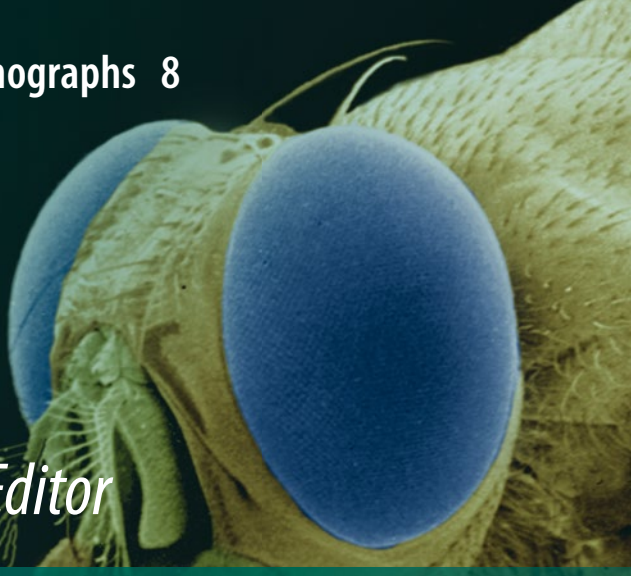
Parasitology Research Monographs 8



Heinz Mehlhorn *Editor*

Nanoparticles in the Fight Against Parasites

Springer

Parasitology Research Monographs

Volume 8

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Heinz Mehlhorn
Department of Parasitology
Heinrich Heine University
Düsseldorf
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Heinz Mehlhorn
Department of Parasitology
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Düsseldorf, Germany
June 2015

Heinz Mehlhorn

Contents

Contributors

Abdullah A. Alarfaj Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

Saleh Al-Quraishy Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

Duraisamy Amaresan Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

C. Balasubramanian Postgraduate and Research Department of Zoology and Microbiology, Thiagarajar College (Autonomous), Madurai, Tamil Nadu, India

A. Najitha Banu Postgraduate and Research Department of Zoology and Microbiology, Thiagarajar College (Autonomous), Madurai, Tamil Nadu, India

Amira A. Bauomy Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt

Giovanni Benelli Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

Angelo Canale Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

Balamurugan Chandramohan Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Marwa S.M. Diab Molecular Drug Evaluation Department, National Organization for Drug Control & Research (NODCAR), Giza, Egypt

Devakumar Dinesh Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Mohamed A. Dkhil Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt

Marimuthu Govindarajan Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Chidambaram, Tamil Nadu, India

Jiang-Shiou Hwang Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan

Kalimuthu Kovendan Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Palanisamy Mahesh Kumar Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Pari Madhiyazhagan Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Heinz Mehlhorn Department of Parasitology, Heinrich Heine University, Düsseldorf, Germany

Kadarkarai Murugan Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Dina A. Nafady Faculty of Architectural Engineering and Digital Design, Dar Al Uloom University, Riyadh, Saudi Arabia

The Higher Institute of Applied Arts, 6th of October City, Cairo, Egypt

Devaraj Nataraj Department of Physics, Bharathiar University, Coimbatore, Tamil Nadu, India

Thiyagarajan Nataraj Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Marcello Nicoletti Department of Environmental Biology, Sapienza University of Rome, Rome, Italy

Chellasamy Panneerselvam Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Abdul Abdul Rahuman Unit of Nanotechnology and Bioactive Natural Products, Post Graduate and Research Department of Zoology, C. Abdul Hakeem College, Melvisharam, Vellore District, Tamil Nadu, India

Govindasamy Rajakumar Unit of Nanotechnology and Bioactive Natural Products, Post Graduate and Research Department of Zoology, C. Abdul Hakeem College, Melvisharam, Vellore District, Tamil Nadu, India

Jayapal Subramaniam Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Udaiyan Suresh Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Chapter 1
Nanoparticles – Definitions

Heinz Mehlhorn

The prefix *nano* has its origin in the Greek and Roman terms nannos/nanus, which describe dwarfs or dwarf-like stages of living organisms respectively similarly sized non-living structures, which of course at the time of their origin had been visible by help of naked eyes. The invention, ameliorations and use of light microscopes (e.g. Antony van Leeuwenhoek; 1632–1723) opened insights in the world of tiny structures, which were later enormously deepened by help of peculiar microscopes. Thus Ernst Ruska (1906–1988) invented 1931 the transmission electron microscope (honored by the Nobel Prize in 1986), Manfred von Ardenne (1907–1997) developed the scanning electron microscope in 1937 and finally Gerd Binnig and Heinrich Rohrer developed the so-called scanning tunneling microscope and were also honored by the Nobel Prize in Physics in the year 1986 (von Ardenne 1938a, b; Binnig et al. 1982a, b; Ruska 1987; Ruska and Knoll 1931; Knoll and Ruska 1932; Goldstein et al. 2003). These and several other new microscopical technologies (Table 1.1) made it possible to discover, to describe and to use a broad range of new very tiny structures of only a few nanometers in size, which at first were named as "ultrafine particles" (Granqvist et al. 1976; Hayashi et al. 1997).

However, soon afterwards these "*ultrafine particles*" were named *nanoparticles* (Kiss et al. 1999; Buzea et al. 2007; Fahlman 2007; Khan 2012; Reiss and Hutten 2010; Gubin 2009). According to recent definitions these ultrafine particles and nanoparticles measure between 1 and 100 nm, whereby 1 nm is 1×10^{-9} m. Tubular structures and fibers with a size of below 100 nm are also termed nanoparticles. In literature there are described further categories of microparticles: *coarse particles* covering a range between 2500 and 10,000 nm, while *fine particles* measure between 100 and 2500 nanometers (nm). On the other hand the so-called nanoplancton in the different water biotopes is defined by a body size measuring between 7 and 15 μm.

H. Mehlhorn
Department of Parasitology, Heinrich Heine University, Building 26.13.01 Room 42, Universitätsstr. 1, Düsseldorf D-40225, Germany
e-mail: mehlhorn@uni-duesseldorf.de

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Table 1.1 Size examples of cellular structures/components and viruses measured in nanometers

Structure/object	Size (nm)
H^+ (hydrogen)	0.2
K^+ (potassium)	0.3
Na^+ (sodium)	0.36
O_2 (oxygen)	0.45
Mg+ (magnesium)	1.08
Ribosomes (80 s)	25 × 16
Ribosomes (70 s)	20 × 15
Cell membrane (diameter)	5–10
Microtubules in cilia, flagella	25
Variola = pox virus (DNA)	250
Papilloma virus (DNA)	53
Herpes virus (DNA)	150
Influenza virus (RNA)	100
Poliomyelitis virus (RNA)	20
Spring summer virus (Flaviviridae) (RNA)	70
Crimean-Congo haemorrhagic fever (CCHF) virus (Bunyaviridae) (RNA)	100
Chikungunya virus (Alpha-virus) (RNA)	100
Dengue virus (Flaviviridae) (RNA)	100
Yellow fever virus (Flaviviridae) (RNA)	100
Japanese encephalitis virus (Flaviviridae) (RNA)	100
West nile virus (Flaviviridae) (RNA)	100
American horse encephalitis virus (Alphavirus, Togaviridae) (RNA)	60–65
California encephalitis virus (Bunyaviridae) (RNA)	80–120
Rift valley virus (Bunyaviridae) (RNA)	80–120
Pappataci fever virus (Phleboviridae)	90–110

Thus the trypanosomatid agent of sleeping sickness in cattle and ruminants, which occurs in the blood is now called *Trypanosoma congolense* and measures 7–18 μm in length, was formerly described as *Trypanosoma nannomonas* or *T. nanum* (Wiesner and Ribbeck 1978; Mehlhorn 2016).

The term nanoparticles is only used for a special group of particles. Although they may exhibit size-related properties that differ not significantly from those seen in *fine particles* or *bulk materials*, the term is used only for a special group of particles. Individual molecules – even when ranging in the same size group – are never referred to as nanoparticles (Salata 2004; Taylor et al. 2013; Vert et al. 2012).

So-called *nanoclusters* have at least one dimension between 1 and 10 nm and show in general a very narrow size variation. The term *nanopowder* is given for agglomerates consisting of *ultrafine particles*, defined amounts of *nanoparticles* or *nanoclusters*. If *single crystals* are nanometer-sized, they were described as *nanocrystals*. The same term is used for single-domain *ultrafine particles*.

Nanoparticles may be dissolved as suspensions, since interactions between the particle surface and the solvent is strong and thus may overcome density differences, which would lead to sinking or floating effects in the liquid.

There are different types of nanoparticles, which may either be solid or semi-solid, e.g. *liposomes* are of semi-solid nature and can be used as delivery systems for drugs and vaccines to enter the tissues of patients. Liposomes, which possess one hydrophilic and another hydrophobic half, are called **Janus** particles (being named after the Greek double-headed god of the beginning and the end and which was often placed at doors when used as entrance and exit). These *Janus particles* stabilize emulsions and may self-assemble e.g. at water/oil interfaces thus possibly acting as solid surfactants.

Nanoparticles can be produced by help of different methods including *hydrothermal synthesis*, *attrition* or *pyrolysis*. Inert gas condensation is used to create nanoparticles from several metals with low melting points. Another method of nanoparticle formation originates from radiation chemistry, where radiolysis occurs, when gamma rays lead to the creation of very active free radicals in a solution. In total there exists already a broad spectrum of different methods, which are used depending on the purpose, for which these nanoparticles will be used. Depending on their components the nanoparticles may appear as globules, nanospheres, nanoreefs, nanotubules, rods, fibres, cups, nanoboxes etc.

For biological applications nanoparticles are used, which have a polar surface coating that is able to provide a high aquaeous solubility and prevents nanoparticle aggregation inside skin, blood or lymph vessels of patients treated by a peculiar type of drug containing nanoparticles.

To protect nanoparticles (when being used in drug trafficking into a body) from attacks of the human immune system, they can be charged with red blood cell coatings. Today more and more nanoparticles are used in applications for medical purposes. In these cases common carriers are liposomes, iron oxide nanoparticles, polymeric nanoparticles, dendrimeres etc. To develop and to test nanoparticles a broad spectrum of chemical and physical methods had been developed as well as sophisticated microscopical techniques (Tables 1.1 and 1.4).

When studying by help of various techniques viruses (Fig. 1.1), bacteria (Figs. 1.2 and 1.3), eukaryotic cells such as parasites (Figs. 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 1.10, 1.11, 1.12, 1.13, 1.14, and 1.15), it was found, that many very important cell/cytologic components range in the world of nanometers (Tables 1.2, 1.3, and 1.4). Even several hydrated chemicals reach into the size range of nanometers ($1\ nm = 10^{-9}$). The size of the wavelength of light is measured in Ångström (Å) (honoring a Swedish scientist, 1814–1874), whereby 1 Å corresponds to 10^{-10} m. As can be seen in Tables 1.1, 1.2, and 1.3 viruses, bacteria and the typical parasites range in different size leagues so that parasites may even contain and transmit viruses and bacteria (Mehlhorn 2010).

Furthermore parasitic protozoans contain and/or excrete vesicles called exosomes, ectosomes, microvesicles or microparticles in the nano range. Thus excretion of exosomes measuring 40–100 nm is described in *Plasmodium berghei*,

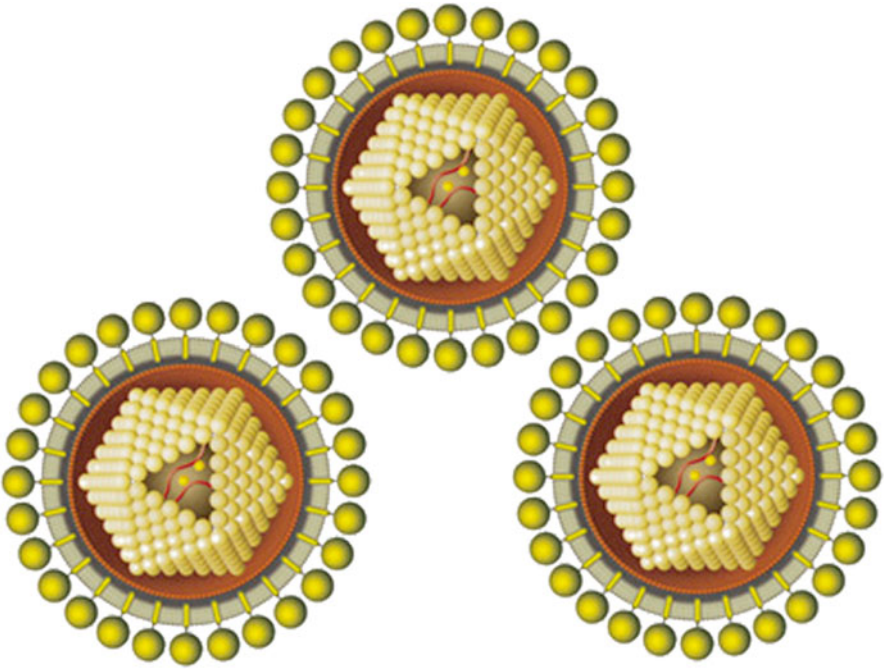

Fig. 1.1 Diagrammatic representation of the tick-transmitted Spring-summer meningoencephalitis viruses measuring only 70 nm

