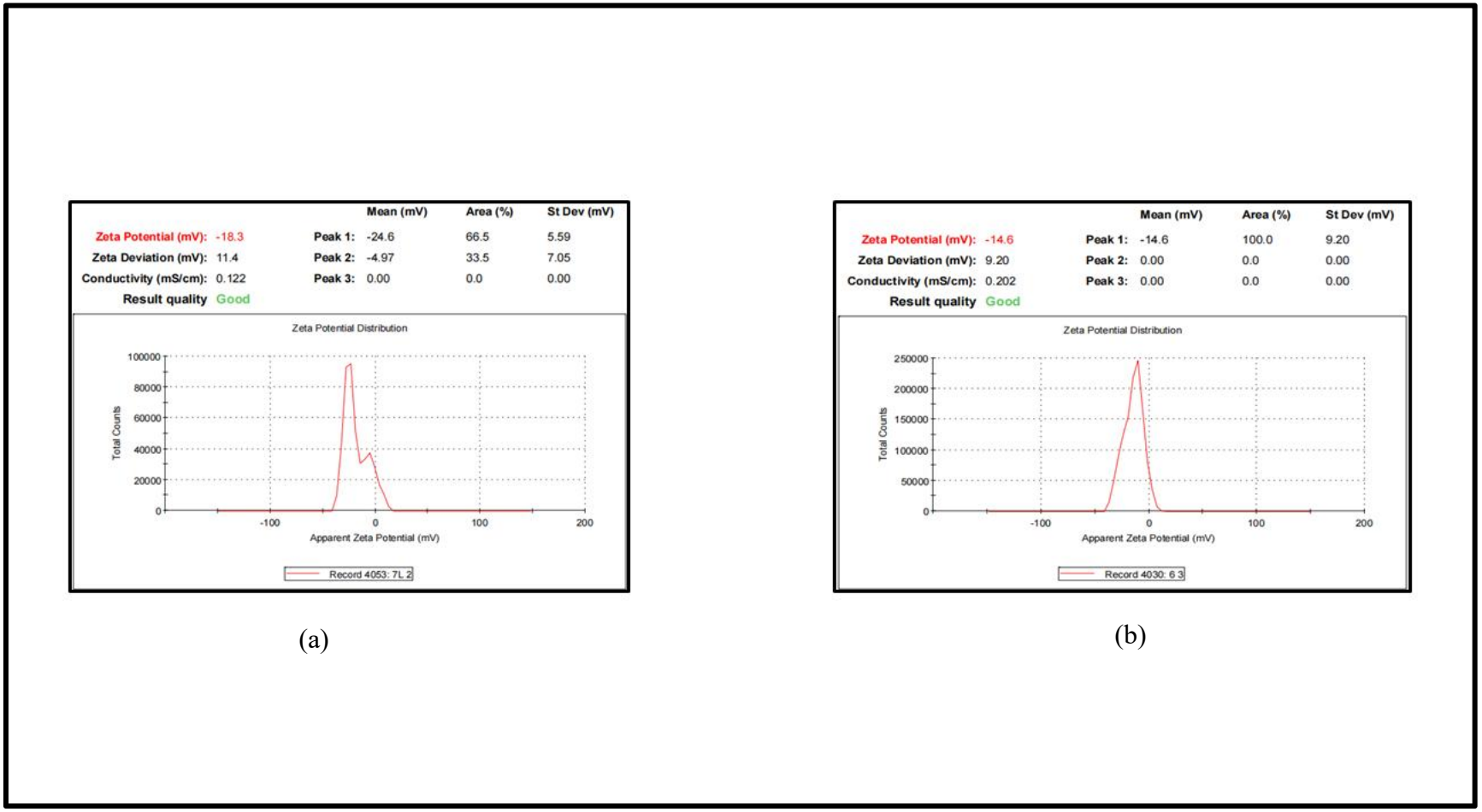


Fig 6.17: (a) Zeta potential of C1(AgNPs) (b) Zeta potential of F3A(AgNPs)

6.2.3 Laboratory evaluation of mycological synthesized AgNPs against *Aedes aegypti*

6.2.3.1 Larvicidal bioassay

The larvicidal efficacy of C1(AgNPs) and F3A(AgNPs) was assessed in third-instar larvae of *A. aegypti* under laboratory conditions. The mosquito larvae were exposed to various test concentrations and a control to determine the activity range of the compounds under test. To determine LC50 and LC90 values, a narrow range of 4-5 concentrations yielding 10% to 95% death in 48hrs was utilized after confirming larvae mortality throughout a wider range.

Third instar larvae of Dengue vector mosquitoes, *A. aegypti* was subjected to larvicidal bioassay in a range of concentrations of 1 ppm to 5ppm. The percentage mortality observed after treatment of C1(AgNPs) was 31.2% after 48 hrs at the concentration of 1 ppm. There was an increase in the mortality rate at the higher concentration of 2ppm, 3ppm an 4ppm which was 48%, 71% and 86%. The highest mortality of 96.8% was observed at the concentration of 5ppm. On contrary to this F3A(AgNPs) treatment showed lesser mortality in the larvae. Mortality percentage observed was 19.2%, 34.4%, 44%, 46.4% and 58.4% at the concentration range from 1ppm to 5ppm. It has been observed that AgNPs synthesized using different fungus *Chrysosporium keratinophilum*, *Verticillium lecanii*, and *Fusarium oxysporum* posses larvicidal activity against *Culex quinquefasciatus.* Results confirmed that silver nanoparticles produced by the fungus *F. oxysporum* caused moderate toxic effect with the LC50 value of 0.1 µl/cm^2. However, *C. keratinophilum* (AgNPs) and *V. lecanii* (AgNPs) were much more toxic due to higher percent mortality of 90 % within 22 hours of treatment. The LC50 value was found to be 0.4 µl/cm^2 (Soni & Prakash, 2012).

Probit analysis was used to determine the LC50 value for C1(AgNPs) and F3A(AgNPs) third-instar larvae of *Aedes aegypti* (Table 6.1). A concentration-dependent response was observed, with AgNPs ranging from 1 to 5 ppm. Results indicated significantly higher susceptibility of *A. aegypti* larvae to C1 (AgNPs), with mortality exceeding 90% at the highest concentration (Fig 6.18). Based on these preliminary findings, C1(AgNPs) was selected for further research.

The larvicidal potential of C1(AgNPs), C1 extract, and $AgNO_3$ was also evaluated against third-instar *Aedes aegypti* larvae (Fig 6.19). Concentration-dependent mortality rates were observed for all treatments, ranging from 1 to 5 ppm. C1 extract exhibited moderate larvicidal activity, with a maximum mortality of 52.8% at 5 ppm after 48 hours. $AgNO_3$ demonstrated 81.6% larval mortality at the highest concentration within 48 hours. In contrast, C1(AgNPs), exhibited superior larvicidal properties, resulting in 73.6% and 96.8% mortality at 5 ppm after 24 and 48 hours, respectively. AgNPs demonstrated efficacy in controlling *Aedes* larvae populations at relatively low dosages. Similarly, AgNPs synthesized using *Metarhizium anisopliae* exhibited significant toxicity towards *Anopheles culicifacies,* a primary Malaria vector in rural areas. Notably, a mortality rate exceeding 90% was observed at an AgNP concentration of 70 ppm (Amerasan et al., 2016). *Trichoderma harzianum* mediated AgNPs were found to be exhibit larvicidal and pupicidal properties

against Dengue vector. The highest mortality of 100 percent was observed within 24 hrs of exposure to the AgNPs (Sundaravadivelan & Padmanabhan, 2014).

Statistical analysis confirmed a significant dose-dependent increase in larval mortality following C1(AgNPs) exposure. The calculated LC50 and LC90 values for C1(AgNPs), were 1.719 ppm and 4.728 ppm, respectively (Table 6.2). Whereas the LC50 and LC90 values for C1 extract and $AgNO_3$ were recorded to be 5.162 ppm (36.758 ppm) and 2.221 ppm (9.196 ppm). The results of upper confidential level (UCL), lower confidential level (LCL) values are mentioned in Table 6.2. It was observed that the larvicidal efficacy of C1(AgNPs) increased with prolonged exposure time. Similarly the AgNPs synthesized using *Eupatorium odoratum* were found to be toxic against *Culex* larvae with the lower LC50 value of 90.9 ppm after 24hrs hrs of treatment. On contrary to this the value of LC50 value of *Eupatorium odoratum* extract was found to be higher with the value of 996.8 ppm, thereby confirming that silver nanoparticles are more effective that plant extract (Elemike et al., 2017). The fourth instar larvae of the three mosquito species tested were highly susceptible to *Leucas aspera* AgNPs. The concentration which caused 50% and 90% mortality in fourth instar larvae of *A. stephensi* after 24 h post treatment was 26.08; 147.6 mg/L and 17.16; 101.42 mg/L against *A. aegypti* and 33.68; 207 mg/L against *C. quinquefasciatus* was observed, while LC50 and LC90 values of synthesized AgNPs treated against fourth instar larvae of *A. aegypti* was 4.02; 11.22 mg/L against *A. stephensi* was 4.69; 12.09 mg/L and *C. quinquefasciatus* was 5.06; 12.74 mg/L. Therefore it was confirmed green synthesized AgNPs of *L. aspera* showed high toxicity against the treated larvae at very low concentrations (Elumalai et al., 2017).

Test solution	Exposure period	Concentration (ppm)	Percent mortality ± Standard error
C1(AgNPs)	48hours	1ppm	31.2±0.8
		2ppm	48.0±0.9
		3ppm	71.2±0.6
		4ppm	86.4±0.6
		5ppm	96.8±0.5
F3A(AgNPs)	48hours	1ppm	19.2±0.4
		2ppm	23.2±0.6
		3ppm	27.2±0.2
		4ppm	34.4±0.4
		5ppm	35.2±0.4

Table 6.1: Percent mortality observed in *Aedes* larvae after treatment with C1(AgNPs), and F3A(AgNPs)

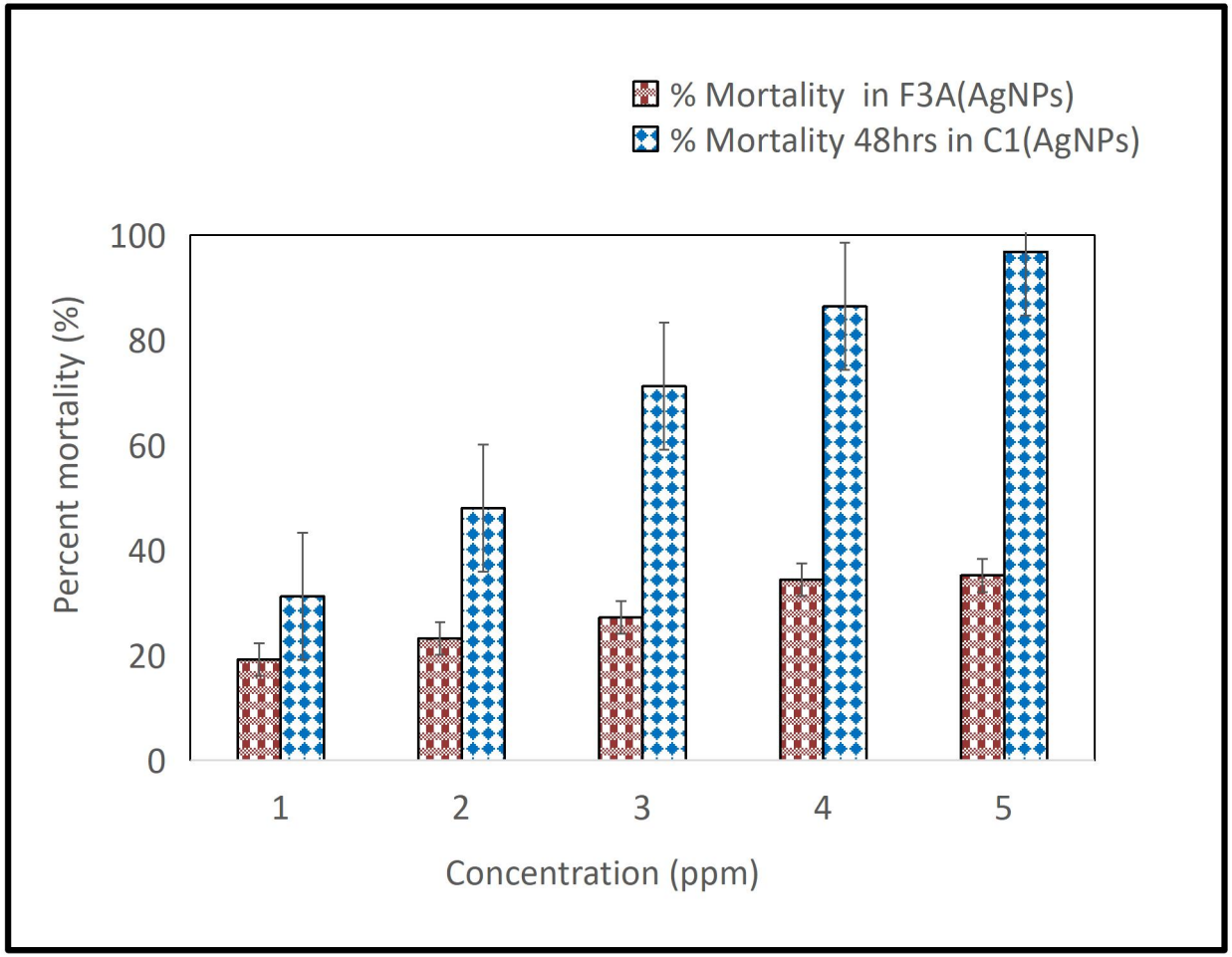


Fig 6.18: (a) Mortality percentage of C1(AgNPs) and F3A(AgNPs) in *Aedes aegyptii* larvae

Table 6.2. The toxicity of C1-AgNPs, C1 extract and $AgNO_3$ against 3rd instar larvae of *Aedes aegypti.*

Treatment	LC_{50} (LC_{90}) (24hrs)	95% Confidence limit LC50 (LC90)	LC_{50} (LC_{90}) (48hrs)	95% Confidence limit LC50 (LC90)
C1AgNPs	2.625ppm (12.068ppm)	LCL 1.907 (6.956) UCL 3.577 (57.190)	1.719ppm(4.728ppm)	LCL 1.280(3.661) UCL 2.104(7.539)
$AgNO_3$	4.665ppm(25.097ppm)	LCL 3.378 (10.833) UCL 10.777 (563.682)	2.221ppm(9.196ppm)	LCL 1.573(5.825) UCL 2.884(29.294)
C1 Extract	5.583ppm(21.855ppm)	LCL 4.100 (10.521) UCL 12.899 (258.058)	5.162ppm(36.758ppm)	LCL 3.511(12.647) UCL 20.332 (6983.409)

LC50 and LC90 are the concentration of C1Ag-NPs, $AgNO_3$ and C1 extract killed 50% and 90% of individuals, respectively. LCL and UCL are lower confidence and upper confidence limits, respectively.