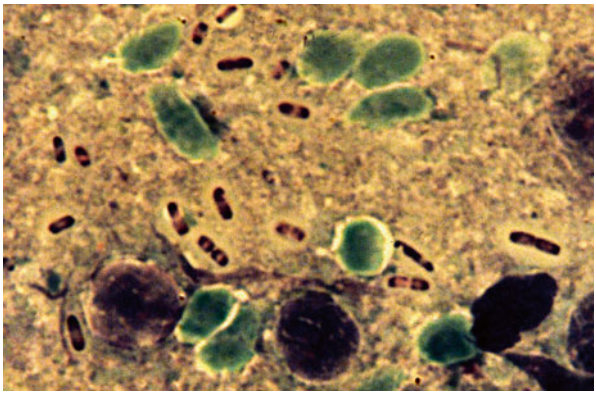

Fig. 1.2 Light micrograph of the gram-negative plague bacteria (*Yersinia pestis*), which are transmitted by fleas and characterized by their bipolar staining. Their length is 1.5×0.5 µm

P. yoeli, *Toxoplasma gondii*, *Trypanosoma cruzi*, T. *brucei*, *Leishmania* species, *Giardia intestinalis*, *Trichomonas vaginalis*. Excretion of microvesicles measuring 0.1–1 µm is described in *Plasmodium vivax*, *P. falciparum*, *Trypanosoma cruzi* as well as in *Leishmania* species and in *Giardia intestinalis*. Even apoptotic remnants of cells range in the general size measuring 1–3 µm in diameter (Mantel et al. 2013; Regev-Rudzki et al. 2013; Nantakomol et al. 2011; Cestari et al. 2012; Silverman et al. 2010; Deolindo et al. 2013; Twu et al. 2013; Geiger et al. 2010; Evans-Osses et al. 2015).

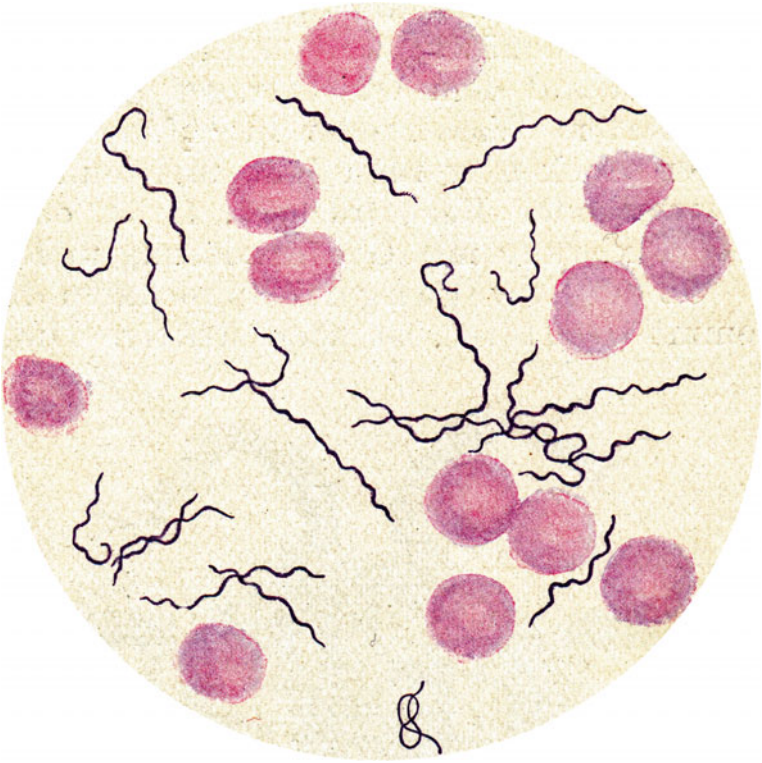

Fig. 1.3 Light micrograph of a Giemsa stained blood smear showing stages of *Borrelia recurrentis*, the agent of tick-borne relapsing fever (measuring 8–30×0.2–0.5 µm)

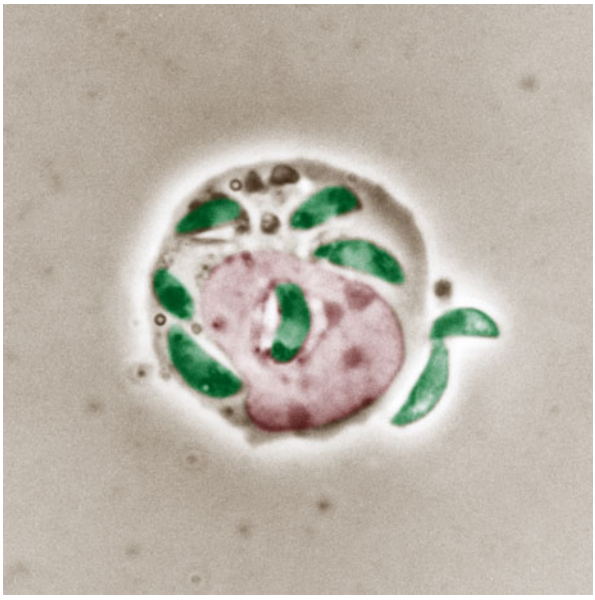

Fig. 1.4 Light micrograph of a host cell containing tachyzoites of *Toxoplasma gondii* (7×1.5 µm)

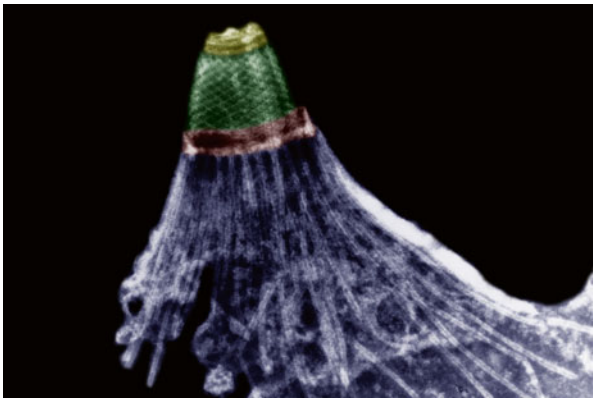

Fig. 1.5 Electron micrograph of a *Toxoplasma gondii* tachyzoite showing the typical subpellicular microtubuli and the conoid, which also consists of (twisted) microtubules (ø 25 nm)

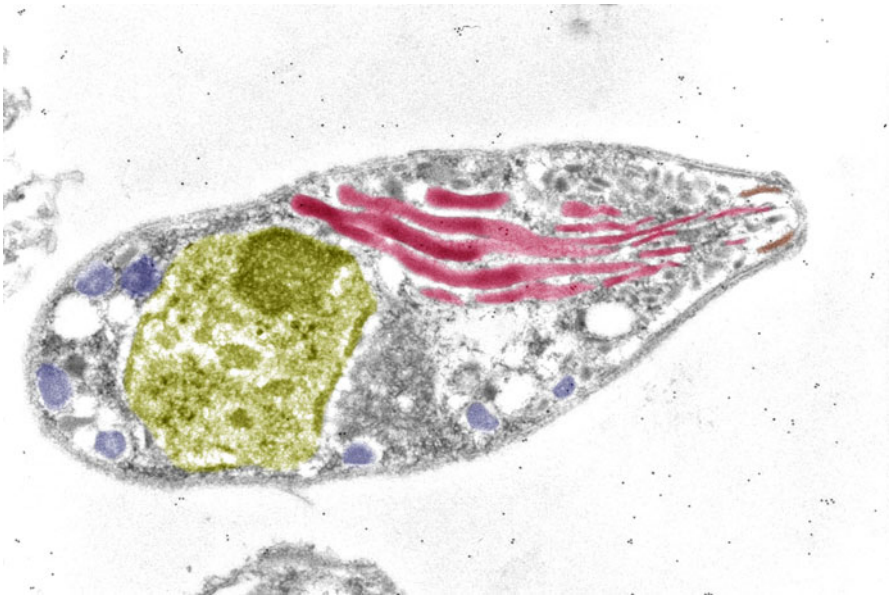

Fig. 1.6 Transmission electron micrograph of a longitudinal section of a tachyzoite of *Toxoplasma gondii* (7 µm). Note that the interior is filled by small spherical ribosomes. The rhoptries (*red*) have diameters of less than 0.1 µm. The nucleus is yellowish-green stained and has diameters of 1.5 µm

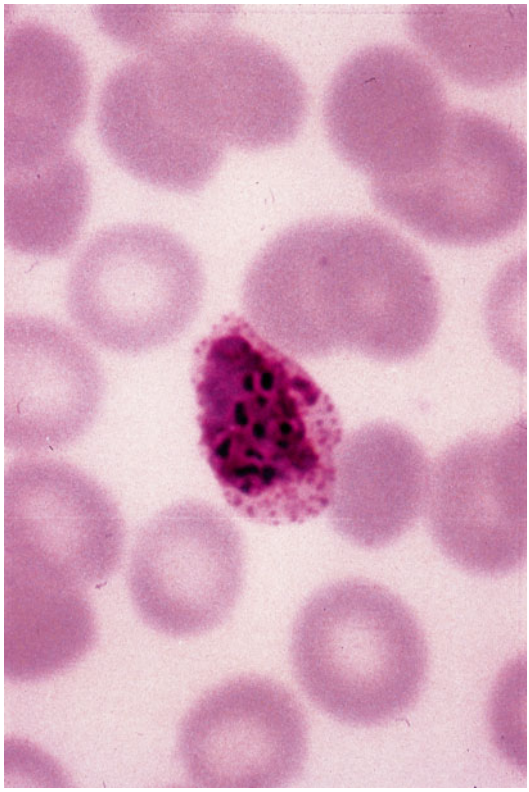

Fig. 1.7 *Plasmodium vivax*. Light micrograph of a rather large Giemsa stained schizont inside a red blood cell measuring about 6 µm in diameter

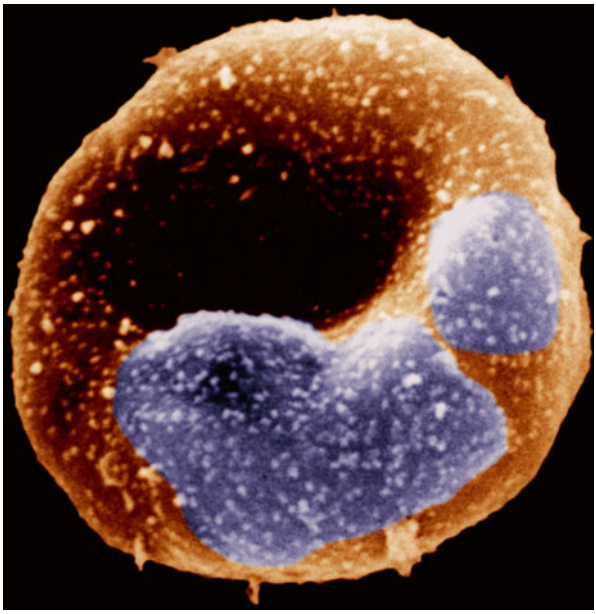

Fig. 1.8 *Plasmodium falciparum*. Scanning electron micrograph showing two protruded schizonts (~2–3 µm) and the typical whitish appearing surface knobs as a characteristic of an infected red blood cell

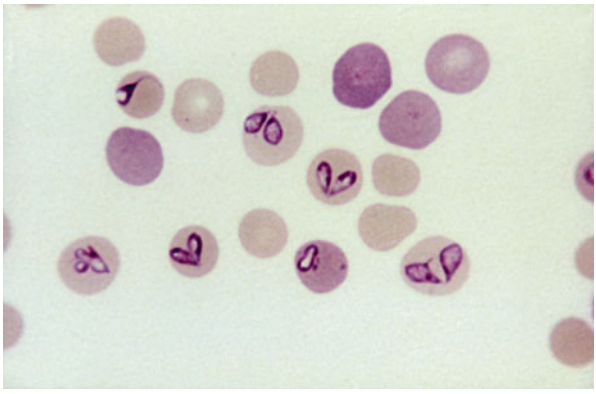

Fig. 1.9 Light micrograph of a Giemsa stained smear preparation of *Babesia canis* stages (mostly in final stage of divisions) in dog red blood cells. They measure about 4–5 µm in length

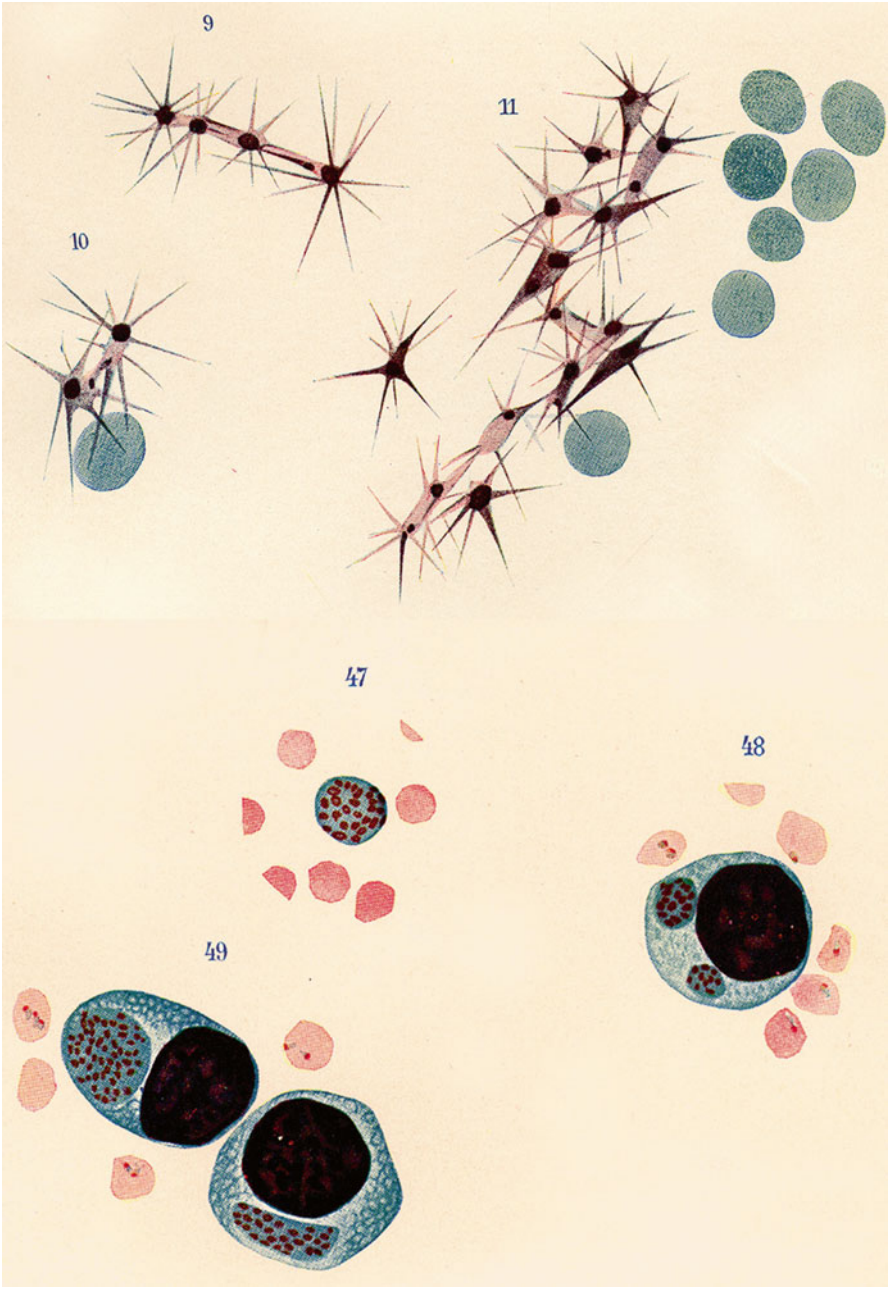

Fig. 1.10 Representation of a drawing of Robert Koch showing in Figs. *9*, *10*, *11* smear preparations of ray bodies = gametes of *Theileria parva* besides erythrocytes in tick intestine. Figures *47*, *48*, *49* show growing schizonts in lymphocytes besides erythrocytes (From Grüntzig and Mehlhorn 2010)

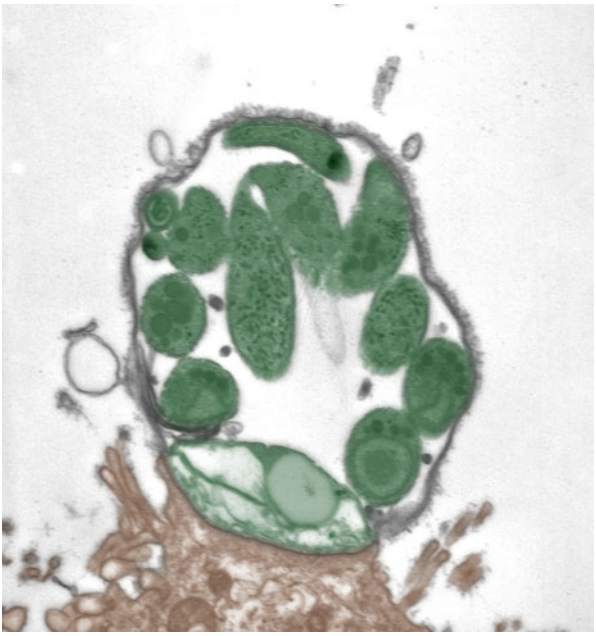

Fig. 1.11 Transmission electron micrograph of a section through a schizont of *Cryptosporidium parvum* (Apicomplexa) showing merozoites with cross sections less than 0.8 µm in diameter

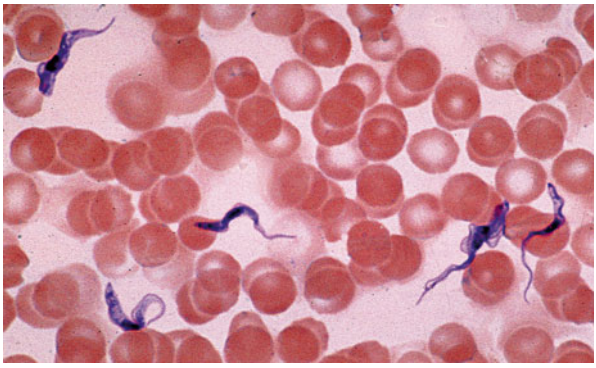

Fig. 1.12 *Trypanosoma brucei rhodesiense*. Light micrograph of Giemsa stained flagellates scattered among human red blood cells (the latter have diameters of about 7–8 µm)

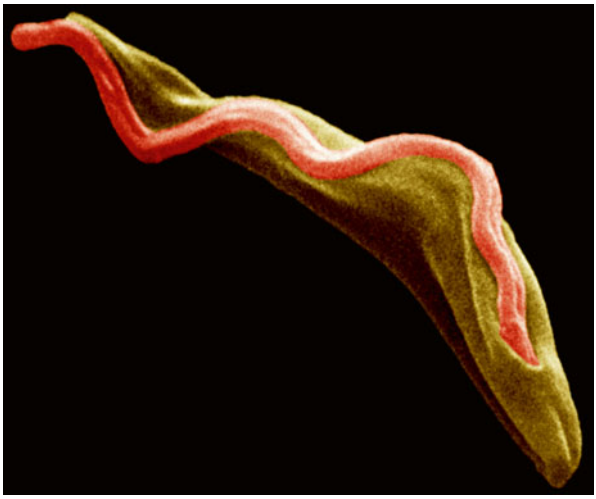

Fig. 1.13 *Trypanosoma brucei*. Scanning electron micrograph of a blood stage showing the typical flagellum, which protrudes at the posterior end of the parasite and becomes free at the anterior one. These stages measure about 20 μm in length

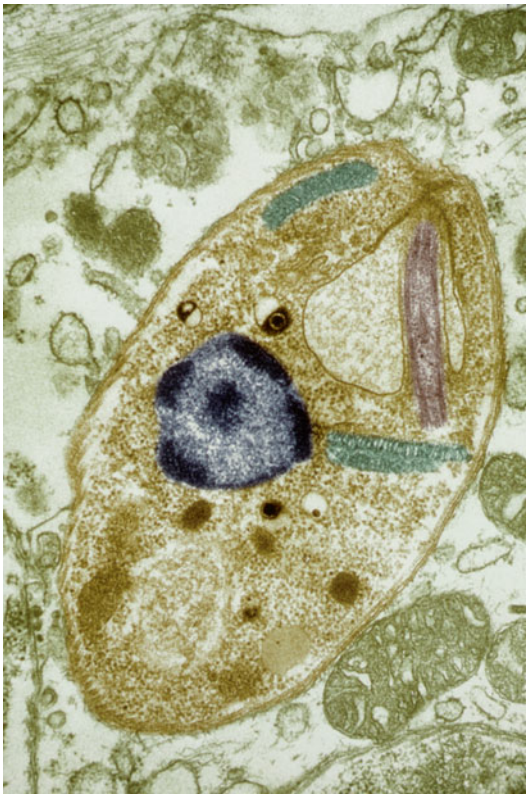

Fig. 1.14 *Leishmania tropica*. TEM of a longitudinal section through a 2 μm sized amastigote stage. Note the blue colored nucleus of the parasites and the reduced flagellum (therefore the correct description is: micromastigote stage)

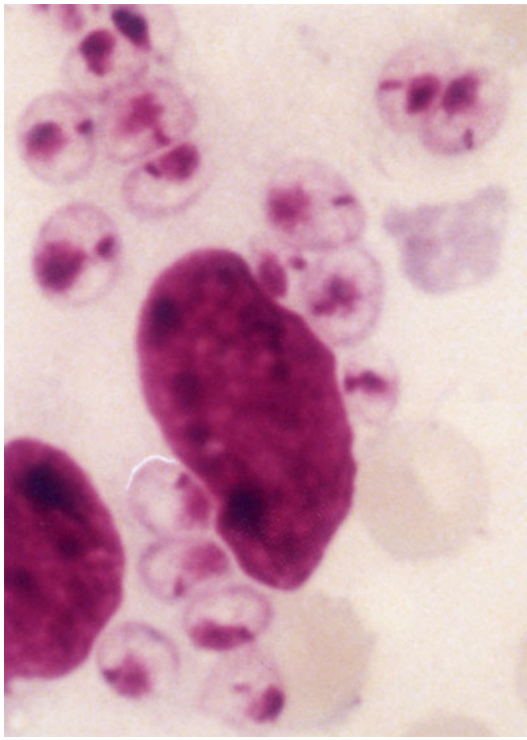

Fig. 1.15 *Leishmania tropica*. Light micrograph of the remnants of a Giemsa stained macrophage, which was destroyed by 2 µm sized *Leishmania* parasites now surrounding the large host cell nucleus

Table 1.2 Size examples of Rickettsiales and bacteria

Organism	Vector	Size (µm)	Disease
Rickettsia prowazeki (R)	Lice	0.8–2×0.3–0.5	Spotted fever
Rickettsia typhi (R)	Fleas	0.8–2×0.3–0.5	Murine spotted fever
Rickettsia rickettsi (R)	Ticks	0.8–2×0.3–0.5	Rocky Mountain spotted fever
Rickettsia conori (R)	Ticks	0.9–1.6×0.3–0.5	Mediterranean fever
Rickettsia africae (R)	Ticks	1×0.4	African tick bite fever
Orienta tsutsugamushi (B)	Mites	1–1.5×0.5	Tsutsugamushi fever
Ehrlichia chaffeensis (B)	Ticks	0.3–0.5	Ehrlichiosis
Anaplasma phagocytophilium (B)	Ticks	0.3–0.5	Anaplasmosis
Coxiella burneti (B)	Ticks	0.3–0.5	Q–Fever
Borrelia burgdorferi (B)	Ticks	20–30×0.2–0.3	Borreliosis
Yersinia pestis (B)	Fleas	1–2×0.5	Plague

R Rickettsia stage, *B* bacteria

Table 1.3 Size of protozoan parasites

Species	Stage	Vector	Size (µm)	Disease
Trypanosoma brucei gambiense	Flagellated blood stage	*Glossina* fly	~18	Sleeping sickness
Trypanosoma cruzi	Flagellated blood stage	Bugs, e.g. *Triatoma* spp.	~18	Chagas disease
Leishmania tropica	Amastigote	Sand fly	~2–4	Leishmaniasis
Plasmodium species	Erythrocytic merozoite	*Anopheles* mosquitoes	~2	Malaria
Babesia species	Erythrocytic merozoite	Ticks	~2–6	Babesiosis
Theileria species	Erythrocytic merozoite	Ticks	~0.7–2.0	Theileriosis
Toxoplasma gondii	Trophozoite	–	7	Toxoplasmosis

Table 1.4 Methods, microscopes and techniques to detect and describe nano-sized particles (selection)

Optical methods	Electron microscopy	Scanning probe microscopy	Other techniques
Light microscopy Confocal microscopy X-ray microscopy UV/Infrared microscopy Spectroscopy Surface enhanced raman spectroscopy	Transmission electron microscopy (TEM) Scanning electron microscopy (SEM)	Atomic force microscopy (AFM) Scanning near-field optical microscopy (SNOM) Scanning tunneling microscopy	Low energy electron diffraction (LEED) Nuclear magnetic resonance (NMR) Electron paramagnetic resonance (EPR)

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Chapter 2
Bloodsucking Parasites as Vectors of Agents of Diseases Endangering Human and Animal Health

Heinz Mehlhorn

2.1 Modes of Blood Uptake

Blood sucking arthropods (ticks, mites, insects) inject saliva into the skin of their hosts that has three main tasks:

1. to avoid feeling pain of the host during the injection of the mouthparts and during the meal of the arthopods;
2. to enlarge the host's blood vessels in order to get an optimum amount of blood during sucking;
3. to avoid coagulation and thus to keep the blood fluid during the engorging process.