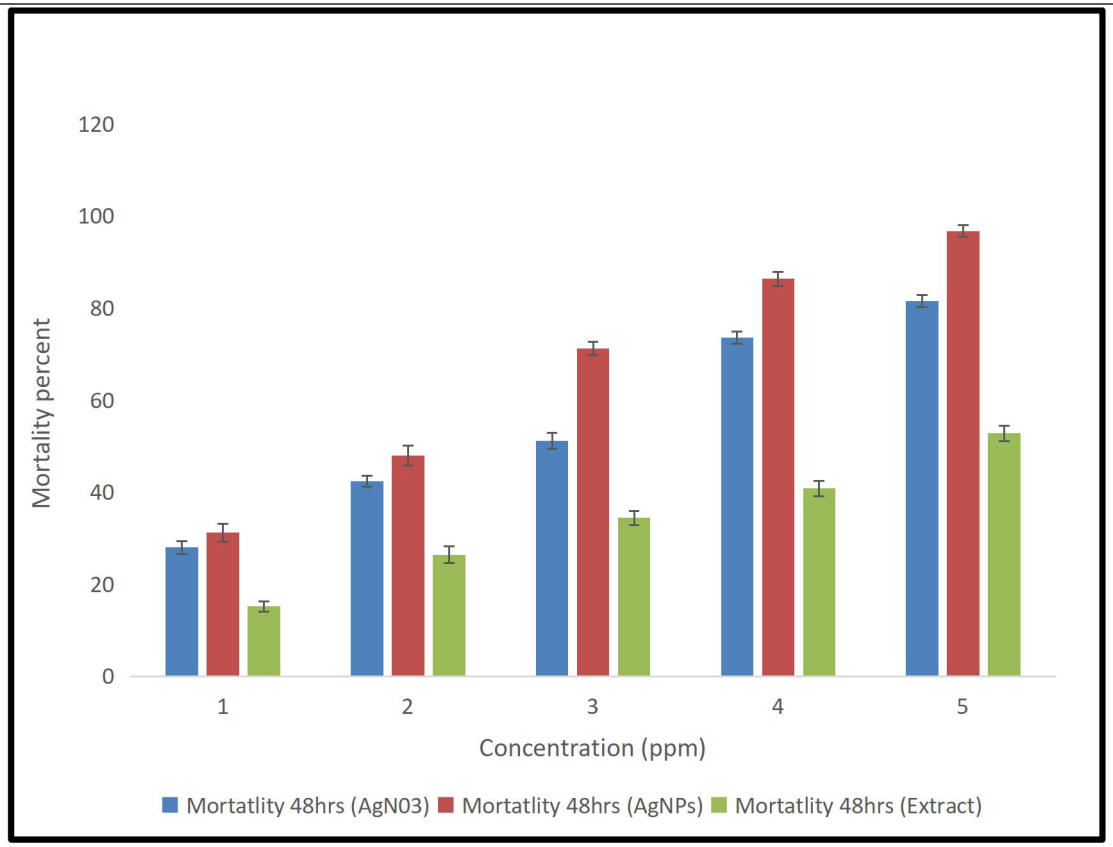


Fig 6.19: Percent mortality in *Aedes* larvae after treatment with C1-AgNPs, C1extract, $AgNO_3$ in 48 h

The larvae treated with C1(AgNPs) showed behavioral changes such as forceful self-biting compared to the other treatment groups. Within 24 hrs of exposure, self-biting of anal papillae was observed that led to the development of a circular body structure (Fig 6.20). Sterio-microscopic studies revealed loss of external hairs and damage in the epithelial layer after treatment of $AgNO_3$ and C1 extract. Similar effects were observed with C1(AgNPs) but damage was more pronounced. A complete breakdown of the midgut region was observed along with the collapse of the larval structure. In addition, increased blackening of the gut along with the shrinkage of the anal papillae was also observed (Fig 6.21). During the treatment period the larvae also suffered deformities and were not able to reach other developmental stages of their life cycle. It was also observed that the application of C1(AgNPs) extended the larval stage in a few larvae at a higher concentration in comparison to the control. These treated larvae failed to go through normal development and finally died.

Silver nanoparticles significantly influence the antioxidant and detoxifying enzymes of insects, leading to oxidative stress and cell death. AgNPs disrupt vital insect genes, leading to decreased protein synthesis and gonadotropin release, ultimately resulting in developmental abnormalities and reproductive dysfunction. The toxicity of nanoparticles is caused by their adhesion to the insect cuticle, leading to the physico-sorption of waxes and lipids causing insect dehydration (Kapil Singh et al., 2013). It is confirmed that biosynthesized AgNPs cause neurobehavioural toxicity in mosquito larvae. In the study of Benelli (2018) and Soni & Prakash (2015) fungal metabolites of *Penicillium* and *Aspergillus* were highly toxic to the *Aedes* larvae which led to forceful self-biting of mouth parts and papillae that eventually caused damage in the cuticle layer. The 2 main neurohormones responsible for insect metamorphosis are 20-hydroxyecdysone and juvenile hormone. The molting process is initiated with the release of Prototho racicotropic hormone (PTTH) by the neurosecretory cells of the brain. The PTTH hormone induces the release of ecydsone which is later modified into active molting hormone 20-hydroxyecdysone. Whereas juvenile hormone released from the secretory cells of corpus allata is only active during larval molts. In the presence of juvenile hormone, 20-hydroxyecdysone stimulates the formation of larvae instars. During the last larval instar coprus allata inhibits the synthesis of juvenile hormone and stimulates the prothoracic gland to release ecdysone. The hormone 20-hydroxyecdysone in the absence of juvenile hormone leads to pupal development by activating the pupa-specific genes (Ragavendran et al., 2019). Various research such as that of Gilbert (2000) have confirmed that dietary exposure to AgNPs interferes with the growth and molting process in larvae. AgNPs further contributed to the elongation in the larval stage leading to the delay in pupal formation. Finally, it was confirmed that AgNPs cause developmental toxicity in insect larvae by inhibiting the activity of hormones.

Fig 6.20: (a) Forceful self-biting observed in *Aedes* larvae after silver nanoparticles treatment (b) Control

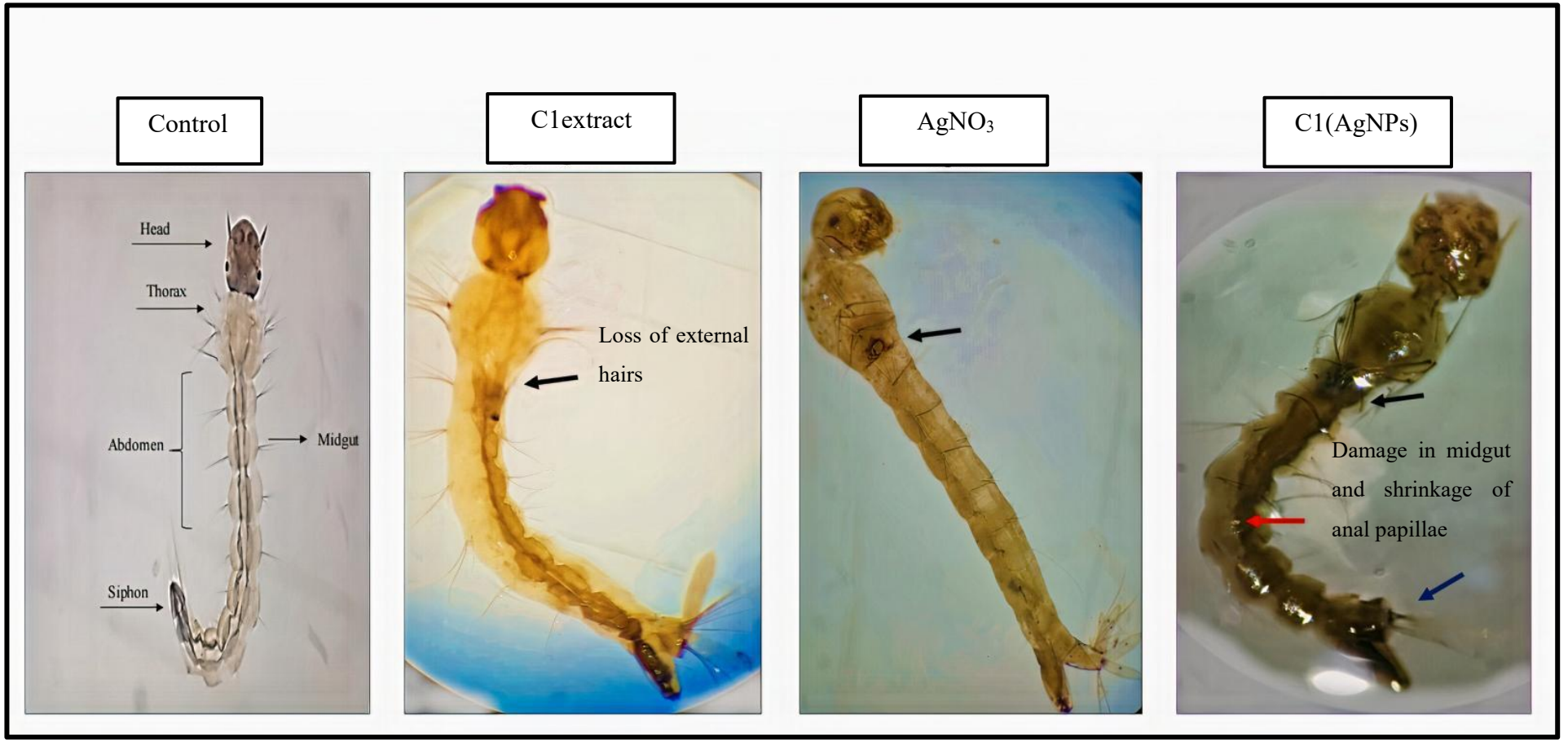


Fig 6.21: Microscopic study of *Aedes aegyptii* larvae treated with C1 extract, $AgNO_3$ and C1(AgNPs). Black, Red, and Blue arrows depicts the loss of external hairs, damage in the midgut and shrinkage of anal papillae

6.2.3.2 Histopathological analysis of Aedes larvae

Histopathology is an in vivo technique employed to examine morphological changes in tissues or organs following nanoparticle exposure. The process involves tissue collection from exposed animals, and fixating, embedding in paraffin, finally sectioning into thin slices using a microtome. Subsequent staining with hematoxylin and eosin (H&E) enhances tissue contrast, allowing for microscopic evaluation of cellular and tissue architecture. Histopathology remains a valuable tool for identifying morphological alterations in target organs such as the brain, eyes, lungs, liver, kidneys, spleen, and heart (Marquis et al., 2009).

Histopathology of *Aedes* larvae after treatment with biosynthesized AgNPs, $AgNO_3$ and fungal extract

Histopathological analysis of *Aedes aegypti* larvae exposed to C1(AgNPs), C1 extract, and $AgNO_3$ demonstrated significant morphological alterations. However the control group remain unchanged. The body of the *Aedes* larvae consists of the head, thorax and abdomen. Gastric caeca is present centrally as well as laterally in the body. The gastric caeca cells are large, slightly flattened, with clear edges. The epithelium is composed of a rectangular, uniform, single layer of cubical and cylindrical cells, highlighting the brush border along with the nucleus which is globular and centrally placed (Fig 6.22). Treatment groups exhibited pronounced damage. Exposure to C1 extract led to the damage of epithelial layer. The midgut region was affected severely. Undistinguished parts of gastric caeca were also observed. Damage in the the cuticle of hindgut was also prominent (Fig 6.23). The $AgNO_3$ treated larvae revealed that the midgut region was characterized by nuclear degeneration and loss of brush border integrity at the highest concentration of 5ppm. Third instar larvae exposed to $AgNO_3$ displayed disrupted epithelial cell layers and damaged midgut. Also severe lesions were seen in the midgut epithelium cells, including rupture of the epithelial cells, and broken membranes (Fig 6.24). Larvae treated with C1(AgNPs) suffered severe abdominal damage, including complete disintegration of the midgut and caeca. Additionally, a pronounced loss of head and body setae was observed in this group. Destruction of the epithelial cells with the degenration of nuclei was also observed in the gut region. Silver nanoparticles deposition was evident within the midgut region as seen in the figure 6.25.

These findings align with previous research which confirmed the toxicity of *Artemisia vulgaris*-synthesized AuNPs in *Aedes* larvae. The study reported histological alterations in the midgut, digestive tract, and larval integument. Exposure of *A. aegypti* to AuNPs at 400 ppm for 24 hours induced significant morphological changes in the midgut, epithelial cells, and integument of third and fourth instar larvae. AuNP accumulation was notably higher in the midgut compared to the other groups (Sundararajan & Ranjitha Kumari, 2017). The effect of *Pedalium murex* seed extract and *P. murex*-based silver nanoparticles (Pm-AgNPs) has also

been evaluated on *Aedes* larvae. Histopathological analysis revealed that fourth instar *Aedes aegypti* larvae exposed to *P. murex* seed extract exhibited loss of head and body setae. Pm-AgNP treatment resulted in epithelial layer disruption and damage to the outer cuticle. Histopathological images further demonstrated epithelial damage and cellular vacuolization in larvae exposed to *P. murex* seed extract. The hindgut epithelium was also affected, with compromised intercellular membranes but intact nuclei. Therefore it was confirmed that Pm-AgNPs had detrimental effects on the hindgut and musculature of larvae (Ishwarya et al., 2017).

The toxicity of *Euphorbia rothiana* leaf extract-synthesized silver nanoparticles (Er-AgNPs) was assessed against *Aedes* larvae. Larvae were exposed to $AgNO_3$, *E. rothiana* leaf extract, and Er-AgNPs. $AgNO_3$ treatment induced disintegration of the outer cuticle's epithelial layer and loss of external setae. Er-AgNP-treated larvae exhibited shrinkage and complete loss of external setae. Histological examination revealed disorganized and damaged epithelial cells in larvae exposed to *E. rothiana* extract and $AgNO_3$. In contrast, Er-AgNP-treated larvae suffered complete disintegration of the midgut and caeca, resulting in collapse of the larval body (Banumathi et al., 2017). Consistent with these findings, a recent study demonstrated that bacterially synthesized silver nanoparticles caused damage to larval epithelial cells, food bolus, basement membrane, musculature, and midgut. The severity of these injuries was directly correlated with nanoparticle concentration, with the larval gut lumen being the primary target of damage (Wilson et al., 2023). Histological analysis of *Ae. albopictus* larvae exposed to *Sonneratia caseolaris*-synthesized AgNPs for 24 hours revealed significant cell damage in epithelial and muscle layers compared to the normal appearance of head, thorax, and abdomen tissues in the control group. The treated larvae also showed swelling in organelles and a disrupted midgut with accumulated cellular components. This damage is likely due to AgNPs' ability to inhibit key enzymes, disrupting normal larval functions (Roni et al., 2024). Exposure of fourth instar larvae of *Aedes aegypti* to *Gracilaria acerosa*-synthesized AgNPs for 24 hours resulted in significant mortality. Histopathological analysis revealed notable tissue disruption in the treated larvae. Microscopic examination demonstrated significant histological aberrations, characterized by severe damage to the midgut epithelial cells, manifesting as cellular disorganization and compromised structural integrity, in contrast to the intact epithelium observed in control specimens. The primary target tissues were the alimentary canal and musculature, with notable lesions observed in the midgut and muscle fibers, underscoring the larvicidal mechanism of *G. acerosa*-derived AgNPs through the induction of severe tissue pathology in *Aedes aegypti* (Aravinth et al., 2025).