In order to get the blood in a most easiest way these arthropods have developed two principally different methods. There are two groups of blood ingestion:

2.1.1 Vessel Feeders

Vessel feeders, e.g. mosquitoes, which inject their double channel mouthparts directly into the blood vessel of the host. One channel contains the above described saliva, while the other is used to engorge the fluid kept blood (Figs. 2.16, 2.17, 2.18, and 2.19).

H. Mehlhorn
Department of Parasitology, Heinrich Heine University, Building 26.13.01 Room 42, Universitätsstr. 1, Düsseldorf D-40225, Germany
e-mail: mehlhorn@uni-duesseldorf.de

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2.1.2 Pool Feeders

The members of this group belong to many different groups of arthropods including many genera of *ticks* and *mites* (Figs. 2.1, 2.2, and 2.3), *lice* (Figs. 2.4a–c, 2.5, and 2.6), *bugs* (Figs. 2.7a, b, 2.8, and 2.9), *bloodsucking flies* (Figs. 2.10, 2.11, 2.12, and 2.13), *black flies* (simuliids) (Figs. 2.20a–c, 2.21, and 2.22), *midges* (Figs. 2.23 and 2.24) *tabanids* (Fig. 2.25) and *fleas* (Figs. 2.25, 2.26a, b, 2.27, and 2.28), while typical flies (such as the house fly *Musca domestica* or the wound fly *Lucilia sericata* respectively species of the genera *Sarcophaga* or *Calliphora*) have licking mouthparts (Figs. 2.14 and 2.15). Therefore these (typical) flies cannot pierce the body surface of potential hosts but lick e.g. at surface wounds of their animal or human hosts.

2.2 Shape of Mouthparts

The different mouthparts of the different blood feeding arthropodan species, which are shown on diagrams, light micrographs and on scanning electron micrographs on Figs. 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, 2.12, 2.13, 2.14, 2.15, 2.16, 2.17, 2.18, 2.19, and 2.20 are composed of similar but differently structured appendages at the lower side of the head (Table 2.1; Figs. 2.1, 2.3, 2.4b, c, 2.7a, 2.10, 2.12a, 2.14, 2.16, 2.20a–c, 2.24, and 2.26a, b). The size and shape of these mouthparts have been adapted during evolution according to the specialization with respect to the site of blood uptake and to their own body size.

2.3 Host Finding

Recognition of potential hosts is differently achieved by the members of the various bloodsucker groups. While eyeless ticks (such as *Ixodes* species, Figs. 2.1, 2.2, and 2.3) use their so-called Haller's organ, which is placed at the anterior portion of their two anterior legs and which contains sensilla for the registration of the movements of smells and soil vibration of approaching potential hosts, to attach themselves at this host, ticks with eyes (e.g. *Dermacentor reticulatus*, *Hyalomma* species, *Rhipicephalus* species) are able to recognize potential hosts over distances of up to 15 m and to crawl to them in a rather short time (often within a few minutes).

Mosquitoes (Fig. 2.16, 2.17, 2.18, and 2.19) possess large compound eyes and thus may recognize potential hosts from far, however, final approach and touch down to the skin depends on host-derived volatile smelling compounds, which are as individually specific that the hungry females show clear preferences not only with respect to different groups of hosts (e.g. animals and humans), but differentiate even among individuals of humans so that one person sleeping next to another may be attacked and the second not.

Table 2.1 Differentiation of mouthparts and their function in stages of blood sucking arthropods (insects, ticks)

Taxa	Sucking apparatus			Function during sucking		
Groups, families Blood sucking stages	**Snout sheath**	**Food channel**	**Salivary channel**	**Piercing system**	**Attachment support**	**Piercing unit**
Hemiptera (Bugs)						
Reduviidae **Cimicidae** (All stages)	Labium Labium	Lacinia Lacinia	Lacinia Lacinia	Mandibles Mandibles	Teeth of Mandibles	Lacinia Mandibles
Siphonaptera (Fleas) (Adult ♀,♂)	Labium, Labial palps	Labrum, epipharynx, labium	Lacinia	Lacinia	Lacinial palps	Lacinia
Phthiraptera (Lice)						
Phthiridae **Pediculucidae** (All stages)	Invagination of labium	Hypopharynx	Hypopharynx	Epipharyngial teeth	Epipharyngial teeth	Hypopharynx
Diptera (Flies, mosquitoes)						
Culicidae (Females)	Labium	Labrum, mandibles	Hypopharynx	Labrum, mandibles, lacinia	Lacinial teeth	Lacinia
Ceratopogonidae (midges) (Females)	Labium	Labrum, Lacinia, Mandibles	Mandibles, Hypopharynx	Mandibles	Lacinia	Mandibles
Simuliidae (Black flies) (Females)	Labium	Labrum, Hypopharynx	Hypopharynx	Labrum, Mandibles	Lacinial teeth	Mandibles, Lacinia
Psychodidae, **Phlebotomidae** (Females)	Labium	Labrum, Mandibles, lacinia	Mandibles, Hypopharynx	Mandibles	Lacinia	Mandibles
Tabanidae (Females)	Labium	Labrum, mandibles	Hypopharynx	Mandibles, Lacinia	Lacinial teeth	Mandibles, Lacinia

(continued)

Table 2.1 (continued)

Taxa	Sucking apparatus			Function during sucking		
Glossinidae (**Tsetse flies**) (Males, females)	Maxillar palps	Labrum	Hypopharynx	Praestomal teeth	Praestomal teeth	Praestomal teeth
Muscidae (***Stomoxys***) **Stable fly** (Males, females)	Absent	Labrum	Hypopharynx	Praestomal teeth	Praestomal teeth	Praestomal teeth
Lepidoptera (**Butterflies**, **genus *Calyptra***) (Males)	Absent	Galeae	Galeae	Tips of galeae	Galea sensillae	Galeae
Acari (**Ticks**) **Ixodidae** **Argasidae** (All stages)	Pedipalps	Base of cheliceres, hypostome	Base of cheliceres, hypostome	Hypostome	Hypostomal teeth	Cheliceres

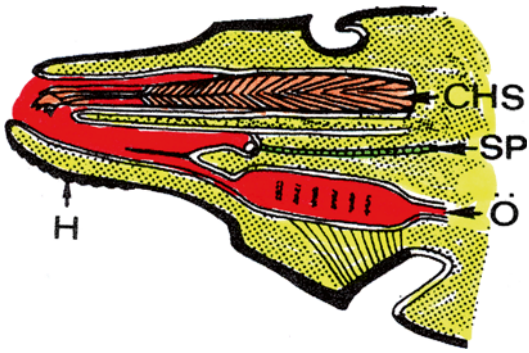


Fig. 2.1 Diagrammatic representation of a longitudinal section through the mouthparts of a tick (e.g. *Dermacentor* sp.). *CHS* muscle system of the sheath of the retractable cheliceres, which cut a hollow into the skin of the host, *H* hypostome, *Ö* esophagus filled with ingested blood, *SD* salivary duct

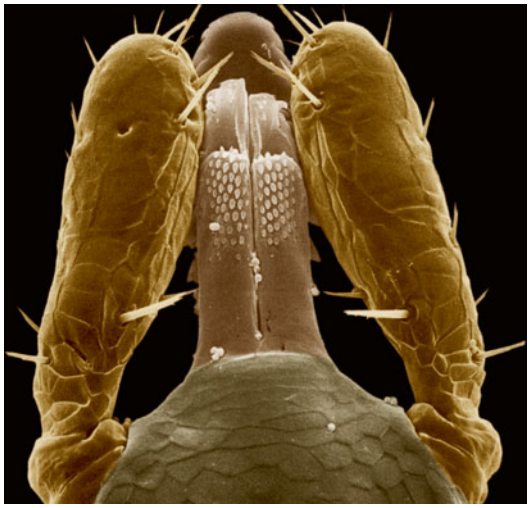


Fig. 2.2 Scanning electron micrograph of the anterior portion of an adult female of *Ixodes ricinus*

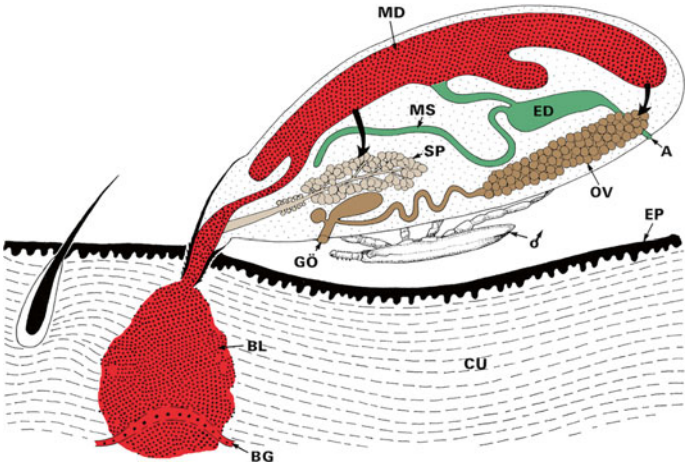


Fig. 2.3 Diagrammatic representation of a female and male ixodid tick in copulation during attachment at a host. *A* anus, *BG* blood vessel, *BL* blood lacune, *CU* cutis, *ED* terminal portion of intestine, *EP* epidermis, *GÖ* female genital opening, *MS* channel of excretion system, *OV* ovary, *SP* salivary gland

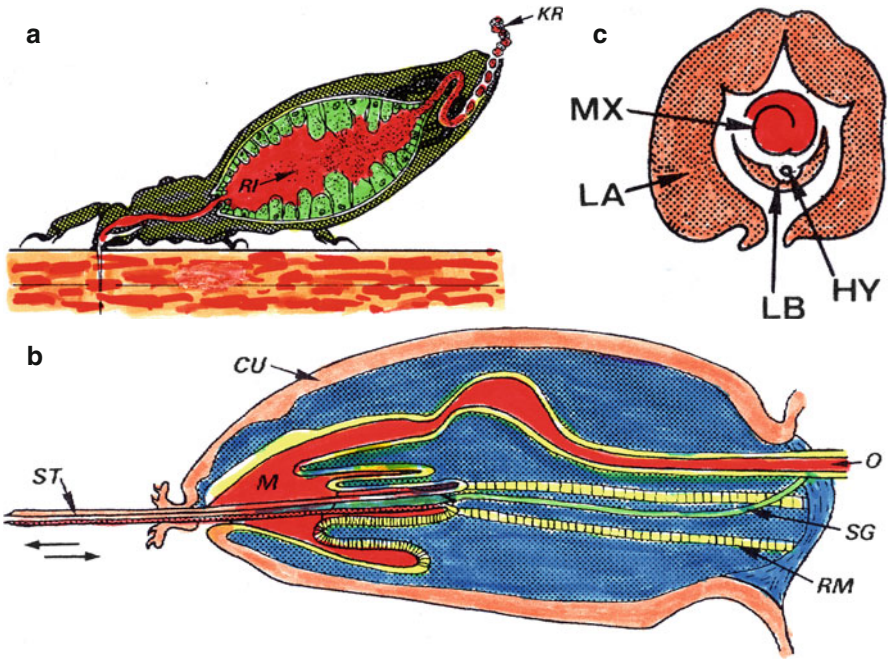


Fig. 2.4 (**a**–**c**) Sucking apparatus of lice. (**a**) Diagrammatic representation of a body louse during blood sucking. (**b**) Longitudinal section through the head of lice showing the sucking apparatus. (**c**) Cross section through the anterior portion of the head of a louse. *CU* cuticle, *HY* hypopharynx, *KR* fecal particle, *LA* labrum (upper lip), *LB* labium, *M* mouth hollow, *MX* maxilla (surrounding ingested blood (*red*)), *O* esophagus, *RI Rickettsia* stages (only in *Pediculus humanus corporis*), *RM* retractor muscles, *SG* salivary gland excretion channel, *ST* sting-like system formed by labium, maxilla and hypopharynx

Fig. 2.5 Light micrographs of females (**a**, **b**) and a male (**c**) of *Pediculus humanus capitis*

Fig. 2.6 Scanning electron micrograph of a female adult louse of *Pediculus humanus corporis* and eggs attached at cloths

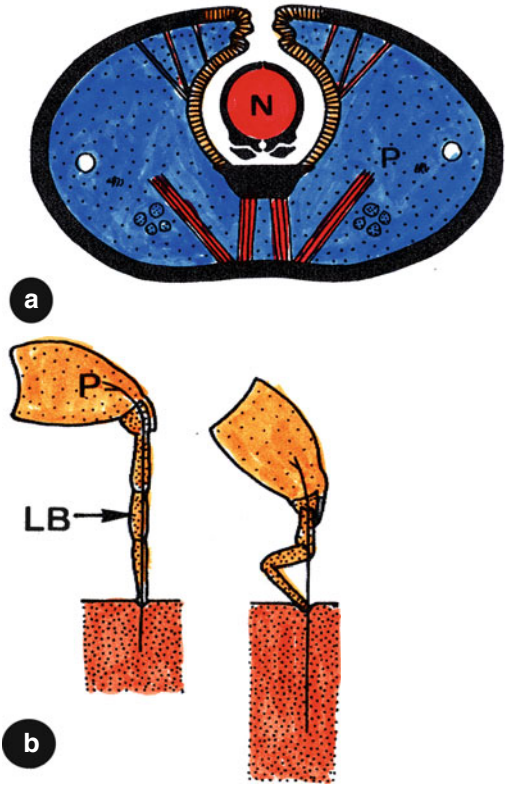


Fig. 2.7 Bed bug (*Cimex lectularius*). (**a**) Cross section through the proboscis, which includes the mouthparts in a channel. Both maxillae form together two channels: the larger upper one is used for transportation of the ingested blood (*N*), while the small one (below) introduces/transports the saliva into the wound during sucking. Below these channels the maxillae are cross-sectioned. *N* food channel, *P* proboscis. (**b**) Diagrammatic representation of the phases of injection of the mouthparts into the host's skin. *LB* labium (erectable mouthpart channel), *P* proboscis

Fig. 2.8 Light micrograph of a bed bug (*Cimex lectularius*) and two eggs just excreted

Fig. 2.9 Light micrograph of a raptor bug (*Triatoma* sp.)

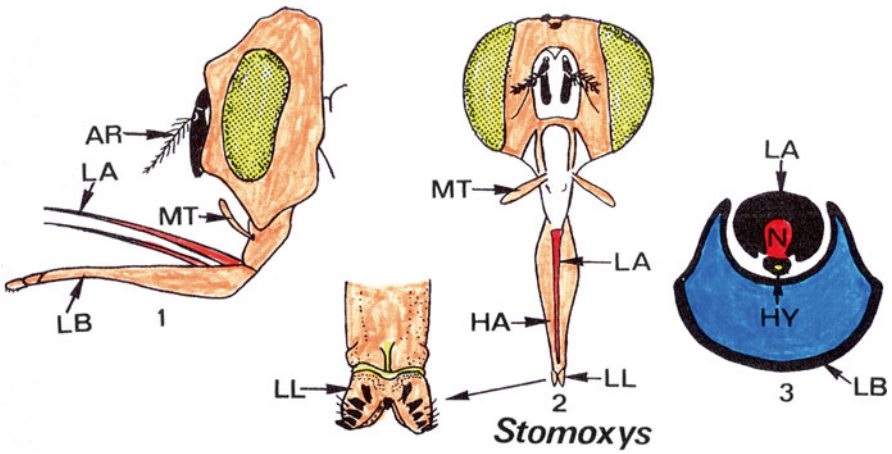


Fig. 2.10 Diagrammatic representation of the mouthparts of *Stomoxys calcitrans* (stable fly). *1* Lateral aspect of the head. *2* Frontal aspect of the head. *3* Cross-section through the labium (*LB*) containing the blood sucking system. *AR* arista and antenna, *HA* haustellum, *HY* hypopharynx with saliva channel, *LA* labrum, *LB* labium, *LL* labellum with grooves to distribute the saliva, *MT* maxillar toucher, *N* food channel

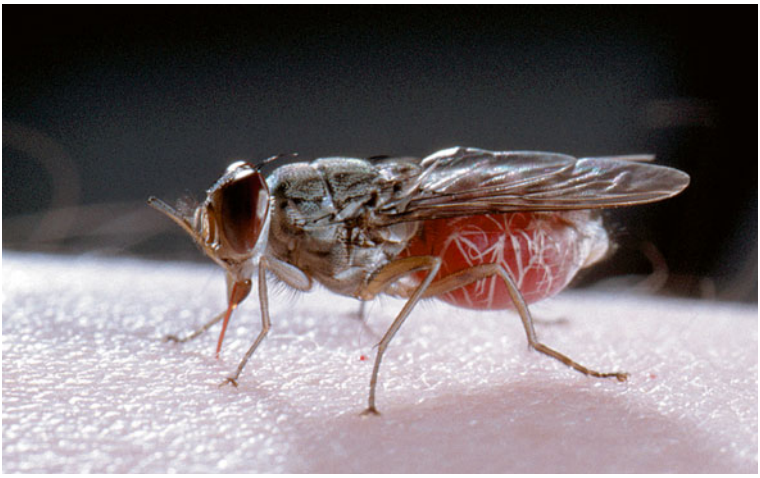


Fig. 2.11 Macrophoto of a stable fly (*Stomoxys calcitrans*)

Fleas (Figs. 2.26a, b, 2.27, and 2.28) possess a single ocellum at both sides of their laterally depressed head, but their viewing capacity is rather limited. Thus soil/floor shakings due to movements of potential hosts are the main registration signs for their host selection. Their second famous ability – jumps of up to 30 cm in length and height – then help them to reach the host. However, also the

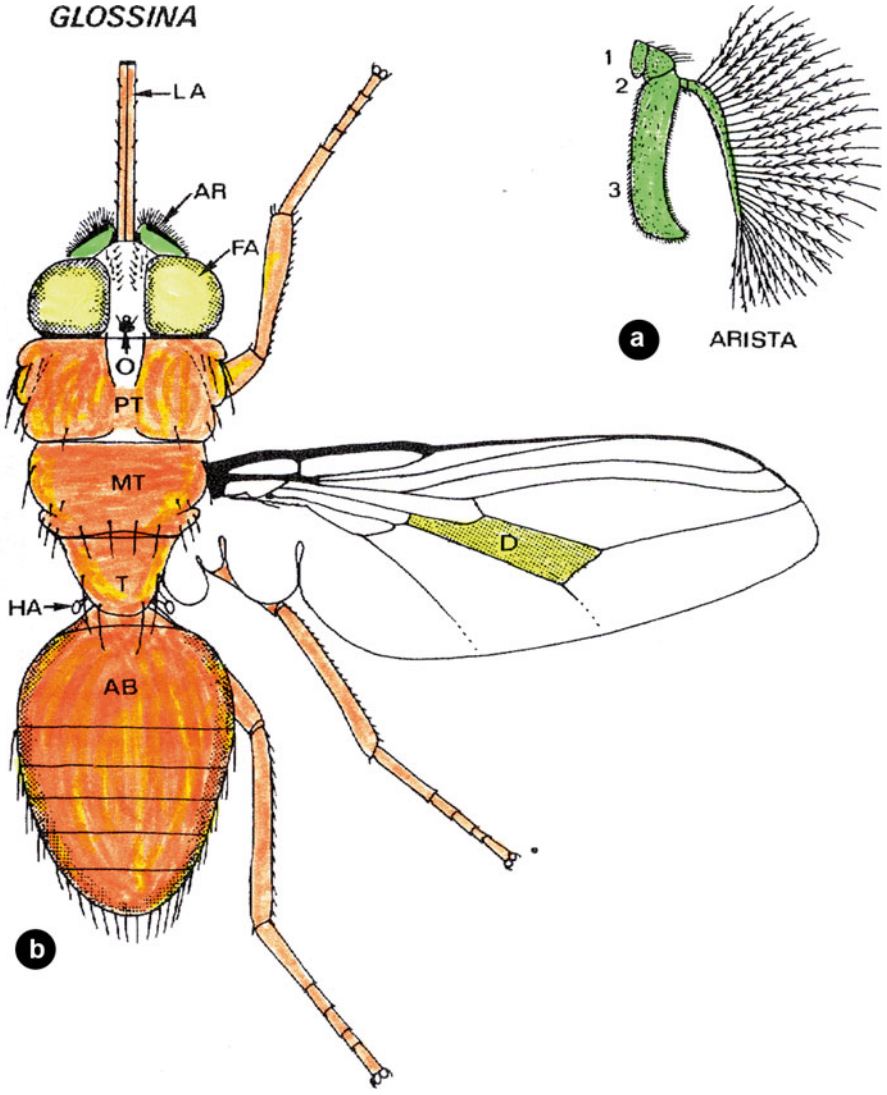
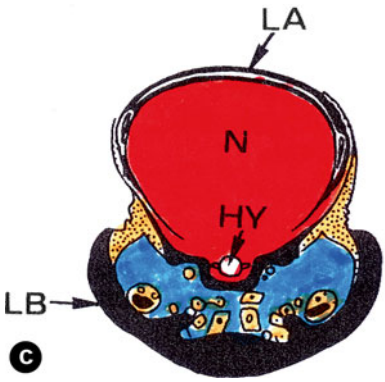


Fig. 2.12 Diagrammatic representation of a tsetse fly and its mouthparts. (**a**) Total dorsal aspect; (**b**) Antenna with three segments and arista; (**c**) cross-section through blood sucking system. *AB* abdomen, *AR* arista, *D* discoidal field of wings, *FA* compound eye, *HA* haltere, *HY* hypopharynx with saliva channel, *LA* labrum, *LB* labium, *MT* mesothorax, *N* food channel, *O* ocellus, *PT* prothorax, *T* metathorax

Fig. 2.12 (continued)



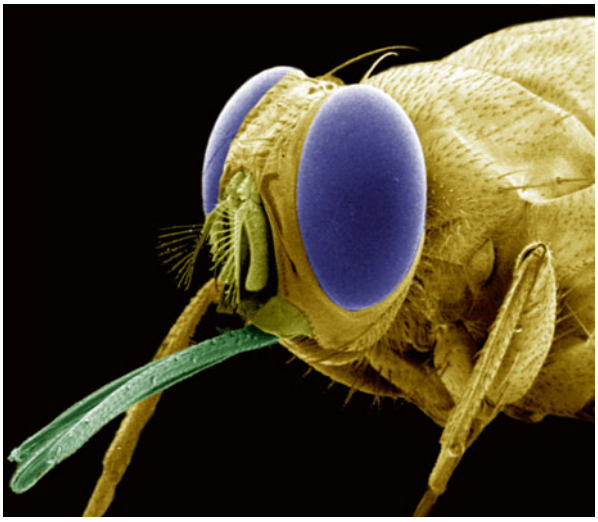


Fig. 2.13 Scanning electron micrograph of the sucking apparatus of a tsetse fly (*Glossina* sp.)

odor of a host is a very important criterion, since there are clear preferences: a cat flea will enter predominantly the hair of the cat if there is a choice between man and cat.

Female black flies (simuliids) (Figs. 2.21 and 2.22) and midges (Figs. 2.23 and 2.24) as well as tabanids (Fig. 2.25) and males and females of biting flies (Figs. 2.11, 2.12, 2.13, and 2.25) possess each two large compound eyes and thus are able to recognize their potential hosts mainly by aspects of their movements at rather large distances. Thus it is common that they follow cyclists or moving hosts or recognize even from far tail movements of cattle and horses (Figs. 2.26, 2.27, and 2.28).