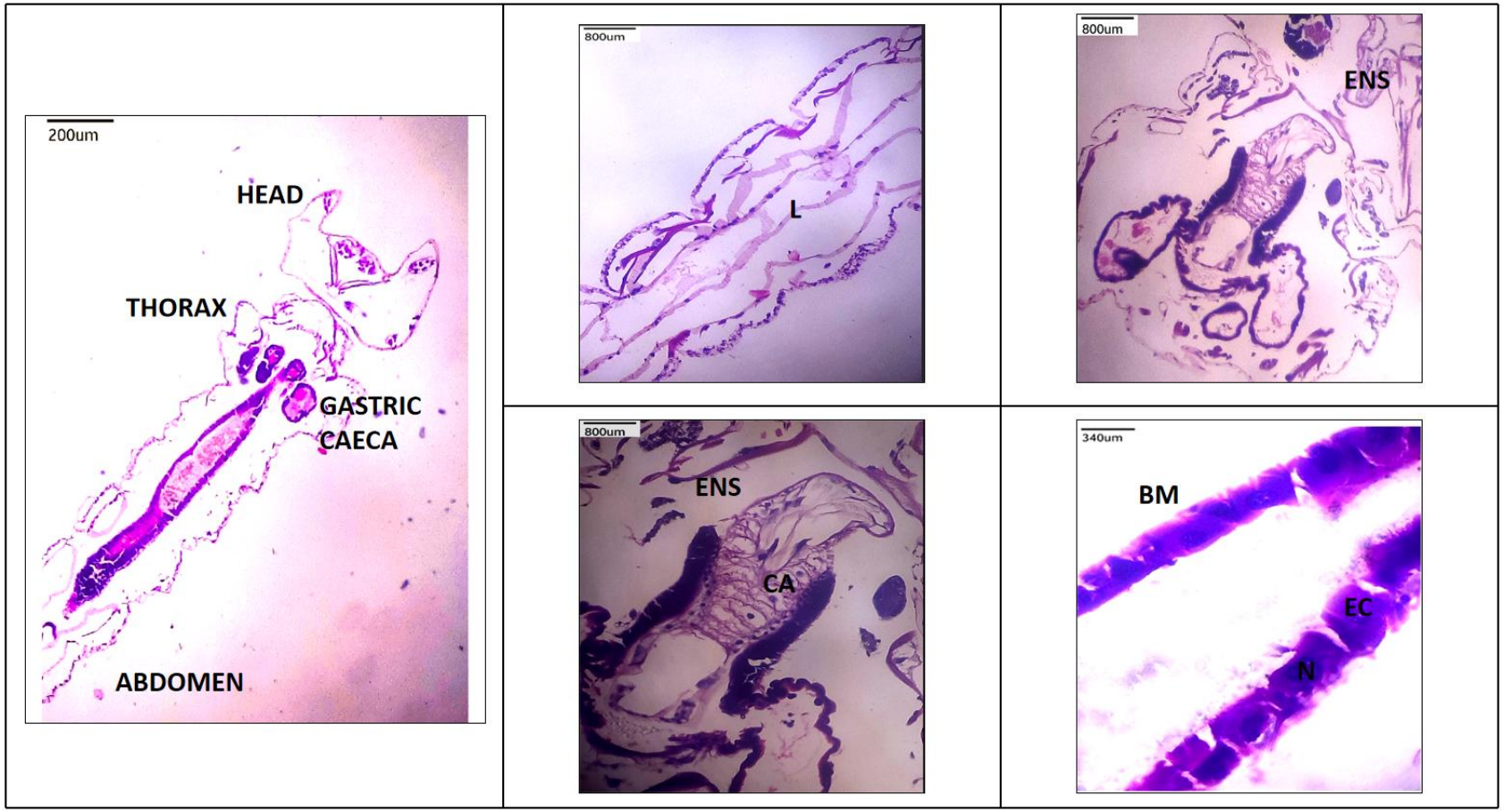


Fig 6.22:Stereo microscopic studies of third instar larvae of *Aedes aegypti* (Control). L, Lumen; ENS, Endoperithophic space; CA, Cardia cells; BM, Basement membrane; EC, Epithelial cells

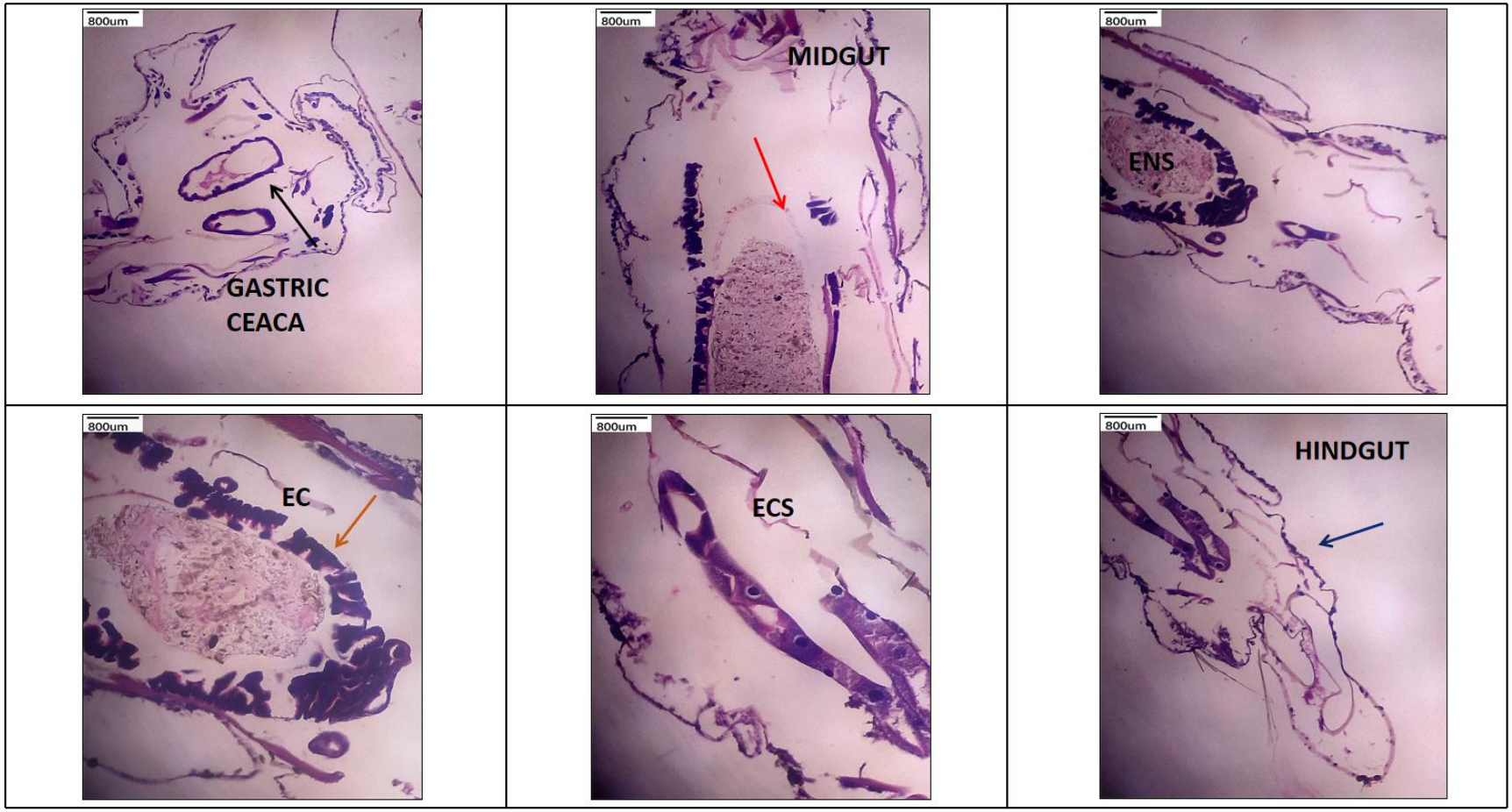


Fig 6.23: Stereo microscopic studies of third instar larvae of *Aedes aegypti* treated with C1 extract. *Black*, *red, brown* and *blue arrow* indicates the damaged gastric caeca, broken midgut, epithelial layer, and disordered hindgut respectively. ENS, Endoperithophic space; EC, Epithelial cells.

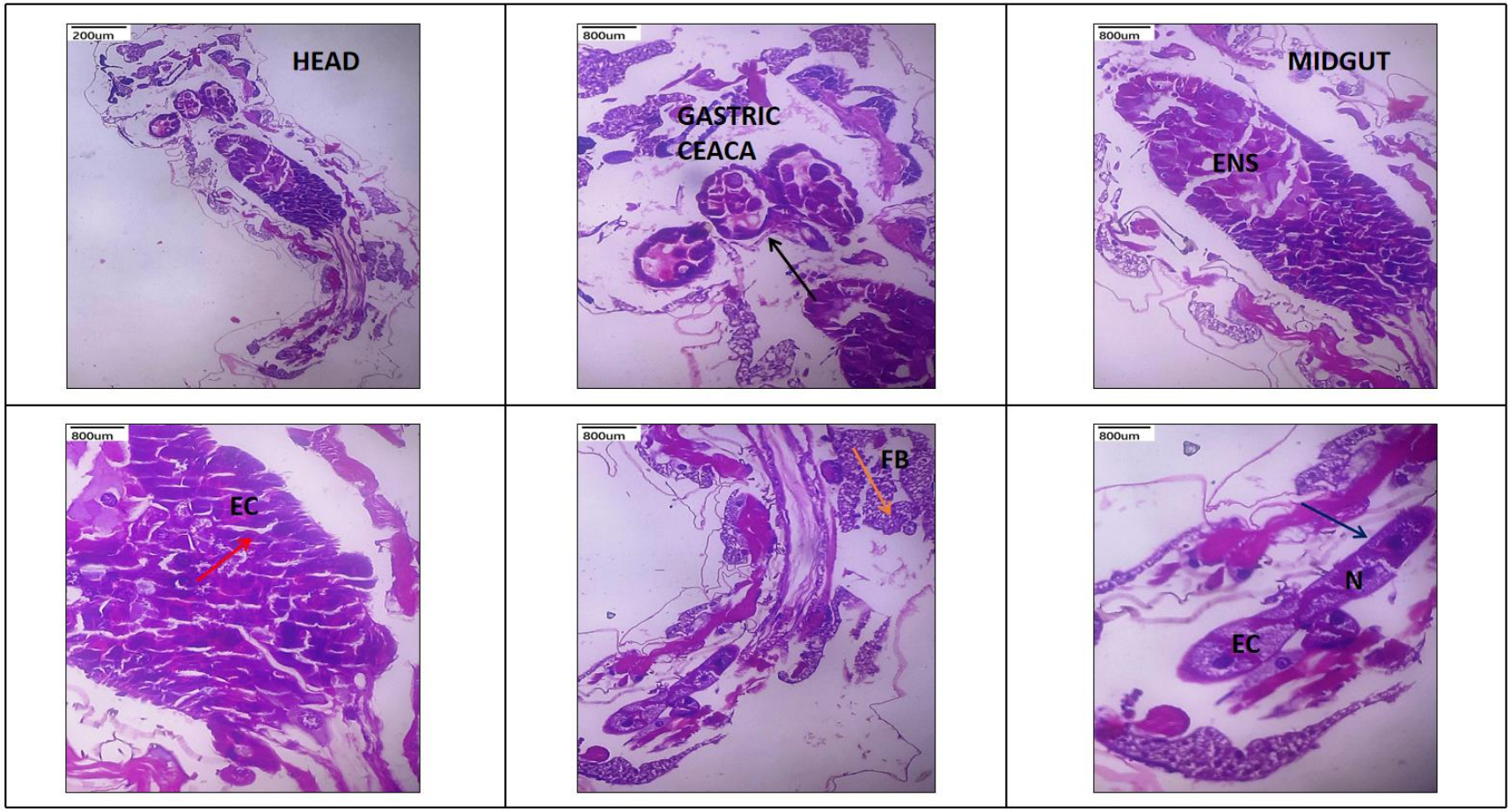


Fig 6.24:Stereo microscopic studies of third instar larvae of *Aedes aegypti* treated with $AgNO_3$. *Black*, *red, brown* and *blue arrow* indicates the damaged gastric caeca, degeneration of epithelial cells, disrupted fat body and nucleus damage respectively. ENS, Endoperithophic space; EC, Epithelial cells; FB, Fat body; N, Nucleus

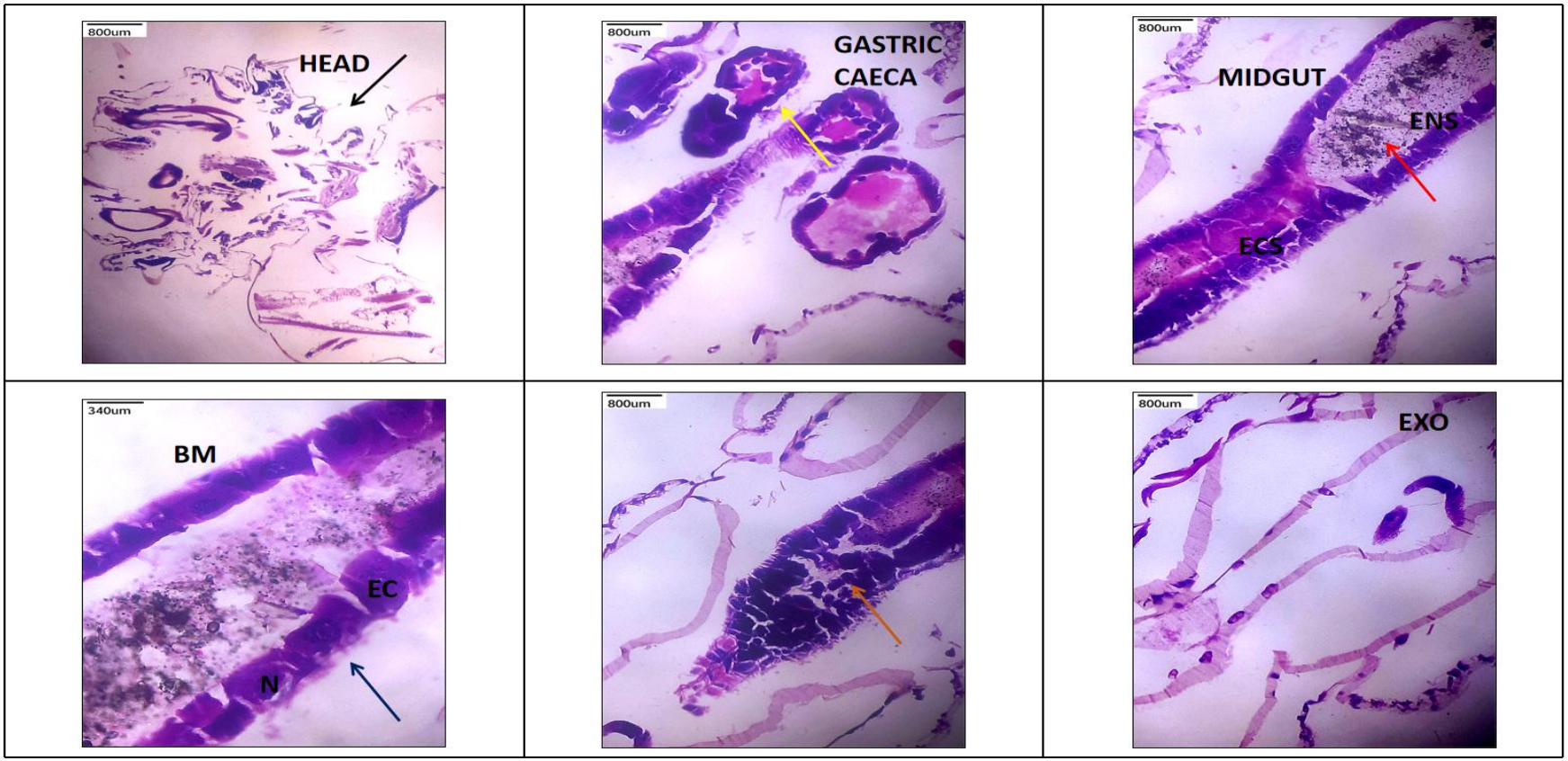


Fig 6.25:Stereo microscopic studies of third instar larvae of *Aedes aegypti* treated with C1 (AgNPs). *Black*, *yellow, red, blue and brown arrow* indicates the loss of head region, toxicity in gastric caeca, accumulation of AgNPs in gut, damaged epithelial layer, and collapse of midgut respectively. ENS, Endoperithophic space; EC, Epithelial cells, N, Nucleus; BM, Basement membrane; Exoskeleton; EXO