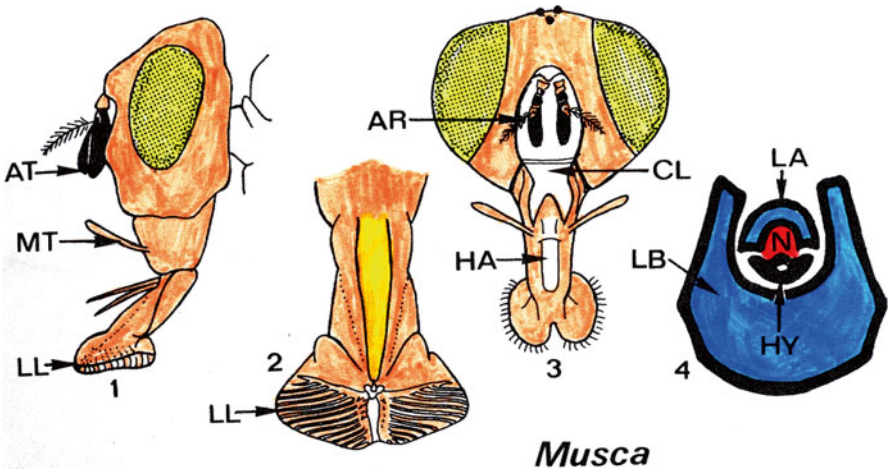


Fig. 2.14 Diagrammatic representation of the mouthparts of *Musca domestica* (house fly). *1* Lateral aspect of the head and mouthparts; *2* Labellum; *3* Frontal aspect of head; *4* Cross-section of the mouthpart channel system. *AR* arista, *AT* antenna, *CL* clypeus, *HA* haustellum, *HY* hypopharynx with saliva channel, *LA* labrum, *LB* labium, *LL* labellum with grooves to distribute the saliva, *MT* maxillar toucher, *N* food channel

Fig. 2.15 Light micrograph of a fly (*Musca domestica*)

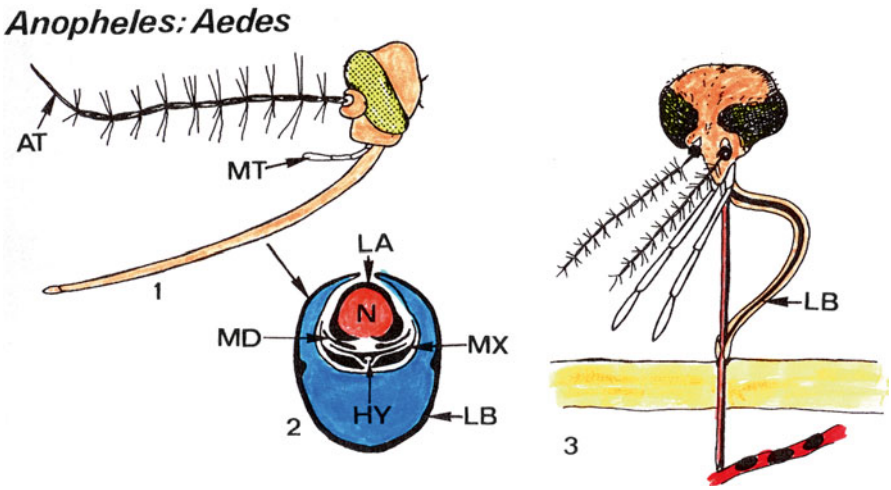


Fig. 2.16 Diagrammatic representation of the mouthparts of a mosquito. *1* Lateral aspect of head (female); *2* Cross-section through blood sucking mouthparts: *3* Injection of the mouthparts into a blood vessel of the host. *AT* antenna, *HY* hypopharynx with saliva channel, *LA* labrum, *LB* labium, *MD* mandible, *MT* maxillar toucher, *MX* maxilla, *N* food channel

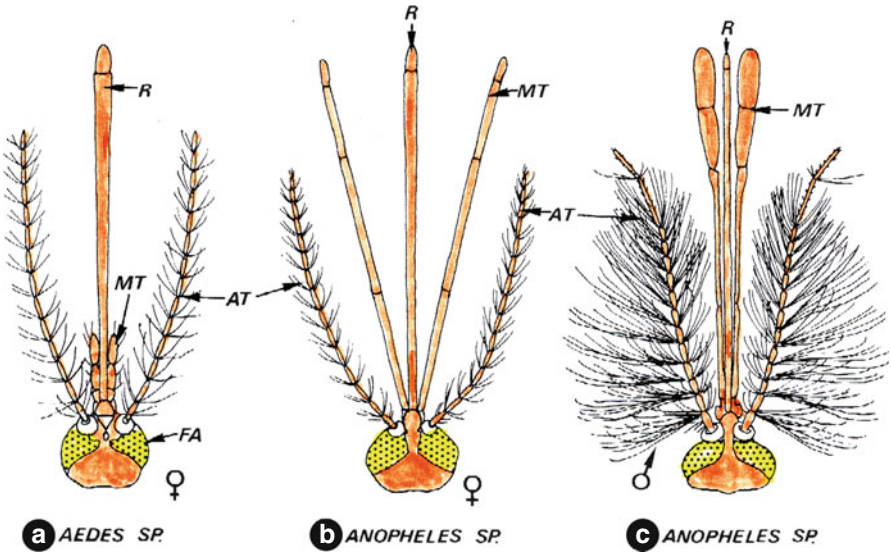


Fig. 2.17 Diagrammatic representation of the head and mouthparts of mosquitoes (**a**–**c**). Note that the antennae of males have a bushy aspect (**c**). *AT* antenna, *FA* compound eye, *MT* maxillar toucher, *R* labium

Fig. 2.18 Light micrograph of a female *Anopheles stephensi* mosquito, a potential vector of agents of malaria

Fig. 2.19 Scanning electron micrograph of a female *Anopheles stephensi* mosquito

2.4 Types of Transmission of Agents of Disease

Since the mouthparts of all blood feeders, when retracted from the skin of a host after a successful blood meal, contain outer contaminations with remnants of the host blood, a mechanical transmission of agents of diseases is rather easy, especially in cases of a potential transmission of agents of diseases belonging to the groups of small sized viruses, bacteria and protozoans. Of course the success of such an accidental transmission depends on the amount of viable agents of disease inside the

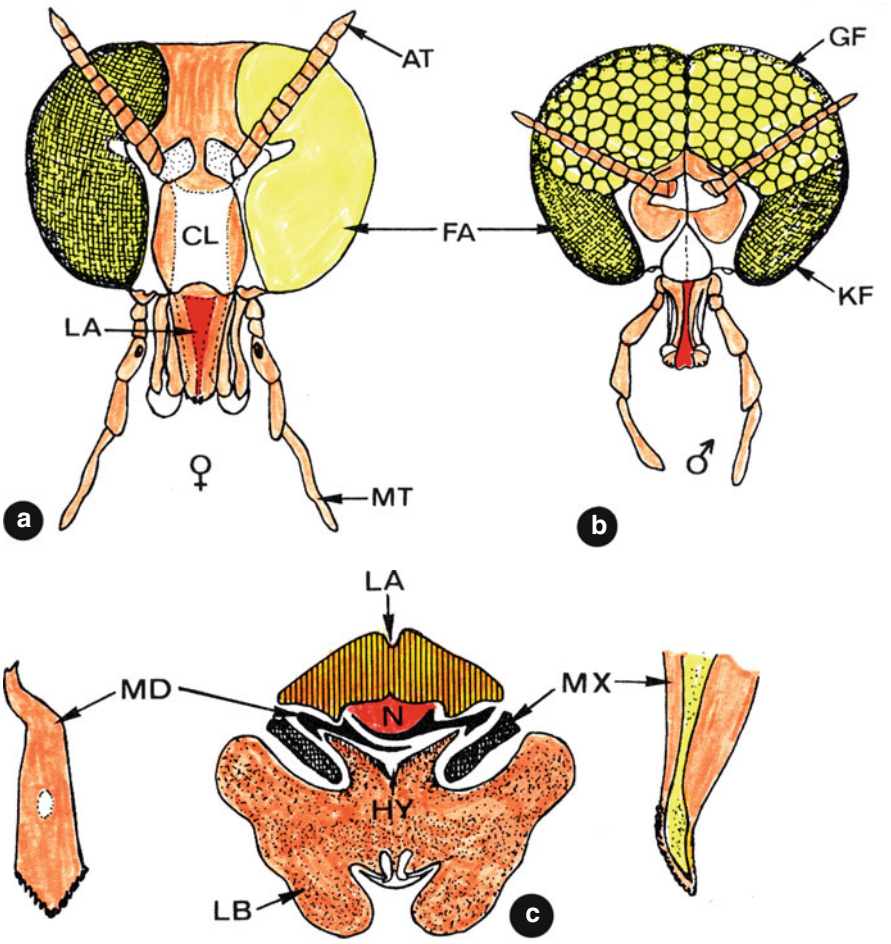


Fig. 2.20 Diagrammatic representation of the head and mouthparts of black flies (*Simulium* sp.). (**a**) Head of female. (**b**) Head of a male. (**c**) Cross-section through mouthparts. *AT* antenna, *CL* clypeus, *FA* compound eye, *GF* large facettes, *HY* hypopharynx with saliva channel, *KF* small facettes, *LA* labrum, *LB* labium, *MD* mandible, *MT* maxillar toucher, *MX* maxilla, *N* food channel

attacked host. But in regions with high rates of endemicity of viruses, bacteria and/or parasites risks due to **accidental transmission** is high (Table 2.2) as can be seen in the recommended literature listed at the end of this chapter.

Cyclic transmission (Table 2.2) of parasites, bacteria and viruses is very common as it is shown in Table 2.2, where, however, only a few examples of the very common and widely spread diseases are listed. Thus billions of humans and their house animals are endangered by arthropod-transmitted diseases. Therefore the individual protection from bites of bloodsucking arthropods by use of repellents is highly needed on one side, but also on the other side trials to eradicate or to reduce the number of arthropod vectors in the surroundings of humans and house animals must be done at the same time (see Chap. 3).

Fig. 2.21 Light micrograph of a black fly (*Simulium* sp.)

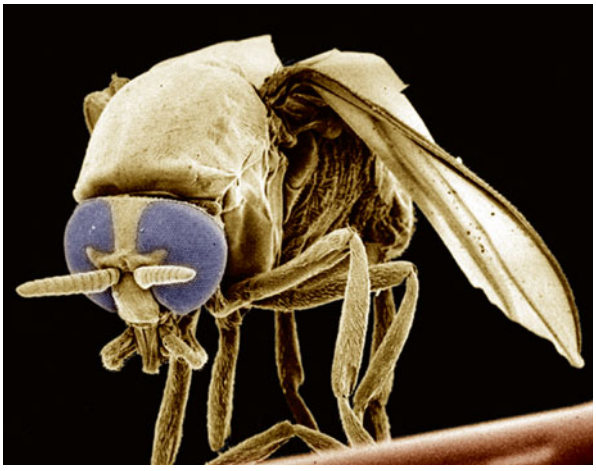


Fig. 2.22 Scanning electron micrograph of a female black fly (*Simulium* sp.)

Fig. 2.23 Light micrograph of a midge (*Culicoides obsoletus*)

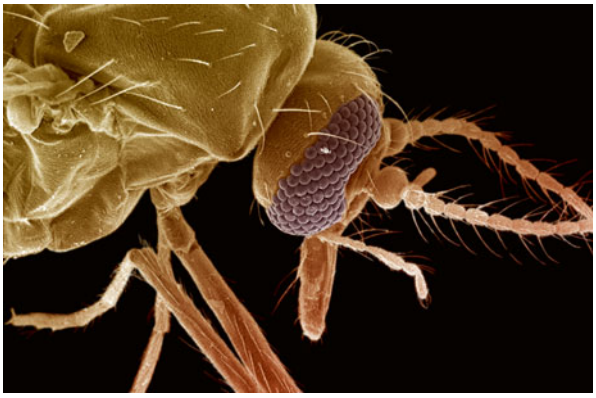


Fig. 2.24 Scanning electron micrograph of a midge (*Culicoides obsoletus*)

Fig. 2.25 Light micrograph of a horsefly (*Chrysops* sp.)

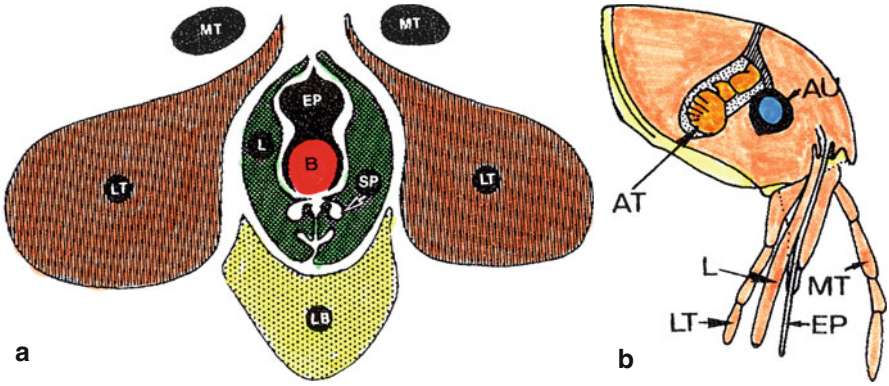


Fig. 2.26 Diagrammatic representation of the head (**a**) and mouthparts (**b**) of fleas. *AT* antenna, *AU* eye, *B* blood inside food channel, *L* lacini, *LB* labium, *LT* labial toucher, *MT* maxillar toucher, *SP* saliva channels

Fig. 2.27 Light micrograph of the human flea (*Pulex irritans*)

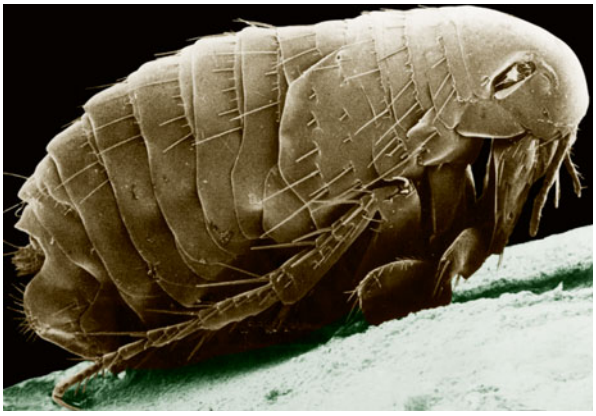


Fig. 2.28 Scanning electron micrograph of the human flea (*Pulex irritans*)

Table 2.2 Transmission of agents of diseases by arthropods (important examples)

Vectors	Cyclic transmission	Accidental by mechanical contamination
Reduviidae (BU)	*Trypanosoma cruzi* (P)	?
Pediculus humanus capitis, *Phthirus pubis* (L)	– –	? –
Pediculus humanus corporis (L)	*Rickettsia prowazeki* (B), *Bartonella quartana* (B), *Borrelia recurrentis* (B)	Hepatitis A, B; other viruses
Anopheles species (M)	*Plasmodium* species (P), Filarial worms (N), Viruses (V)	?
Aedes species (M)	Filarial worms (N), Viruses (V)	?
Culex species	Filarial worms (N), Viruses (V)	?
Simulium species	Arboviruses (V), Filarial worms (N)	Viruses
Culicoides species (MI)	Viruses (e.g. Bluetongue virus in cattle)	Viruses, bacteria
Phlebotomidae (S)	*Leishmania* species (P), Arboviruses (V), *Bartonella bacilliformis* (B)	?
Glossinidae (T)	*Trypanosoma brucei* (P)	Bacteria, viruses
Muscidae (F)	–	Bacteria, viruses, protozoans, worm eggs, fungi
Stomoxyidae (F)	–	Bacteria, viruses, protozoans
Pulicidae (FL)	*Yersinia pestis* (B), *Rickettsia* species (B)	Bacteria, viruses
Noctuidae (BF), males	–	Viruses (hepatitis B)
Argasidae (TI)	*Borrelia* species (e.g. *Borrelia duttoni*) (B)	Viruses, bacteria
Ixodidae (TI)	*Anaplasma* species (B), *Rickettsia* species (B), *Borrelia* species (B), Viruses (e.g. Flavivirus, Nairovirus, Coltivirus, Bunyavirus) (V)	*Coxiella burneti* (B)
Dermanyssidae (MIT)	*Rickettsia acari* (B)	Viruses
Trombiculidae (MIT)	*Orienta tsutsugamushi* (B)	Viruses

According to Krenn and Aspöck (2010)
B bacteria, *BF* butterflies, *BU* bugs, *F* flies, *FL* fleas, *L* lice, *M* mosquitoes, *MI* midges, *MIT* mites, *N* nematode, *P* protozoa, *RI* rickettsiales, *S* sandflies, *T* tsetse fly, *TI* ticks, *V* viruses

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Chapter 3
Available Means to Control Bloodsucking Ticks, Mites and Insects – An Overview

Heinz Mehlhorn

3.1 Introduction

Ticks, mites and insects are important members in the pyramid of living organisms on earth, whereby most species serve as food for predators, while others control the amounts of predators by weakening them or even killing them due to blood sucking or by transmission of agents of diseases. On the other side plants, which belong to the food of numerous insects, have also developed measurements to survive in the struggle for life. These measurements were mainly based on the production and release of substances, which have either repellent or killing effects. Some of the available plant-derived compounds have both properties: repellency and insecticidal/killing activity. At the early days of mankind humans learnt to use the plant-derived compounds for their own protection (Faulde 2010; Nentwig 2003; Amer and Mehlhorn 2006a, b, c, d). However, since these rather specific products have mostly only rather poor or short-lasting effects on insects and other arthropods attacking humans, the latter started to develop chemical repellents and insecticides/arthropodocides, the efficacy of which, however, nowadays is threatened by increasing resistancy among the billion-headed aggressors (Figs. 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6). Thus development of new approaches in the fight against arthropods that endanger health of humans and/or their food production, are highly needed. Therefore the introduction of nanoparticles as carriers of insecticides seem to be a very promising method to overcome recent lacks in arthropod control (Rai et al. 2014).

Humans have developed two main strategies in the fight against arthropods:

(a) Use of so-called repellents (from Latin repellere = to repulse, to push away, reject).

H. Mehlhorn
Department of Parasitology, Heinrich Heine University, Building 26.13.01 Room 42, Universitätsstr. 1, Düsseldorf D-40225, Germany
e-mail: mehlhorn@uni-duesseldorf.de

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(b) Development of so-called arthopodocides/insecticides = products that kill aggressors belonging to the group of ticks, mites or blood-feeding respectively food or material destroying insects.

3.2 Repellents

Especially mosquitoes and flies can be kept away from their blood donors by help of plant-derived repellent compounds or by chemical substances, which both hide the hosts from attacking bloodsuckers by covering the attractive skin odor, which consists of a clearly individual mixture of about 40 compounds such as carboxyl ammonia, lactic acid, carbon dioxide etc. (Smallegange et al. 2005). The special odor of each person or animal, which is more or less intense in the air surrounding a host (and the clothes of humans, too) are noted by a huge number of receptors of an extremely high number of at least five types of sensilla (olfactoric chemoreceptors), which are situated densely on the antennae of blood sucking insects or at the pedipalps and forelegs (Haller's organ) of ticks (Davis 1985; Mehlhorn 2015; Sonenshine 1991, 1992) (Figs. 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6).

3.2.1 Plant Derived Repellents

This group of active compounds includes:

- essential oils,
- short chain fatty acids,
- coconut fatty acids.

Fig. 3.1 Light micrograph of a female mosquito of *Aedes* sp. Note the tiny antennae, which bear, however, numerous very short sensilla

Amer and Mehlhorn (2006c) tested 41 essential oils and summarized as Nentwig (2003) the findings in this field. However, depending on the concentrations rather very short lasting protection rates were found. Increasing the concentrations of

Fig. 3.2 Scanning electron micrograph of the frontal aspect of a female *Anopheles* mosquito showing the long antennae

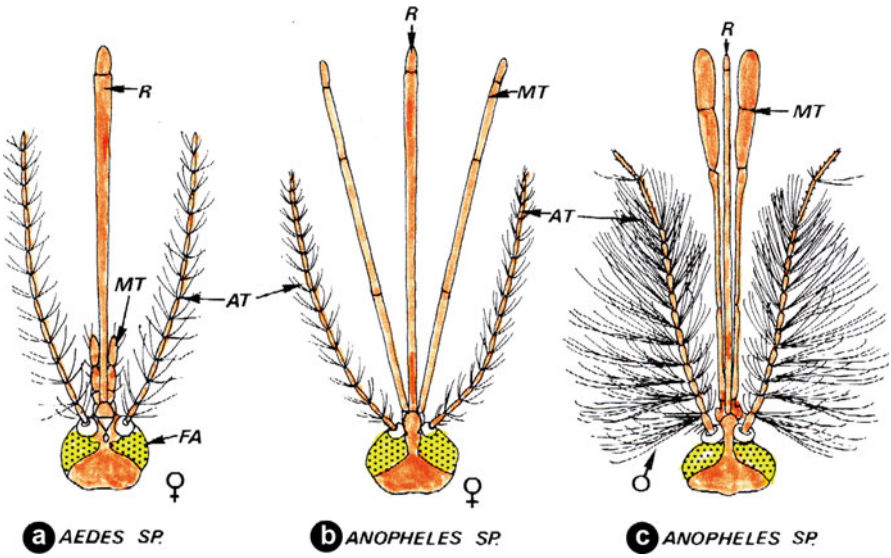

Fig. 3.3 Diagrammatic representation of the head and antennae of male and female mosquitoes. *AT* antennae, *FA* compounds eye, *MT* maxillar toucher, *R* labium

Fig. 3.4 Scanning electron micrograph of the anterior end of a nymph of *Ixodes ricinus* showing the thorny injection system and the pedipalps

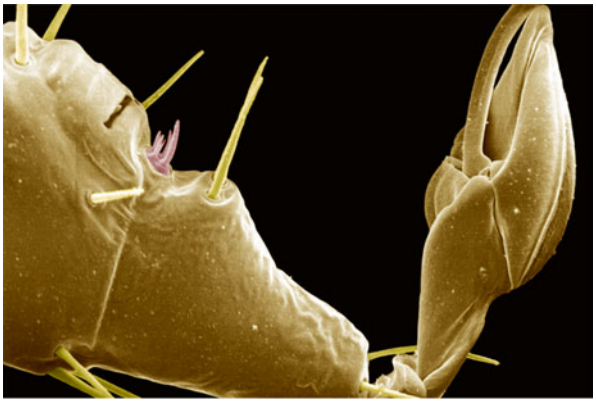

Fig. 3.5 Scanning electron micrograph of the foreleg of *Ixodes ricinus* showing the depression of the so-called Haller's organ with the *reddish* painted tips of sensillae

these plant extracts also increases considerably the risk of development of allergic reactions of the user's body. Thus products based on such extracts leave a considerable risk of the transmission of agents of diseases in regions, where malaria, dengue fever, yellow fever etc. are endemic.

3.2.2 Chemical Derived Repellents

These products, the number of which was recently considerably reduced by legal obligations of the European Community. Thus there are rather few compounds left: e.g. DEET (di-ethyl-toluamid), IR 3535 ((N-n-butyl-N-acetyl)-aminoproprionacidethylester), Icaridin/Saltidin (2-(2-hydroxyethyl)-piperidin-1-carbonic acid-1-methylpropylester), perhaps also an *Eucalyptus* derivative (paramenthan-diol)

Fig. 3.6 Diagrammatic representation of the Haller's organ and its nerves of an *Ixodes* tick. *Blue* nerves, *yellow* glands, *other colors* different types of sensitive nerve cells

Dimethylphthalate (DMP) was the first relevant compound being introduced in 1929 in a wider spectrum of countries. Its activity = protection period, however, was rather short and also only a limited number of arthropod species were kept away from treated bodies. Today this compound is considered as harmful for the skin, but is still used as means to soften and/or dilute other compounds (Legrum 2011; Lorz et al. 2007). Further research activities in this field led to the launching of indol and 2-ethyl-1,3-hexandiol (Rutgers 612) as new repellents (wikipedia.org/wiki/indol). Since these products had also rather weak repellent effects, research went on – especially in the laboratories of the Army of the United States of America. In large series of experiments the substance Diethyltoluamid (DEET) was detected in the year 1942 and first used by American soldiers in Asia during the so-called Korea war (1950–1953). Further intense research activities made it possible that DEET was offered in the year 1955 to the civil population in the USA and in other countries worldwide, where it is up to now the most commonly used repellent (Barnard 2000; Barnard et al. 2012; Nauke et al. 2006; Nentwig 2003), although wrong applications led to severe health damages – even death cases have been reported in relevant scientific journals (Reuveni and Yagupski 1982; Stinecipher and Shaw 1997; Tenebein 1987; Faulde 2010).

These potential health problems due to DEET and also the facts, that this compound leads to a glueing feeling on the skin combined with a bad smelling, that it destroys plastics (e.g. bracelets of watches, belts, shoes etc.) and that it has only a rather poor activity against ticks, made it necessary that many governmental institutes and private chemical companies started intense research projects.

In the year 1969 scientists of Merck company synthetized the chemical compound (N-n-butyl-N-acetyl)-amino-propionacidethylester (IR 3535).

Bayer (Germany) succeeded after long series of thousands of tests by the finding of KBR 3021, which now is named Saltidin, after passing a long list of different compound names (Bayrepel, Propidin, Picaridin, Icaridin etc.). This product was tested worldwide and showed a broad and safe efficacy against an extremely widely spectrum of bloodsuckers besides offering very high health safety parameters (Semmler et al. 2011).

The chemical compound p-menthane-3,8-diol was isolated from oil of the citron-eucalyptus tree (*Eucalyptus maculata citridon*), but has a less long and less broad efficacy against blood suckers compared to DEET and Saltidin/Icaridin.

3.3 Insecticides and Acaricides

If it is needed to control insects, ticks and mites by diminishing their number (and not only to keep them away from human and animal bodies) at given surroundings, the use of insecticides and/or acaricides is obligatory. Most of those biocidal acting compounds have polytoxic effects showing combinations of the following effects (Faulde 2010; Koren et al. 2003; Perrotey et al. 2002; Amer and Mehlhorn 2006a–d):

- killing effects,
- knock-down effects,
- flushing-out effects,
- hot feet effects,
- detaching effects,
- antifeedant effects and even
- repellent effects.

These biocides may be based on

- natural and/or
- synthetized chemical compounds.

3.3.1 Natural Compounds

Natural compounds are included in plant oils (e.g. neem oil), fungal extractions (abamectins), bacterial contents (toxins of *Bacillus thuringensis*) or in dusts produced from diatomeans respectively based on natural pyrethroids obtained e.g. from *Chrysanthemum* plants.

3.3.2 Synthetic Compounds

Synthetic compounds have their origin in

- amorgic substances (e.g. boreal acid) or
- organic chemicals such as pyrethroids (e.g. permethrin, allethrin), carbamates, organophosphates etc.

All these products act primarily neurotoxic on different regions of the peripheral nerve system and/or on the "brain" ganglia of the target organisms by different mechanisms such as e.g.:

– blocking of neurotransmitters,
– blocking of GABA receptors or their antagonists,
– blocking of the acetylcholine esterase.

These synthetical insecticidal compounds have, however, several disadvantages, since

– there exist already a broad spectrum of resistances with respect to many substances,
– several compounds have a very high toxicity,
– rain can wash them down from walls or from fur of animals thus requiring costful repeated treatments,
– they may become transported by hand-mouth contacts into the intestinal tract of humans and thus leading to intoxication,
– may be licked off from walls, fences etc. by cattle, sheep, horses etc. – also increasing the risk of intoxication.