6.3 Objective 3:Toxicity impact of AgNPs on aquatic organism *Daphnia* and *Labeo rohita* (fingerlings)

6.3.1 Bio-efficacy of AgNPs against non-target organisms

6.3.1.1 Acute toxicity study of bio-synthesized AgNPs on Daphnia magna

In order to evaluate the biotoxicity of C1(AgNPs) on non target organisms acute toxicity studies were conducted. *D. magna*, a key zooplankton species, was collected from a pond near LPU campus. It was identified using an image-based identification key of Ebert (2005). The culture was maintained in the laboratory of 56 block LPU. The *Daphnia* species is characterized by a distinct rostrum, lack of cervical sinus, and a carapace extending from the head shield to the sinuate post abdomen. Its unique carapace, which forms a continuous strip with the head shield, readily differentiates it from other *Daphnia* species (Fig 6.24). As a sensitive filter feeder, *Daphnia* plays a crucial role in aquatic ecosystems. These small crustaceans, often referred to as water fleas, are a primary food source for aquatic predators. Their short lifespan and susceptibility to various toxins and heavy metals make them valuable indicators of water quality (Tatarazako & Oda, 2007). Due to these characteristics, *Daphnia* is widely used in toxicity testing, such as the OECD guideline 202, which recommends 48-hour acute toxicity tests on neonates.

Bioassay are toxicity tests used to determine if a chemical or its residue poses a sufficient environmental threat to harm animals. These tests are categorized into acute and chronic types. Acute toxicity tests are rapid, inexpensive, and provide preliminary toxicity assessments or comparative species sensitivity data. Chronic toxicity studies are more complex and time-consuming. While many acute bioassays focus on animals, they also employ a diverse range of organisms, including algae, daphnia, fish, bacteria, and even cell cultures. Non-mammalian species are particularly valuable for acute toxicity studies due to their short lifespans, and rapid environmental responses (Lomba et al., 2020).

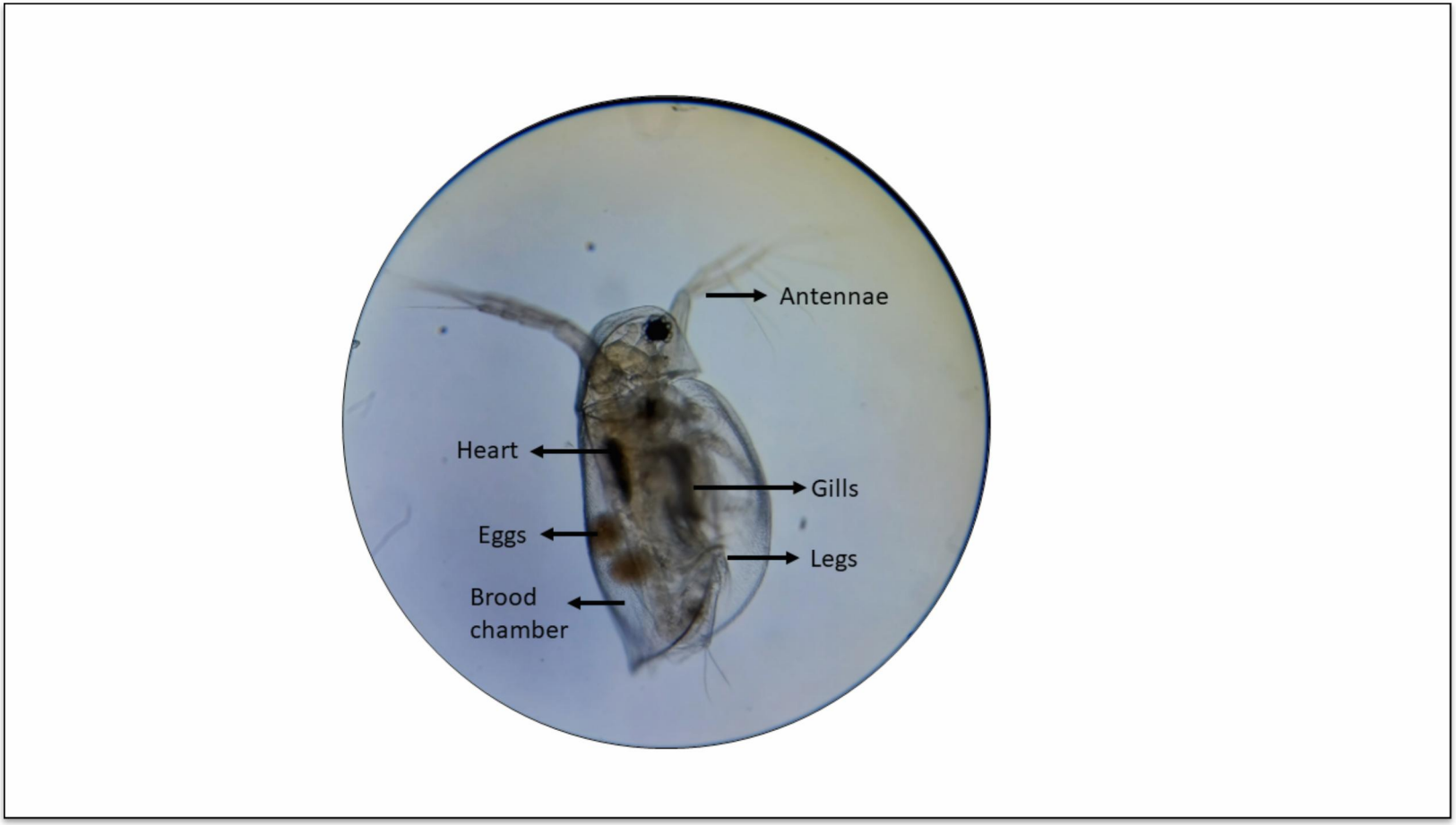


Fig 6.26 : Microscopic image of *Daphnia magna* at 40X

An acute toxicity assessment of C1(AgNPs), C1(AgNPs), and $AgNO_3$ was conducted on *D. magna* following protocol OECD (2004) with minor modification. The *Daphnia* culture used in this research were from a laboratory-maintained colony established two months prior to the study. *D. magna* were exposed to the same concentration range of 1ppm to 5ppm as was used for *Aedes* larvae. The percent mortality of *Daphnia* after the acute toxicity study of 48 hrs was found to be least in C1(AgNPs) treatment. At the lowest concentration of 1 ppm no mortality was seen. On increase in the concentration to 4 ppm the mortality slightly increased to 2% which remained constant till the highest concentration of 5 ppm. In contrast to this $AgNO_3$ demonstrated significant toxicity at higher concentrations (5 ppm), causing 80% mortality. Table 6.3 highlights the percent mortality observed in *Daphnia magna* after being exposed to treatment of C1(AgNPs), C1 extract and $AgNO_3$ for 48 hrs. Studies have shown that the exposure to silver nitrate have caused 100% mortality when *Daphnia magna* were exposed to the concentration of 0.006, 0.00325, 0.275, and 0.0032 mg/L (Asghari et al., 2012b). *Daphnia* were exposed to AgNPs where a sublethal doses of 1 μg/L for 21 days was used. It was observed that beginning from day 13, least mortality of 20% was recorded which remained constant until the end of the test for AgNPs exposure (Falanga et al., 2020).

The survival percentage recorded in C1(AgNPs) treatment was 100% after 48 hours of study. At the concentration of 4ppm and 5ppm survival was maintained above 95% which remain consistent throughout the treatment period of 48hrs. Similar to this result, 98% survival in *Daphnia* was recorded in C1 extract. While it was observed that $AgNO_3$ showed least survival of 68% at 1 ppm concentration which drastically decreased to 20% at the highest concentration of 5ppm (Fig 6.27). It is confirmed that during the waterborne $AgNO_3$, the survival of daphnids decreased dramatically from 100% to 30% over a narrow concentration range of 1.5 to 3mg/L. However, for waterborne AgNP, no mortality was observed up to 500mg/L AgNP and all daphnids were alive and appeared normal (Zhao & Wang, 2011b) .

$AgNO_3$ exhibited a high level of toxicity, with a LC50 value of 2.156 ppm, the C1(AgNPs) demonstrated negligible toxicity, with an LC50 of 16.568ppm. The LC50 values recorded during the treatment with C1 extract was 15.213 ppm (Table 6.3). No mortality was observed in the control group. Acute toxicity of $AgNO_3$ is attributed to ionic Ag^+ which leds to the disruption of the Na, K -ATPase located at the basolateral membrane in *Daphnia.* Silver exposure resulted in significant alterations to physiological and biochemical parameters. Whole body sodium concentration was markedly depleted, exhibiting a 65% reduction. Despite a 60% upregulation of Na+,K+-ATPase activity, a substantial 81% inhibition of unidirectional sodium influx was observed, indicating a severe disruption of sodium homeostasis (Bianchini & Wood, 2002).The findings of the current research aligns with previous research highlighting the lower toxicity of green-synthesized silver nanoparticles compared to silver nitrate. To further emphasize this point, study by (Khoshnamvand et al., 2020) revealed a similar result with *Alcea rosea*-based AgNPs. These biogenic AgNPs displayed

significantly higher tolerance limits for *Daphnia* than $AgNO_3$, as evidenced by their 48-hour LC50 values of 1.86 ± 0.12 µg/L and 1.30 ± 0.07 µg/L, respectively. In a another study, the acute toxicity of biologically synthesized gold nanoparticles (AuNPs) using *Saccharina japonica* was assessed. A critical finding was the minimal release of Au(III) ions into the *Daphnia* media, below detectable levels using ICP-MS. This indicates that the biosynthesized AuNPs were highly stable and did not decompose to release toxic ionic forms. Consequently, no acute toxicity was observed in *Daphnia* exposed to these AuNPs. Therefore these results indicate that the AgNPs did not induce substantial physical alterations in the test organisms. Finding confirm that engineered nanoparticles exhibit a higher propensity for ion release compared to their biosynthesized NPs. Ion release is positively correlated with nanoparticle concentration. Conversely, biosynthesized nanoparticles demonstrate enhanced stability attributed to the presence of biomolecular capping agents within the plant extract (Saif et al., 2016).

Concentration (ppm)	Mortality%±SD	
Control	0±0	
1	0±0	**LC50:16.568ppm**
2	0±0	**LC90:36.577ppm**
3	0±0	
4	2±0.44	
5	2±0.44	

a

Concentration (ppm)	Mortality%±SD	
Control	0±0	
1	0±0	**LC50:15.213ppm**
2	0±0	**LC90:28.296ppm**
3	0±0	
4	0±0	
5	2±0.4	

b

Concentration (ppm)	Mortality%±SD	
Control	0±0	
1	32±0.83	**LC50:2.156ppm**
2	46±0.89	**LC90:12.629ppm**
3	54±0.89	
4	64±1.14	
5	80±1	

c

Table 6.3: Mortality percentage and LC50 values observed in crustacean *Daphnia magna* when exposed to different concentrations of (a) C1 extract (b) C1(AgNPs) (c) $AgNO_3$

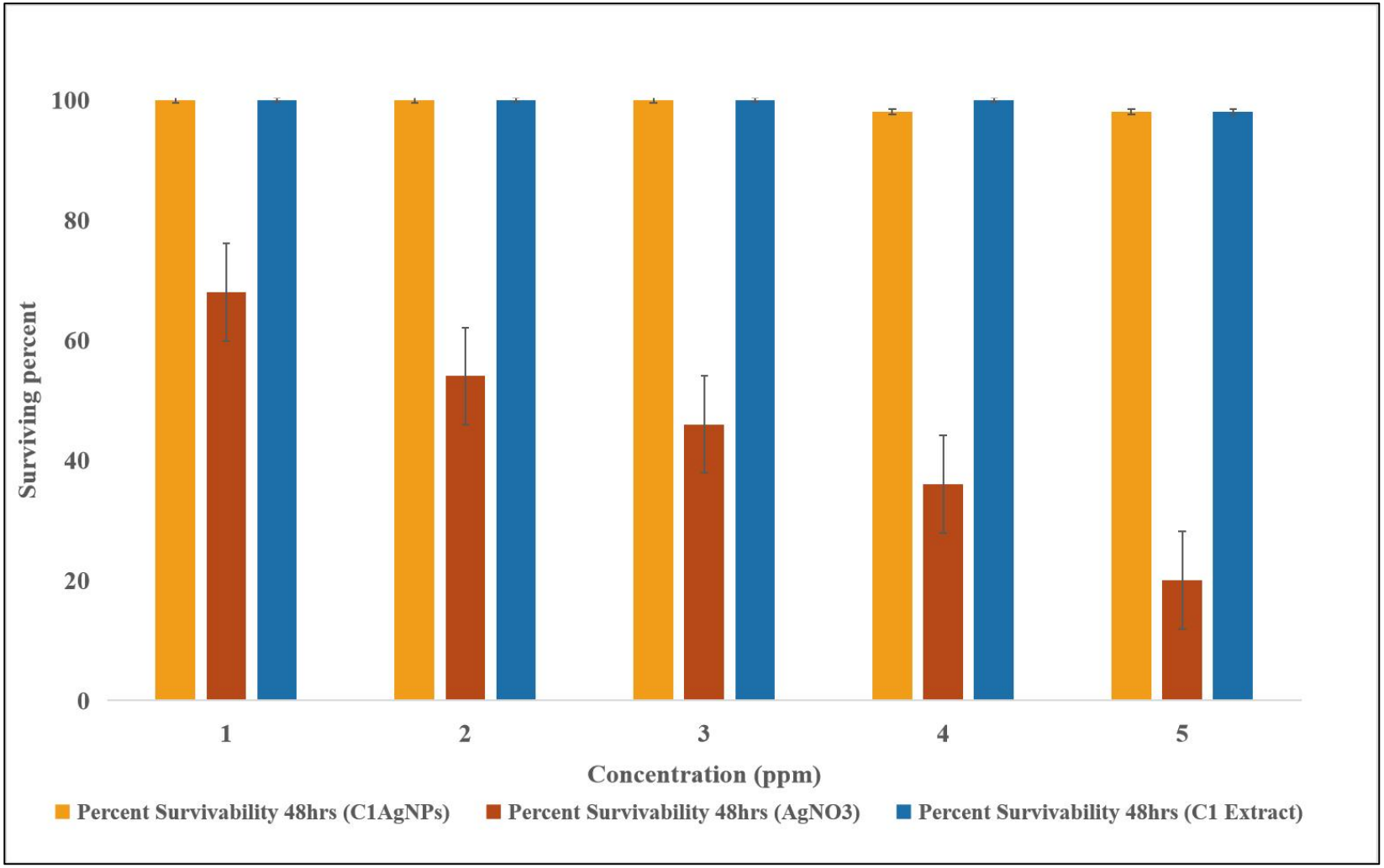


Fig 6.27: Survivability percent of *Daphnia magna* after being exposed to C1AgNPs, C1extract and $AgNO_3$

Light microscope images were taken of *Daphnia magna* taken after 48 hrs of exposure to C1 (AgNPs), C1extract and $AgNO_3$ to study the morphological changes. Control organisms displayed typical neonate morphology without abnormalities. *Daphnia* exposed to C1AgNPs and C1fungal extract exhibited negligible morphological deviations from the control group. Conversely, $AgNO_3$-treated neonates demonstrated marked morphological aberrations, characterized by pronounced deterioration of the carapace and internal structures (Fig 6.28). Study has confirmed that *Daphnia* when exposed to silver for 24 hrs led to the accumulation of Ag^+ in the gut region (Glover & Wood, 2005). The observed differences in toxicity of $AgNO_3$ exposure may be attributed to differential target organ interactions. Metals absorbed from the dissolved phase are likely to accumulate primarily on gill and external epithelial tissues. Conversely, dietary uptake of $AgNO_3$ may preferentially target internal organs, such as those involved in reproductive processes, as evidenced by potential inhibition of vitellogenesis (Zhao & Wang, 2011b). The biosynthesized AgNPs accumulation in Daphnia is found to be least because of its filter feeding nature. Microscopic examination of the gut region revealed linear agglomerations of ingested AgNPs. ICP-OES analysis determined AgNP accumulation and elimination kinetics in exposed organisms. Results indicated a relatively rapid elimination of AgNPs from the intestinal tract within 24 hours post-acute exposure, suggesting a low level of toxicity under these conditions. Finally the toxicological profile of AgNPs is primarily influenced by particle morphology (shape, size, and structure), surface chemistry, the presence of plant extracts, aggregation state, and potential impurities (Muthukrishnan et al., 2017).

Results indicated that C1(AgNPs) exhibited no toxicity to *Daphnia magna* at any concentration tested. Survival and swimming behavior remained unaffected after 48 hours of exposure. These findings clearly demonstrate the non-toxic nature of C1AgNPs compared to the highly toxic $AgNO_3$ in *Daphnia magna*. Multiple studies have consistently shown that biologically synthesized AgNPs pose minimal risk to non-target *Daphnia*. For instance, clove-mediated AgNPs demonstrated a benign impact on *Daphnia*, attributed to the presence of protective phytochemicals in the extract and the capping of AgNP ends (Yadav & Preet, 2023). Finally according to a recent study of Naveenkumar et al. (2023), biologically synthesized AgNPs were highly efficient mosquito larvicidal agents having a nontoxic nature towards non-target *D. magna.*

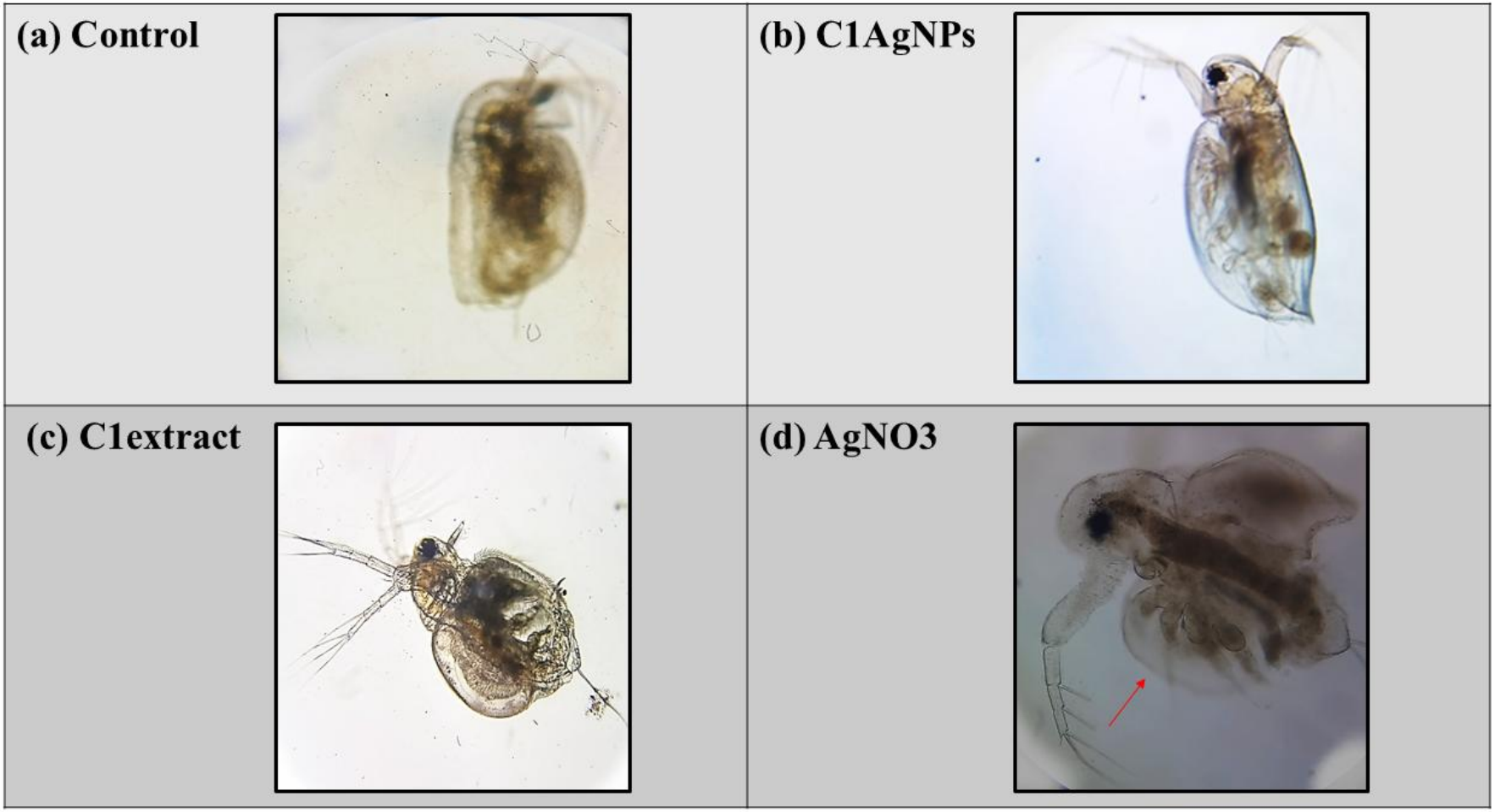


Fig 6.28: Morphological alterations in *Daphnia magna* neonates after 48 h acute toxicity bioassay observed under phase contrast microscope (a) Control (b) C1(AgNPs), (c) C1 extract (d) $AgNO_3$. Red arrow depicts the deterioration of carapace and internal structures due to the toxicity of $AgNO_3$.

6.3.1.2 Acute toxicity study in Labeo rohita fingerlings

Labeo rohita, a popular freshwater fish known for its high protein content, low bone count, and delicious taste, is widely distributed in Indian rivers, lakes, and ponds. Its suitability for laboratory conditions, particularly its tolerance to toxicity testing, makes it an ideal model organism for this study. Rohu fingerlings, weighing an average of 5-12 grams (8-20cm), were procured from a commercial fish farm in Khanna, Punjab. Small sized fingerlings simplifies experiments by requiring less space, water, and chemicals. This leads to more accurate results. It also allows for detailed study of organs like gills and liver.

Acute toxicity was determined following the OECD (1992) guidelines with minor modification. All the treatment tank were cleaned and filled with dechlorinated water. To each of the treatment tank, 200, 400, 600, 800 and 1000ppm concentrations of C1(AgNPs), C1(extract) and $AgNO_3$ suspensions were added, that has been used for mosquito larvae causing LC50 (50 times higher dose). The fingerlings were categorized into 6 groups, each group having 10 organisms (the last group acted as a control). Toxicity assessments conducted on Rohu fingerlings confirms the results indicated that $AgNO_3$ exhibited the highest toxicity, with an LC50 value of 207.95 ppm. The percent mortality observed at the lowest concentration was 50% after exposure to $AgNO_3$. Complete mortality of 100% was recorded at highest concentration of 5ppm. Conversely, biosynthesized AgNPs demonstrated the lowest toxicity, with an LC50 of 29626.778ppm. The mortality percentage was only 6% at the concentration of 5ppm. The toxicity of C1 extract was also negligible, as evidenced by an LC50 value of 35585.376 ppm (Table 6.4)

Recent studies have focused on assessing the toxicity of biologically synthesized silver nanoparticles on non-target organisms. The review of Benelli (2018) highlights the extensive research on plant-based silver nanoparticles and their acute toxicity in aquatic environments. Notably, these studies consistently indicate that biogenic nanosilver poses minimal harm to aquatic life. Exposure to sublethal concentrations of bio-silver nanoparticles has shown no significant adverse effects on non-target species such as *Gambusia affinis* and *Poecilia reticulata.* The potential impact of mosquito control agents on other aquatic organisms has been a subject of investigation. Subarani et al. (2013) synthesized silver nanoparticles using *Vinca rosea* leaf extract and assessed their toxicity on guppy fish (*P. reticulata*). No adverse effects were observed after a 72-hour exposure to 10 mg/mL nanosilver. Subramaniam et al. (2015) employed *Mimusops elengi* leaf extract to produce silver nanoparticles, demonstrating efficacy against *Aedes stephensi* and *Aedes albopictus* larvae and pupae. Exposure of mosquitofish (*G. affinis*) to one-third the LC50 dose for mosquito larvae resulted in increased predation rates without subsequent toxicity, suggesting a potential enhancement of biological control measures. These findings align with those of Govindarajan & Benelli (2016), who confirmed that

Barleria cristata leaf extract based silver nanoparticles were non-toxic to non-target *Gambusia affinis*, with an LC50 value of 866 ppm. Similarly, Govindarajan et al. (2016) investigated the toxicity of *M. sylvestris* leaf extract and *M. sylvestris*-synthesized silver nanoparticles toward *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus* larvae. AgNPs demonstrated superior toxicity to the leaf extract against all three mosquito species, with LC50 values ranging from 10.33 to 12.19 µg/mL. Importantly, these AgNPs were found to be safe for non-target organisms, such as *Gambusia affinis*, with LC50 values ranging from 813.16 to 1044.52 µg/mL. In agreement with these findings, Esan et al., (2021) assessed the larvicidal efficacy and environmental impact of *A. venenata* leaf extract and AgNPs. Ecotoxicity studies on *Gambusia affinis* revealed LC50 values ranging from 734 to 21,140 µg/mL, indicating low toxicity and environmental safety of both the plant extract and synthesized AgNPs. In controlling *Ae. albopictus* mosquitoes, both mangrove extract and its silver nanoparticles (AgNPs) were effective, with AgNPs (LC50: 25.20-101.90 ppm for larvae/pupae) showing greater toxicity than the extract (LC50: 240.34-404.76 ppm). Both also reduced adult lifespan and egg production. Importantly, the study observed that when mosquito larvae were exposed to AgNPs, the predatory efficiency of *Gambusia affinis* fish against these larvae actually *increased* (from 66.46% to 69.98% for III instar, and from 53.99% to 56.05% for IV instar). These results highlight that *S. caseolaris*-derived AgNPs, within these concentration ranges, not only directly control mosquitoes but may also enhance the effectiveness of natural predators like *Gambusia affinis* (Roni et al., 2024).

The present study also revealed that exposure to C1(AgNPs) for ten days had no discernible impact on fingerling swimming behavior. In contrast, fish exposed to silver nitrate exhibited pronounced lethargy, impaired locomotion, and accelerated gill movements. Excessive mucus secretion and darkening of the skin and gills were also evident. This result can be correlated to another study in which fingerlings of Indian major carp *Labeo rohita* were exposed (35 days) to sublethal concentrations of silver nitrate ($AgNO_3$) and bioaccumulation pattern, biochemical and histological alterations were evaluated. A considerable quantity of $AgNO_3$ was accumulated in the gill, liver and kidney tissues of *Labeo rohita*. Accumulation of $AgNO_3$ was high in liver tissue compared to other organs/tissue. Membrane-bound enzyme (Na+/K+/-ATPase) activity was inhibited when compared with the control group (Shobana et al., 2021).

a

Concentration (ppm)	Mortality%± SD	
200	0±0	**LC50:29676.778ppm**
400	2±0.4	
600	4±0.5	
800	4±0.5	
1000	6±0.5	

b

Concentration (ppm)	Mortality (%)±SD	
200	0±0	**LC50 :35585.376ppm**
400	2±0.4	
600	2±0.4	
800	4±0.5	
1000	6±0.5	

c

Concentration (ppm)	Mortality(%) ±SD	
200	50±1.5	**LC50: 207.954ppm**
400	80±0.7	
600	94±0.5	
800	98±0.4	
1000	100±0	

Table 6.4: Biotoxicity of (a) C1(AgNPs) (b) C1extract (c) $AgNO_3$ against non-target Rohu fingerlings

Histopathological analysis of Rohu fingerlings

The liver and gills from control, and all treatment groups were dissected and washed in 0.9% cold saline solution before being fixed in 10% formalin for 24 hours. The fixed tissues were then processed for paraffin embedding, sectioned at 7 μm thickness, and stained with hematoxylin and eosin. Light microscopy was used to examine the changes. Results confirmed that the treatment of C1(AgNPs) and C1 extract exhibited normal gill architecture, characterized by intact primary and secondary gill lamellae, well-defined pillar cells, and healthy epithelial cells. Along with that no toxicity was observed in the hepatocytes. Whereas the $AgNO_3$-treated group exhibited pronounced gill tissue damage, including lamellar degeneration, necrosis, epithelial disruption, lifting and fusion of lamellae, and abnormal cartilage formation. The severity of these histopathological alterations was increased when exposed to higher concentrations of $AgNO_3$ (Fig 6.29).

The observed results align with previous research indicating that $AgNO_3$ group exhibit pronounced fluctuations in silver concentration. This is likely due to the high reactivity and rapid uptake of ionic silver by organisms. The small size of Ag^+ ions facilitate their interaction with cellular components, such as sodium channel (Ferroni et al., 2022). Sodium borohydride synthesized silver nanoparticles, guava leaf extract, along with silver nitrate were evaluated for their acute toxicity in *Danio rerio.* 96 h LD50 dose for silver nitrate was 100 μg L^{-1} where as it was 80 and 400 μg L^{-1} for chemically and biologically synthesized silver nanoparticles respectively. Observations under microscopy depicted the fact that ovary of control group elicited normal character of ooplasm. $AgNO_3$ also elicited medium alterations showed by follicular damage and shrinkage. Chemically synthesized nanosilver showed maximum alterations marked by atresia and follicular dystrophy, but guava synthesized silver nanoparticles exhibited mild atresia. Findings confirm that biologically synthesized AgNPs were least toxic among all (Sarkar et al., 2014b). The toxicological effects of mycogenic silver nanoparticles, synthesized using the fungus *Aspergillus tubingensis* AgNP-AT, were assessed using *Palaemon pandaliformis*, on a shrimp species recognized as a sensitive indicator of environmental quality. A 96-hour toxicity test demonstrated that AgNP-AT exhibited lower toxicity to shrimp compared to $AgNO_3$. This reduced toxicity is likely attributed to the presence of a protein corona on the AgNPs, which inhibits the release of Ag+ ions, mitigating adverse effects on the shrimp (Rebeiro et al., 2023). It was confirmed that compared to control groups, rohu fingerlings exposed to silver nitrate exhibit severe histopathological abnormalities in both gills and liver. Gill tissue displayed hyperplasia, epithelial lifting, and lamellar curling, while the liver showed hepatic nuclear degeneration, vacuolation, and necrosis (Shobana et al., 2018). A study comparing $AgNO_3$ and AgNPs effects in tilapia revealed that both influenced gill health and caused oxidative stress. The findings suggested that $AgNO_3$ had a more pronounced impact than AgNPs on antioxidant enzyme activity at higher concentrations. While both silver forms had biological effects, AgNPs showed comparatively less impact at the tested levels (Sibiya et al., 2022). At lower

concentrations of *Terminalia chebula*-AgNPs, specifically ranging from 100 to 500 µg/mL, observed minimal impact on the zebrafish embryos. The hatching rate remained high, at approximately 94.92%, which was statistically similar to the control group. Furthermore, these lower concentrations did not show any significant toxicity to the embryos throughout the 96-hour observation period. The viability rates remained consistently high across all these lower concentrations, indicating that the embryos survived and developed normally without significant adverse effects (Tharani et al.,2023). Likewise research evaluated the effects of biogenic silver nanoparticles synthesized using *Aspergillus tubingensis* (AgNP-AT) on Nile tilapia (*Oreochromis niloticus*) and compared them to silver nitrate ($AgNO_3$). Notably, AgNP-AT exhibited a substantially higher 96-hour LC50 (8.8 µM) compared to AgNO3 (0.028 µM), indicating that AgNP-AT is significantly less acutely toxic than ionic silver. While exposure to higher concentrations of AgNP-AT (30-40 µM) did induce physiological changes, such as increased oxygen consumption and reduced swimming activity, and caused structural alterations in the gills (reduced lamellar length, increased width), these effects occurred at concentrations considerably higher than the acutely toxic levels of $AgNO_3$. These findings suggest that while biogenic AgNPs are not entirely without impact, they present a lower degree of acute toxicity to Nile tilapia compared to ionic silver, providing important context for assessing the environmental safety of nanotechnology applications (Ribeiro et al., 2025).

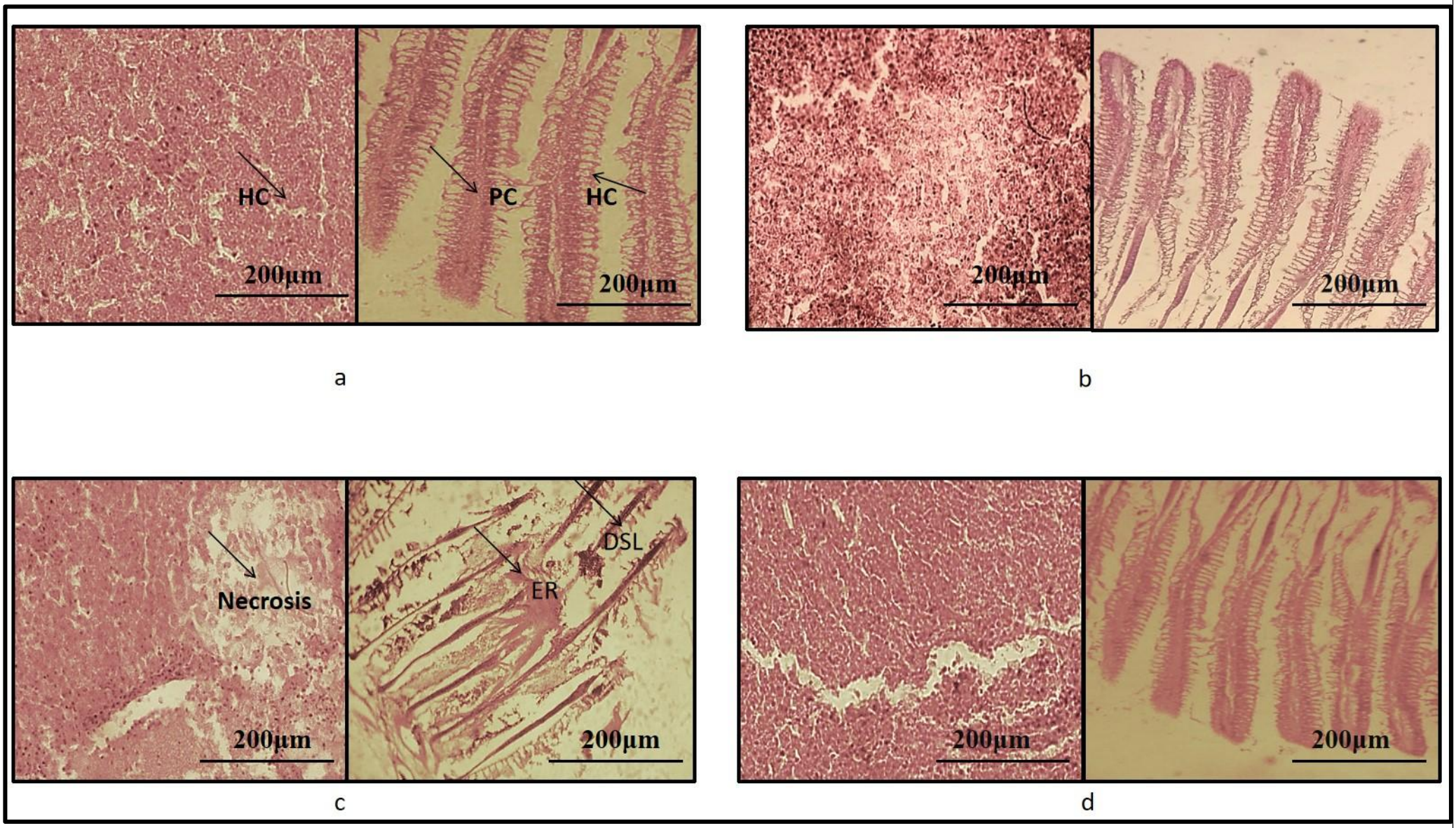


Fig 6.29: Histopathological analysis in rohu fingerlings (a) Control; hepatocytes (HC) in liver; normal gill structure like pillar cells (PC), and epithelial cells (EC) (b) C1(AgNPs) (c) $AgNO_3$; Fish gill (ER;Epithelium rupture,DSL;Disintergrating secondary lamellae); Liver (Necrosis) (d) C1 extract

Molecular identification of fungi colony C1 isolated from insect cadaver

The C1 fungi colony isolated from the insect cadaver was subjected to molecular identification. Molecular sequencing revealed a 100% similarity to *A. fumigatus.* Data was submitted to GenBank under accession number PP177350 and the phylogenetic tree constructed using MEGA software is shown in Fig. 6.30.

Fig 6.30: Phylogenetic tree analysis of *Aspergillus fumigatus*

CHAPTER 7

SUMMARY AND CONCLUSION

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Mosquito vector, which serves as a reservoir for numerous infections that spread severe illnesses including Dengue, Chikungunya, Yellow fever, etc. More than half of the world's population is now at danger of contracting one of these infections due to the sharp rise in the occurrence of these illnesses over the past few years. Although various chemical and biological methods have been undertaken in the past to control vector populations, still drawbacks limit their application. In this scenario using entomopathogenic fungi acts as a novel way out for eradication of the vector. Therefore the central idea of this review is to highlight the importance of entomopathogenic fungi as an emerging mosquito larvicidal candidate.

Since prehistoric times entomopathogenic fungi have been used as an effective bio-insecticide agent against a wide variety of insect species. Due to this reason, they have been looked upon as a potent mosquitocidal agent. Studies have proved that spores or conidia and metabolites of entomopathogenic fungus have caused lethal infections in mosquito larvae. Nanoparticles have higher penetration power due to their small size. They are easily able to penetrate through the cuticle of mosquito larvae. Silver nanoparticles have an insecticidal property and currently, it is being used in the pest control strategy. Entomopathogenic fungi secrete a wide variety of metabolites which help in the synthesis of silver nanoparticles. Entomopathogenic fungi-based silver nanoparticles have higher efficacy towards mosquito larvae. Hence this research deals with the synthesis of AgNPs using EPF and testing its larvicidal efficacy against the Dengue vector.

The first aim was concerned with the isolation of fungi from the soil samples and insect cadaver from the tea garden, Palampur (Himachal Pradesh). As per the literature survey the microbes are highly prominent in cultivable land such as tea plantations which have high organic matter and acidity. The objective focused on the isolation of the fungal colonies from the soil samples and insect cadaver. All the colonies were identified using morphologically and microscopically. The total 21 isolated colonies were identified to be belonging to *Mucor, Rhizopus* and *Aspergillus* genera.

The second objective is divided into two parts. Firstly synthesis of AgNPs from all the fungus colonies isolated. Out of all, only 2 colonies successfully synthesized AgNPs which were further studied for their characterization studies such as UV-Vis spectroscopy, FTIR, SEM-EDS, XRD and Zeta potential. The second part of the objective discuss the larvicidal efficacy of *A. fumigatus* based silver nanoparticles against third instar *Aedes aegypti* larvae. Result confirm that the biosynthesized AgNPs solution had a better larvicidal impact (LC50:2.624ppm) thereby confirming its use for vector control for future.

Finally the third objective was based on the effect of *A. fumigatus*-AgNPs on the non target organism like *Daphnia magna* and Rohu fingerlings which occupy the same ecological niche with mosquito larvae. Results confirm that the biologically synthesized silver nanoparticles does not pose any threat to the non target organisms. The histopathological studies also validates this notion.

Nowadays biomediated AgNPs are getting a lot of prominence in various sectors such as agriculture, pest and vector control. Studies conclude that a lower concentration of silver nanoparticles does not induce any toxicity and behavioral change in aqua biota. The current research has also spotlighted the consequences of biogenic silver nanoparticles on aquatic fora and fauna. The study showed that Ag NP produced using *A. fumigatus* is effective at low dosages for controlling mosquito populations that serve as vectors without negatively impacting non-target aquatic organisms, like *Daphnia magna* and *Labeo rohita* fingerlings, are preyed upon. Therefore it can be concluded that the findings of this study indicate the potential of EPF has the potential to facilitate the swift synthesis of silver nanoparticles without causing any harm to other aquatic organisms occupying the same ecological niche.

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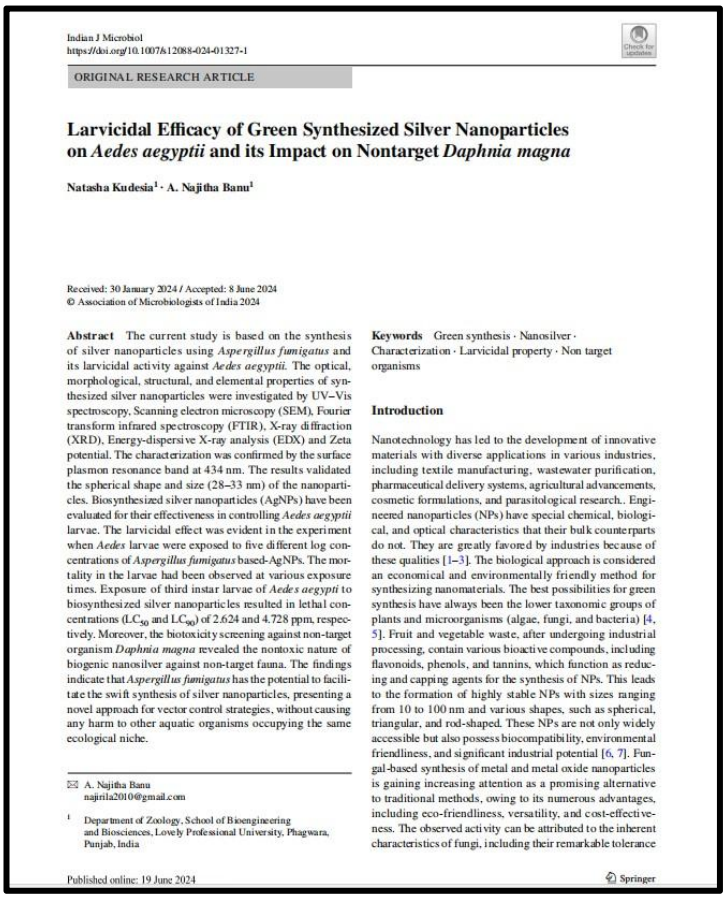
APPENDIX 1

LIST OF PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS (ARTICLES)

Research paper published in Indian Journal of Microbiology titled "**Larvicidal Efficacy of Green Synthesized Silver Nanoparticles on *Aedes aegyptii* and its Impact on Nontarget *Daphnia magna*".** (Kudesia, N. and Banu, A.N., 2024. Larvicidal Efficacy of Green Synthesized Silver Nanoparticles on Aedes aegyptii and its Impact on Nontarget Daphnia magna. Indian Journal of Microbiology, pp.1-13).



Indian J Microbiol
https://doi.org/10.1007/s12088-024-01327-1

ORIGINAL RESEARCH ARTICLE

Larvicidal Efficacy of Green Synthesized Silver Nanoparticles on *Aedes aegyptii* and its Impact on Nontarget *Daphnia magna*

Natasha Kudesia[1] · A. Najitha Banu[1]

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Abstract The current study is based on the synthesis of silver nanoparticles using *Aspergillus fumigatus* and its larvicidal activity against *Aedes aegyptii*. The optical, morphological, structural, and elemental properties of synthesized silver nanoparticles were investigated by UV–Vis spectroscopy, Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Energy-dispersive X-ray analysis (EDX) and Zeta potential. The characterization was confirmed by the surface plasmon resonance band at 434 nm. The results validated the spherical shape and size (28–33 nm) of the nanoparticles. Biosynthesized silver nanoparticles (AgNPs) have been evaluated for their effectiveness in controlling *Aedes aegyptii* larvae. The larvicidal effect was evident in the experiment when *Aedes* larvae were exposed to five different log concentrations of *Aspergillus fumigatus* based-AgNPs. The mortality in the larvae had been observed at various exposure times. Exposure of third instar larvae of *Aedes aegypti* to biosynthesized silver nanoparticles resulted in lethal concentrations (LC_{50} and LC_{90}) of 2.624 and 4.728 ppm, respectively. Moreover, the biotoxicity screening against non-target organism *Daphnia magna* revealed the nontoxic nature of biogenic nanosilver against non-target fauna. The findings indicate that *Aspergillus fumigatus* has the potential to facilitate the swift synthesis of silver nanoparticles, presenting a novel approach for vector control strategies, without causing any harm to other aquatic organisms occupying the same ecological niche.

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Keywords Green synthesis · Nanosilver · Characterization · Larvicidal property · Non target organisms

Introduction

Nanotechnology has led to the development of innovative materials with diverse applications in various industries, including textile manufacturing, wastewater purification, pharmaceutical delivery systems, agricultural advancements, cosmetic formulations, and parasitological research.. Engineered nanoparticles (NPs) have special chemical, biological, and optical characteristics that their bulk counterparts do not. They are greatly favored by industries because of these qualities [1–3]. The biological approach is considered an economical and environmentally friendly method for synthesizing nanomaterials. The best possibilities for green synthesis have always been the lower taxonomic groups of plants and microorganisms (algae, fungi, and bacteria) [4, 5]. Fruit and vegetable waste, after undergoing industrial processing, contain various bioactive compounds, including flavonoids, phenols, and tannins, which function as reducing and capping agents for the synthesis of NPs. This leads to the formation of highly stable NPs with sizes ranging from 10 to 100 nm and various shapes, such as spherical, triangular, and rod-shaped. These NPs are not only widely accessible but also possess biocompatibility, environmental friendliness, and significant industrial potential [6, 7]. Fungal-based synthesis of metal and metal oxide nanoparticles is gaining increasing attention as a promising alternative to traditional methods, owing to its numerous advantages, including eco-friendliness, versatility, and cost-effectiveness. The observed activity can be attributed to the inherent characteristics of fungi, including their remarkable tolerance

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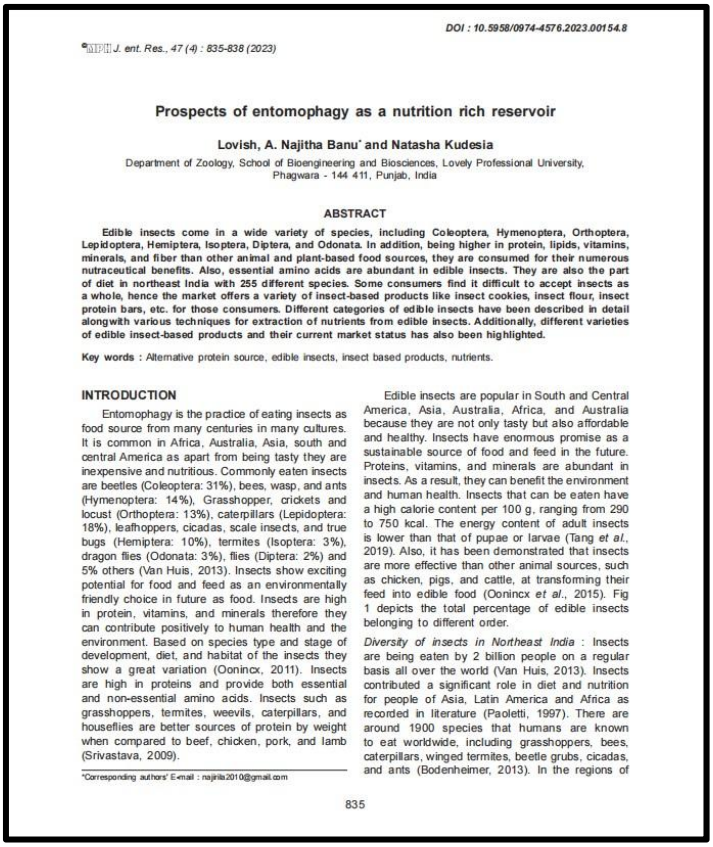

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Prospects of entomophagy as a nutrition rich reservoir

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ABSTRACT

Edible insects come in a wide variety of species, including Coleoptera, Hymenoptera, Orthoptera, Lepidoptera, Hemiptera, Isoptera, Diptera, and Odonata. In addition, being higher in protein, lipids, vitamins, minerals, and fiber than other animal and plant-based food sources, they are consumed for their numerous nutraceutical benefits. Also, essential amino acids are abundant in edible insects. They are also the part of diet in northeast India with 255 different species. Some consumers find it difficult to accept insects as a whole, hence the market offers a variety of insect-based products like insect cookies, insect flour, insect protein bars, etc. for those consumers. Different categories of edible insects have been described in detail alongwith various techniques for extraction of nutrients from edible insects. Additionally, different varieties of edible insect-based products and their current market status has also been highlighted.

Key words : Alternative protein source, edible insects, insect based products, nutrients.

INTRODUCTION

Entomophagy is the practice of eating insects as food source from many centuries in many cultures. It is common in Africa, Australia, Asia, south and central America as apart from being tasty they are inexpensive and nutritious. Commonly eaten insects are beetles (Coleoptera: 31%), bees, wasp, and ants (Hymenoptera: 14%), Grasshopper, crickets and locust (Orthoptera: 13%), caterpillars (Lepidoptera: 18%), leafhoppers, cicadas, scale insects, and true bugs (Hemiptera: 10%), termites (Isoptera: 3%), dragon flies (Odonata: 3%), flies (Diptera: 2%) and 5% others (Van Huis, 2013). Insects show exciting potential for food and feed as an environmentally friendly choice in future as food. Insects are high in protein, vitamins, and minerals therefore they can contribute positively to human health and the environment. Based on species type and stage of development, diet, and habitat of the insects they show a great variation (Oonincx, 2011). Insects are high in proteins and provide both essential and non-essential amino acids. Insects such as grasshoppers, termites, weevils, caterpillars, and houseflies are better sources of protein by weight when compared to beef, chicken, pork, and lamb (Srivastava, 2009).

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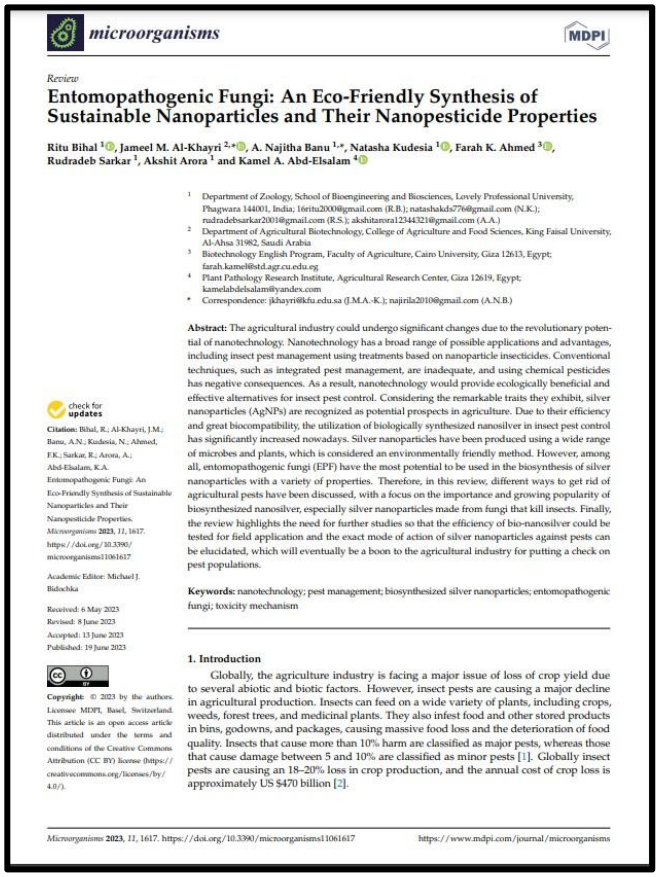
Edible insects are popular in South and Central America, Asia, Australia, Africa, and Australia because they are not only tasty but also affordable and healthy. Insects have enormous promise as a sustainable source of food and feed in the future. Proteins, vitamins, and minerals are abundant in insects. As a result, they can benefit the environment and human health. Insects that can be eaten have a high calorie content per 100 g, ranging from 290 to 750 kcal. The energy content of adult insects is lower than that of pupae or larvae (Tang *et al.*, 2019). Also, it has been demonstrated that insects are more effective than other animal sources, such as chicken, pigs, and cattle, at transforming their feed into edible food (Oonincx *et al.*, 2015). Fig 1 depicts the total percentage of edible insects belonging to different order.

Diversity of insects in Northeast India : Insects are being eaten by 2 billion people on a regular basis all over the world (Van Huis, 2013). Insects contributed a significant role in diet and nutrition for people of Asia, Latin America and Africa as recorded in literature (Paoletti, 1997). There are around 1900 species that humans are known to eat worldwide, including grasshoppers, bees, caterpillars, winged termites, beetle grubs, cicadas, and ants (Bodenheimer, 2013). In the regions of

Review paper published in the journal Microorganisms titled **"Entomopathogenic Fungi: An Eco-Friendly Synthesis of Sustainable Nanoparticles and Their Nanopesticide Properties".** (Bihal, R., Al-Khayri, J.M., Banu, A.N., Kudesia, N., Ahmed, F.K., Sarkar, R., Arora, A. and Abd-Elsalam, K.A., 2023. Entomopathogenic fungi: an eco-friendly synthesis of sustainable nanoparticles and their nanopesticide properties. Microorganisms, *11*(6), p.1617).



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Review

Entomopathogenic Fungi: An Eco-Friendly Synthesis of Sustainable Nanoparticles and Their Nanopesticide Properties

Ritu Bihal [1], Jameel M. Al-Khayri [2,*], A. Najitha Banu [1,*], Natasha Kudesia [1], Farah K. Ahmed [3], Rudradeb Sarkar [1], Akshit Arora [1] and Kamel A. Abd-Elsalam [4]

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Abstract: The agricultural industry could undergo significant changes due to the revolutionary potential of nanotechnology. Nanotechnology has a broad range of possible applications and advantages, including insect pest management using treatments based on nanoparticle insecticides. Conventional techniques, such as integrated pest management, are inadequate, and using chemical pesticides has negative consequences. As a result, nanotechnology would provide ecologically beneficial and effective alternatives for insect pest control. Considering the remarkable traits they exhibit, silver nanoparticles (AgNPs) are recognized as potential prospects in agriculture. Due to their efficiency and great biocompatibility, the utilization of biologically synthesized nanosilver in insect pest control has significantly increased nowadays. Silver nanoparticles have been produced using a wide range of microbes and plants, which is considered an environmentally friendly method. However, among all, entomopathogenic fungi (EPF) have the most potential to be used in the biosynthesis of silver nanoparticles with a variety of properties. Therefore, in this review, different ways to get rid of agricultural pests have been discussed, with a focus on the importance and growing popularity of biosynthesized nanosilver, especially silver nanoparticles made from fungi that kill insects. Finally, the review highlights the need for further studies so that the efficiency of bio-nanosilver could be tested for field application and the exact mode of action of silver nanoparticles against pests can be elucidated, which will eventually be a boon to the agricultural industry for putting a check on pest populations.

Keywords: nanotechnology; pest management; biosynthesized silver nanoparticles; entomopathogenic fungi; toxicity mechanism

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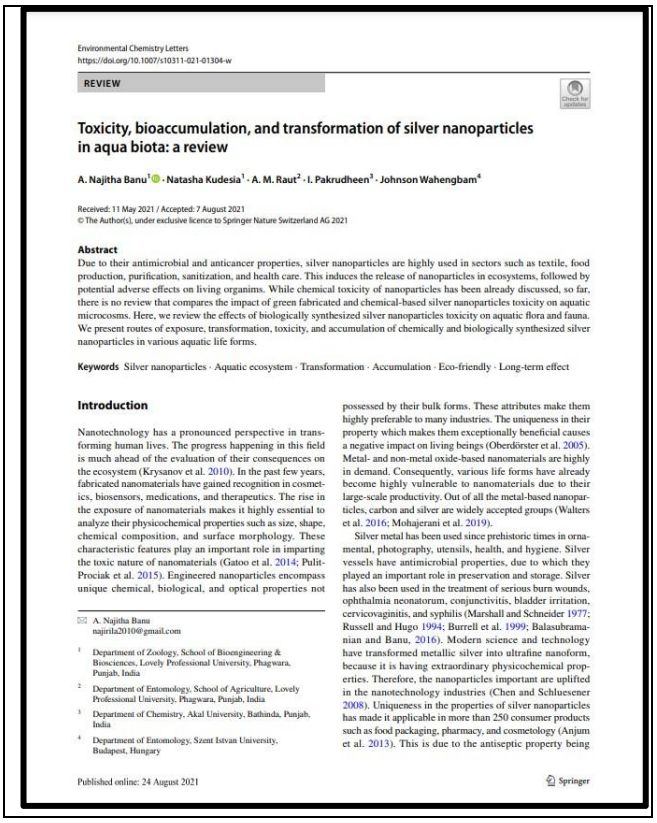
1. Introduction

Globally, the agriculture industry is facing a major issue of loss of crop yield due to several abiotic and biotic factors. However, insect pests are causing a major decline in agricultural production. Insects can feed on a wide variety of plants, including crops, weeds, forest trees, and medicinal plants. They also infest food and other stored products in bins, godowns, and packages, causing massive food loss and the deterioration of food quality. Insects that cause more than 10% harm are classified as major pests, whereas those that cause damage between 5 and 10% are classified as minor pests [1]. Globally insect pests are causing an 18–20% loss in crop production, and the annual cost of crop loss is approximately US $470 billion [2].

Review paper "**Toxicity, bioaccumulation, and transformation of silver nanoparticles in aqua biota: a review**" published in the journal Environmental Chemistry Letters. Banu, A.N., Kudesia, N., Raut, A.M., Pakrudheen, I. and Wahengbam, J., 2021. Toxicity, bioaccumulation, and transformation of silver nanoparticles in aqua biota: A review. Environmental Chemistry Letters, 19(6), pp.4275-4296.



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REVIEW

Toxicity, bioaccumulation, and transformation of silver nanoparticles in aqua biota: a review

A. Najitha Banu[1] · Natasha Kudesia[1] · A. M. Raut[2] · I. Pakrudheen[3] · Johnson Wahengbam[4]

Received: 11 May 2021 / Accepted: 7 August 2021

Abstract
Due to their antimicrobial and anticancer properties, silver nanoparticles are highly used in sectors such as textile, food production, purification, sanitization, and health care. This induces the release of nanoparticles in ecosystems, followed by potential adverse effects on living organims. While chemical toxicity of nanoparticles has been already discussed, so far, there is no review that compares the impact of green fabricated and chemical-based silver nanoparticles toxicity on aquatic microcosms. Here, we review the effects of biologically synthesized silver nanoparticles toxicity on aquatic flora and fauna. We present routes of exposure, transformation, toxicity, and accumulation of chemically and biologically synthesized silver nanoparticles in various aquatic life forms.

Keywords Silver nanoparticles · Aquatic ecosystem · Transformation · Accumulation · Eco-friendly · Long-term effect

Introduction

Nanotechnology has a pronounced perspective in transforming human lives. The progress happening in this field is much ahead of the evaluation of their consequences on the ecosystem (Krysanov et al. 2010). In the past few years, fabricated nanomaterials have gained recognition in cosmetics, biosensors, medications, and therapeutics. The rise in the exposure of nanomaterials makes it highly essential to analyze their physicochemical properties such as size, shape, chemical composition, and surface morphology. These characteristic features play an important role in imparting the toxic nature of nanomaterials (Gatoo et al. 2014; Pulit-Prociak et al. 2015). Engineered nanoparticles encompass unique chemical, biological, and optical properties not possessed by their bulk forms. These attributes make them highly preferable to many industries. The uniqueness in their property which makes them exceptionally beneficial causes a negative impact on living beings (Oberdörster et al. 2005). Metal- and non-metal oxide-based nanomaterials are highly in demand. Consequently, various life forms have already become highly vulnerable to nanomaterials due to their large-scale productivity. Out of all the metal-based nanoparticles, carbon and silver are widely accepted groups (Walters et al. 2016; Mohajerani et al. 2019).

Silver metal has been used since prehistoric times in ornamental, photography, utensils, health, and hygiene. Silver vessels have antimicrobial properties, due to which they played an important role in preservation and storage. Silver has also been used in the treatment of serious burn wounds, ophthalmia neonatorum, conjunctivitis, bladder irritation, cervicovaginitis, and syphilis (Marshall and Schneider 1977; Russell and Hugo 1994; Burrell et al. 1999; Balasubramanian and Banu, 2016). Modern science and technology have transformed metallic silver into ultrafine nanoform, because it is having extraordinary physicochemical properties. Therefore, the nanoparticles important are uplifted in the nanotechnology industries (Chen and Schluesener 2008). Uniqueness in the properties of silver nanoparticles has made it applicable in more than 250 consumer products such as food packaging, pharmacy, and cosmetology (Anjum et al. 2013). This is due to the antiseptic property being

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PUBLICATIONS (CHAPTERS)

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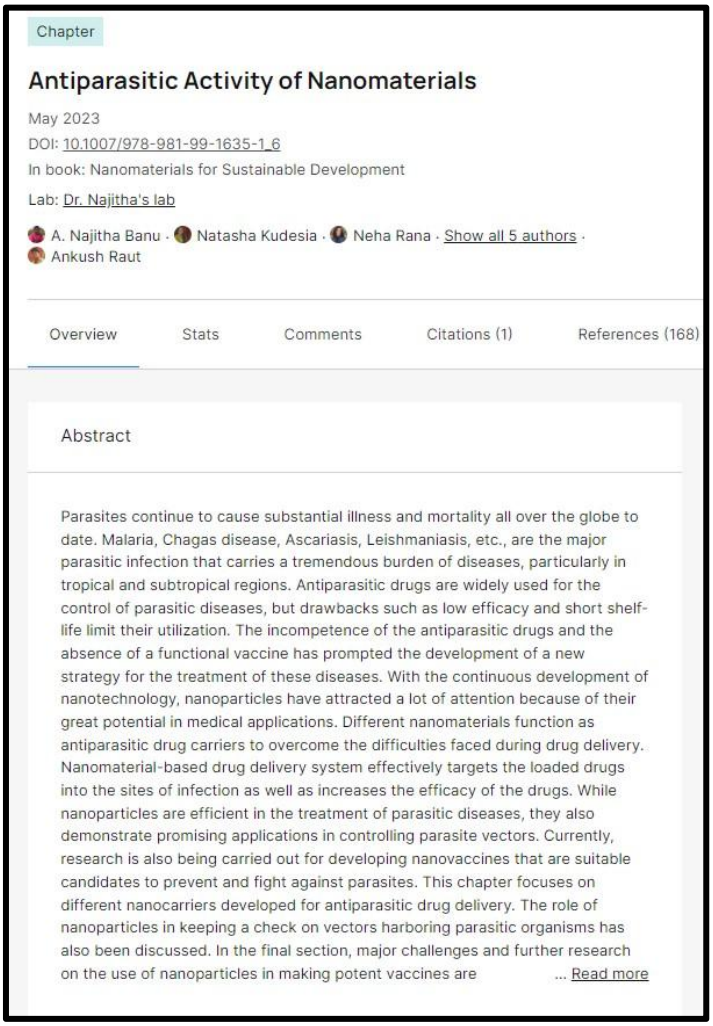

Chapter

Antiparasitic Activity of Nanomaterials

May 2023

DOI: 10.1007/978-981-99-1635-1_6

In book: Nanomaterials for Sustainable Development

Lab: Dr. Najitha's lab

A. Najitha Banu · Natasha Kudesia · Neha Rana · Show all 5 authors · Ankush Raut

Overview Stats Comments Citations (1) References (168)

Abstract

Parasites continue to cause substantial illness and mortality all over the globe to date. Malaria, Chagas disease, Ascariasis, Leishmaniasis, etc., are the major parasitic infection that carries a tremendous burden of diseases, particularly in tropical and subtropical regions. Antiparasitic drugs are widely used for the control of parasitic diseases, but drawbacks such as low efficacy and short shelf-life limit their utilization. The incompetence of the antiparasitic drugs and the absence of a functional vaccine has prompted the development of a new strategy for the treatment of these diseases. With the continuous development of nanotechnology, nanoparticles have attracted a lot of attention because of their great potential in medical applications. Different nanomaterials function as antiparasitic drug carriers to overcome the difficulties faced during drug delivery. Nanomaterial-based drug delivery system effectively targets the loaded drugs into the sites of infection as well as increases the efficacy of the drugs. While nanoparticles are efficient in the treatment of parasitic diseases, they also demonstrate promising applications in controlling parasite vectors. Currently, research is also being carried out for developing nanovaccines that are suitable candidates to prevent and fight against parasites. This chapter focuses on different nanocarriers developed for antiparasitic drug delivery. The role of nanoparticles in keeping a check on vectors harboring parasitic organisms has also been discussed. In the final section, major challenges and further research on the use of nanoparticles in making potent vaccines are ... Read more

Book Chapter published in Springer under the title of "**Fabricated Nanofertilizers: A Clean and Feasible Substitute for Conventional Fertilizers**" in book Nanofertilizers for Sustainable Agroecosystems. Najitha Banu, A., Rana, N., Kudesia, N., Sadaf, D. and Raut, A.M., 2023. Fabricated Nanofertilizers: A Clean and Feasible Substitute for Conventional Fertilizers. In Nanofertilizers for Sustainable Agroecosystems: Recent Advances and Future Trends (pp. 35-59). Cham: Springer Nature Switzerland.

Home > Nanofertilizers for Sustainable Agroecosystems > Chapter

Fabricated Nanofertilizers: A Clean and Feasible Substitute for Conventional Fertilizers

Chapter | First Online: 29 November 2023
pp 35–59 | Cite this chapter

A. Najitha Banu, Neha Rana, Natasha Kudesia, Durdana Sadaf & A. M. Raut

Part of the book series: Nanotechnology in the Life Sciences ((NALIS))

273 Accesses

Abstract

The rising population, leading to the decline in cultivable land and water resources, creates a need in agriculture for greater food production efficiency. Fertilizers have been discovered to play a significant role in enhancing food supply, particularly with the advent of crop types that are receptive to fertilizers and produce a high yield. At the same time, conventional fertilizers are also susceptible to a variety of losses, including leaching, fixation, denitrification, and volatilization. Due to the widespread use of chemical fertilizers and current agricultural techniques, managing the soil's fertility and nutrients for crops will present the biggest challenge in the coming years. On the contrary, nanofertilizers (NFs) are a sustainable alternate crop production practice to enhance food output as well as bring environmental and economic viability. Because of the unique properties of nanomaterials, such as high surface area to volume ratio, controlled-release kinetics of nutrients to specified areas, and absorption capacity, they have become critical in the designing and utilization of new fertilizers. Nanofertilizers primarily prolong nutrient release and boost the fertilizer effect period and have thus created new prospects by ensuring global food security, enhancing input efficiency, lowering prices, and mitigating environmental deterioration. Nanofertilizers, aiming to optimize the flow of nutrients based on crop needs while simultaneously limiting differential losses, offer enormous potential in the field of agriculture. These "smart fertilizers" are increasingly seen as a promising option, to the point that they should be preferred over conventional fertilizers. As a result, the purpose of this chapter is to emphasize nanofertilizers and their effects on crop productivity.

PRESENTATIONS

Presented a research paper on "Toxicity of green synthesized silver nanoparticles on *Aedes* larvae" in an oral presentation at “INTERNATIONAL CONFERENCE ON MICROBIAL BIOPROSPECTING TOWARDS SUSTAINABLE DEVELOPMENT GOALS held on 24th-25th November, 2023, organized by Association of microbiologist in India, LPU Unit and society of chemical and synthetic biology at Lovely Professional University, Phagwara, Punjab.

ICMBSDG
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Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Natasha Kudesia** of **Lovely Professional University** has Participated/Presented a Oral Presentation entitled **Toxicity of green synthesized silver nanoparticles on Aedes larvae** in International Conference on **“Microbial Bioprospecting Towards Sustainable Development Goals”** held on **24th- 25th November 2023** organized by Association of Microbiologist of India-LPU Unit and Society of Chemical and Synthetic Biology at Lovely Professional University, Punjab.

Date of Issue : 12-12-2023
Place : Phagwara (Punjab), India

Prepared by (Administrative Officer-Records)	Dr. Arun Karnwal President AMI-LPU Unit	Dr. Karthik Loganathan President SCSB	Dr. Ashish Vyas Organizing Secretary AMI-LPU Unit	Dr. Neeta Raj Sharma Senior Dean SBEB, LPU

Presented an article on "Assessment of the toxicity effect of biologically synthesized silver nanoparticles on aqua biota" in an oral presentation at “5th INTERNATIONAL CONFERENCE ON ADVANCES IN AGRICULTURE TECHNOLOGY AND ALLIED SCIENCES (ICAATAS 2022) on 4th-5th June, organized by Society of Agriculture Research and Social Development, MS Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Odisha and Association of Rice Research Workers (ARRW), NRRI.

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This is to certify that ***Prof./Dr./Mr./Ms. Natasha Kudesia*** *of* ***Lovely Professional University*** *has participated in* ***Oral Presentation*** *on the topic entitled* ***Evaluation of toxicity impact of green synthesized silver nanoparticles in Daphnia magna*** *in International Conference on* ***Sustainability: Life on Earth 2021 (ICS-LOE 2021)*** *held on 17-18 December 2021 organized by Department of Botany and Zoology, School of Bio-engineering and Biosciences, and Institute of Forest Productivity, Ranchi, Jharkhand, at Lovely Professional University, Punjab.*

Date of Issue: 18-02-2022
Place of Issue: Phagwara (India)

Prepared by
(Administrative Officer-Records)

Dr. Joydeep Dutta
Organizing Secretary, ICS-LOE, 2021

Dr. Neeta Raj Sharma
Convener, ICS-LOE, 2021

Presented a research paper on "Evaluation of toxicity impact of green synthesized silver nanoparticles in *Daphnia magna*" in an oral presentation at International Conference on Sustainability Life on Earth 2021 (ICS-LOE 2021) (Online Conference), Organized by the School of Bioengineering and Biosciences Lovely Professional University Phagwara Punjab & Institute of Forest Productivity Ranchi, Jharkhand held on 17th -18th December 2021.

SARSD/ICAATAS/2022...130

5th INTERNATIONAL CONFERENCE ON
ADVANCES IN AGRICULTURE TECHNOLOGY AND ALLIED SCIENCES
(ICAATAS 2022) on JUNE- 4-5, 2022

CERTIFICATE

This is to certify that Prof./Dr./Mr./Mrs./Ms...NAJASHA.....KAJDESI.A.....
Actively participated/presented a paper (Orál/Poster) entitled Assessment of toxicity effect of biologically synthesized silver nanoparticles on Daphnia Magna

As Delegate/Scholar/Student in the 5th INTERNATIONAL CONFERENCE ON ADVANCES IN AGRICULTURE TECHNOLOGY AND ALLIED SCIENCES (ICAATAS 2022) on JUNE- 4-5, 2022, held at MS Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Gajapati, Odisha – 761211, India

Mr. Akhil Gupta, President SARSD
Dr. Mehjabeen, Oraganizing Convener
Dr. Ashirbachan Mahapatra, Oraganizing Secretary
Dr. S.P. Nanda, Conference Director
Dr. Ankush Raut, Chief Oraganizing Secretary

LIST OF SCIENTIFIC NAMES

S/No	**Abbreviation**	**Full name**
1	*Chlamydomonas reinhardtii*	*C. reinhardtii*
2	*Daphnia similis*	*D. similis*
3	*Ochromonas danica.*	*O. danica.*
4	*Pithophora oedogonia and Chara vulgaris*	*P. oedogonia and C. vulgaris*
5	*Egeria densa and Juncus efusus*	*E. densa and J. efusus*
6	*Caenorhabditis elegans.*	*C. elegans.*
7	*Artemia salina*	*A. salina*
8	*Danio rerio*	*D. rerio*
9	*Labeo rohita*	*L. rohita*
10	*Oncorhynchus mykiss*	*O. mykiss*
11	*Morinda tinctoria*	*M. tinctoria*
12	*Aedes aegyptii*	*A. aegypti*
13	*Bacillus siamensis*	*B. siamensis*
14	*Pithophora oedogonia*	*P. oedogonia*
15	*Hypnea musciformis*	*H. musciformis*
16	*Beauveria bassiana*	*B. bassiana*
17	*Rhynchophorus ferrugineus*	*R. ferrugineus.*
18	*Metarhizium anisopliae*	*M. anisopliae*
19	*Oreochromis niloticus.*	*O. niloticus.*
20	*Trichoderma atroviride*	*T. atroviride*
21	*Poecilia reticulata*	*P. reticulata*
22	*Anopheles stephensi and Anopheles albopictus*	*A. stephensi and A. albopictus.*
23	*Gambusia affnis.*	*G. affnis.*
24	*Culex quinquefasciatus*	*Cx. quinquefasciatus.*
25	*Ceriodaphnia cornuta*	*C. cornuta*
26	*Mucor moelleri, and Mucor heterogamus*	*M. moelleri, and M. heterogamus*
27	*Aspergillus tamarii*	*A. tamarii*

28	*Aspergillus niger, Aspergillus flavus, and Aspergillus clavatus.*	*A. niger, A. flavus, and A. clavatus.*
29	*Pedalium murex*	*P. murex*
30	*Euphorbia rothiana*	*E. rothiana*