With respect to all these above described problems, the nanotechnology based inclusion of insecticides/acaricides within extremely tiny particles is a very promising technique that will help to solve – or at least – to minimize the above cited problems.

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Chapter 4
Available Compounds, Methods and Means to Control Protozoan and Helminthic Parasites

Heinz Mehlhorn

4.1 Introduction

Parasites in a strict sense belong to the animal phylum groups of

- Protozoa
- Metazoa with its subgroups of
  - Myxosporea
  - Platyhelminthes
    - Trematodes (Mono-, Digenea = flukes)
    - Cestodes (tapeworms)
  - Nemathelminthes (roundworms)
  - Acanthocephala (thorny-headed worms)
  - Annelida (sic leeches)
  - Pentastomida (tongue worms)
  - Arachnida (mites, ticks)
  - Insecta (insects)
  - Crustacea (crustaceans)

The members of the animal group of parasites have developed highly sophisticated methods to enter potential hosts (humans, animals), to survive inside their hosts and to give rise to offspring, which is able to survive outside the host. Thus a successful elimination of infections by parasites needs strategies that comprise a broad spectrum of measurements. The following approaches are needed:

– development of broad spectrum and specialized diagnostic methods,

H. Mehlhorn
Department of Parasitology, Heinrich Heine University, Building 26.13.01 Room 42, Universitätsstr. 1, Düsseldorf D-40225, Germany
e-mail: mehlhorn@uni-duesseldorf.de

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- development of medicaments to treat internal parasitosis,
- development of acaricides and insecticides,
- development of repellent compounds, which prevent skin penetration,
- development of immunological protection by vaccines,
- development of methods and tools of cleaning, sterilization respectively disinfection of working places, equipment, dwellings, hospitals, stables etc.,
- development of prophylactic protection from infections by establishment of hygienic standards and their constant control and amelioration as well as in stables, dwellings and in surroundings of humans and farmed animals,
- development of methods of elimination of human and animal feces,
- development of methods to avoid entrance of particles of human and animal feces in lakes, rivers and drinking and bathing water,
- development of urgency plans to minimize consequences in case one of the described targets had been missed.

4.2 Available Medications against Parasites (Selected Compounds)

4.2.1 Chemical Compounds

4.2.1.1 Antiprotozoal Compounds and Target Species and Genera

- Albendazol (*Giardia lamblia, Echinococcus* species)
- Allopurinol (*Leishmania* species)
- Amphothericin (*Acanthamoeba*, *Naegleria*)
- Atovaquon-Proguanil (*Plasmodium* species)
- Chloroquin (*Plasmodium vivax*)
- Clindamycine (*Toxoplasma gondii*, *Neospora*, *Babesia*)
- Cotrimoxazole (*Isospora belli*, *Cyclospora cayetanensis*, *Enterocephalitozoon* sp.)
- Doxycyclin (*Plasmodium* species)
- Diclazuril (Coccidia)
- Eflornithin (*Trypanosoma* species)
- Emodepsid + Toltrazuril (*Isospora*)
- Fenbendazole (*Giardia*, *Encephalitozoon*)
- Furazolidon (*Giardia*)
- Halofuginone (Coccidia, *Theileria* species)
- Imidocarb (*Babesia* species, *Hepatozoon* species)
- Lasalocid (Coccidia)
- Manduramycin (Coccidia)
- Mefloquin (*Plasmodium* species)
- Meglumantimonat (*Leishmania donovani*)
- Metronidazole (*Giardia*, *Blastocystis*, *Trichomonas vaginalis*)
- Miltefosin (*Leishmania* species)

- Monensin (Coccidia)
- Narasin (Coccidia)
- Nifurtimox (*Trypanosoma cruzi*)
- Nitroimidazole (*Balantidium coli*, *Entamoeba histolytica*)
- Pyrimethamine + Sulfonamides (*Toxoplasma gondii*)
- Pyrimethamine (*Toxoplasma gondii*)
- -Robenidin (*Eimeria* species)
- Salinomycin (Coccidia)
- Semduramycin (Coccidia)
- Sodium stibogluconate (*Leishmania donovani*, *Leishmania* species)
- Spiramycin (*Toxoplasma gondii*)
- Sulfadiazin (*Toxoplasma gondii*)
- Suramin (*Trypanosoma cruzi*)
- Toltrazuril (*Eimeria* species)

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Chapter 5
Phytosynthesis of Metal and Metal-Oxide Nanoparticles – Technological Concepts and Their Biomedical Applications

Govindasamy Rajakumar and Abdul Abdul Rahuman

Abbreviations

AFM	Atomic force microscopy
Ag NPs	Silver nanoparticles
Au NPs	Gold nanoparticles
Cu NPs	Copper nanoparticles
DNA	Deoxyribonucleic acid
FTIR	Fourier transform infrared spectroscopy
MNPs	Metal nanoparticles
MONPs	Metal oxide nanoparticles
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
NPs	Nanoparticles
PCR	The polymerase chain reaction
Pd NPs	Palladium nanoparticles
ROS	Reactive Oxygen Species
TiO_2 NPs	Titanium dioxide nanoparticles
UV	Ultraviolet-visible
ZnO NPs	Zinc oxide nanoparticles

G. Rajakumar • A.A. Rahuman (✉)
Unit of Nanotechnology and Bioactive Natural Products, Post Graduate and Research Department of Zoology, C. Abdul Hakeem College,
Melvisharam, Vellore District, Tamil Nadu 632 509, India
e-mail: abdulrahuman6@hotmail.com

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5.1 Introduction

The field of nanotechnology is a standout amongst the most dynamic ranges of in cutting edge materials science. New uses of nanoparticles and nanomaterials are rising quickly (Jahn 1999; Dickson 1999; Naiwa 2000). Nanotechnology is perceived as a progressive assembling innovation of the twenty-first century, including multidisciplinary exploration issues that depend on the comprehension and control of substances at the nano-scale length. Human hair and red blood cell, are pretty nearly 80,000 and 7000 nm wide, are littler than 1 nm, though numerous particles including a few proteins range somewhere around 1 nm and bigger (Whitesides 2003). Nanotechnology includes the customizing of materials at the atomic level to achieve remarkable properties, which can be suitably manipulated, controlled for the craved applications (Gleiter et al. 2003). The center for phytosynthesis has moved from physical and compound procedures towards green science and bioprocesses (Vigneshwaran et al. 2007). As of now, there is a developing need to grow naturally big-hearted nanoparticle union procedures that don't utilize dangerous chemicals in blend convention (Whitesides 2003). Nanotechnology has made a sort of upheaval as this new zone includes physical science, science, materials science, building, science and prescription. A few applications are imagined from these intriguing materials in the field of sensors (Haes and Van Duyne 2004), catalysis (Roucoux et al. 2002), symptomatic instruments (Rosi and Mirkin 2005), remedial operators (Chen and Wang 2006), medication/quality conveyance vehicles (McAllister et al. 2003), sun based cells (Anderson and Lian 2005), plasmonics gadgets (Zou and Schatz 2006), beautifiers (Cengiz et al. 2006), covering materials (Baglioni and Giorgi 2006), phone imaging (El-Sayed et al. 2005), power modules (Fichtner 2005) and photonic band crevice materials (Moran et al. 2004).

5.1.1 Nanoscaled Materials

The general vision of nanoscience depends unequivocally on the capacity of making and controlling matter at the nanoscale (Murphy 2002). The examination in nanoscaled matter started to become exponentially when it got to be perceived that the mass properties of materials change radically as their sizes diminish from the mass material to small clusters of atoms (Service 2004). Suitable control of the properties of nanometer-scale structures can prompt new science and in addition new items, gadgets and advancements (Rao and Cheetham 2001). Two important variables are in charge of bringing on the properties of nanoscaled materials to vary fundamentally from their conduct in mass condition. The expanded relative surface zone and size ward properties start to rule when the matter is lessened to the nanoscale. These impacts can't just change the synthetic reactivity and quality definitely, additionally the electrical, optical and warm attributes.

5.1.2 Synthesis of Nanoparticles

The synthesis of inorganic nanomaterials has been exhibited by a few systems including physical, concoction and organic. A portion of the physical courses prompting fruitful combination of nanophase materials, particularly the noble metal nanoparticles include vapour deposition (Choi et al. 2002; Perekrestov 2005), thermal decomposition (Hou et al. 2004; Lee and Kang 2004), spray pyrolysis (Kim et al. 2002; Suh and Suslick 2005), photo irradiation (Esumi et al. 1995; Sakamoto et al. 2006; Jia et al. 2006), laser removal (Cai et al. 1998; Zhu et al. 2006), ultrasonication (Suslick et al. 1996; Wu et al. 2006), radiolysis (Doudna et al. 2003) and solvated metal molecule scattering (Davis and Klabunde 1982; Stoeva et al. 2002). Nonetheless, substance techniques for synthesis of metal nanoparticles have been better known and have increased wide acknowledgement. A portion of the regular concoction courses incorporates a sol-gel system (Devarajan et al. 2005; Shukla and Seal 1999), solvothermal (Gao et al. 2005) and galvanic replacement reaction (Liang et al. 2004; Shukla et al. 2005). Chemical reduction has been the most popular famous course towards blend of metal nanostructures because of simple conventions and the fine shape and size control gave by this strategy. The control over size, shape, dependability and the gathering of nanoparticles is accomplished by fusing distinctive topping specialists, solvents and layouts. Topping operators that have been utilized, range from straightforward particles to polymeric atoms and even biomolecules (Toshima et al. 1991; Toshima and Wang 1994).

Albeit, in the course of recent decades, physical and chemical methods have commanded the amalgamation of nanostructures, as of late significant consideration has been paid towards the utilization of organic frameworks. Natural frameworks have been known not mind boggling structures at the small scale and nano-scales with exact control in typical ecological conditions. The wonderful siliceous exoskeletons of the diatoms and radilarians (Mann 1993; Kröger et al. 1999) and calcareous structures orchestrated by the coccoliths (Young et al. 1999) were micro scale materials, which have pulled in enormous hobby. This has attracted researchers to comprehend the hidden instruments utilized by the organic frameworks and consequently, investigate the biomimetic methodology towards combination of nanomaterials. Opened up the doors for the utilization of organic frameworks for the combination of silver nanoparticles (Ag NPs) accounted for by Klaus et al. (1999) utilizing the periplasmic space of the microbes *Pseudomonas stutzeri* (AG259). A few microscopic organisms *Escherichia coli*, *Pseudomonas aeruginosa* (Sondi and Salopek-Sondi 2004; Konishi et al. 2004; Husseiny et al. 2007; Hernández-Sierra et al. 2008), S-layer microorganisms like *Pseudomonas stutzeri* AG259 (Pum and Sleytr 1999; Sleytr et al. 1999), fungi like *Fusarium oxysporum*, *Colletotrichum* sp., *Aspergillus fumigatus* (Mukherjee et al. 2002; Duran et al. 2005; Mandal et al. 2006; Bhanska and D'Souza 2006), algae, *Padina pavonica* (Hosea et al. 1986) and plants like *Pelargonium graveolens*, *Piper longum*, *Desmodium triflorum* and *Andrographis paniculata* (Armendariz et al. 2004; Ahmad et al. 2011; Jacob et al. 2012; Suriyakalaa et al. 2013) have been utilized for the amalgamation

of diverse nanoparticles with distinctive shapes, sizes and compositions. Cell-free culture supernatants of five psychrophilic bacteria, *Pseudomonas antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis* and *Arthrobacter gangotriensis* and two mesophilic bacteria, *Bacillus indicus* and *Bacillus cecembensis* have been used to synthesize Ag NPs (Shivaji et al. 2011). Synthesis of gold nanoparticles using seed aqueous extract of *Abelmoschus esculentus* (Jayaseelan et al. 2013b). An ecofriendly, ambient temperature protocol for size controlled synthesis of gold nanoparticles, using the fungus, *Aspergillus terreus* (Priyadarshini et al. 2014). Two model organisms have primarily been used to study the production and application of bio- Palladium like the sulfate reducing bacterium, *Desulfovibrio desulfuricans* (Lloyd et al. 1998) and the metal-respiring bacterium, *Shewanella oneidensis* (De Windt et al. 2006). Synthesis of copper oxide nanoparticles using Serratia sp., a Gram-negative bacterium was reported (Hasan et al. 2007). Biosynthesis of ZnO NPs using reproducible bacteria, *Aeromonas hydrophila* as eco-friendly, reducing and capping agent (Jayaseelan et al. 2012). Eco-friendly and reproducible microbes, *Bacillus subtilis* mediated biosynthesis of TiO_2 nanoparticles (Kirthi et al. 2011).

5.1.3 Properties of Nanoparticles

Bulk materials have generally steady physical properties paying little mind to their size, but at the nanoscale this is regularly not the situation. As the material gets to be littler, the rate of molecules at the surface builds with respect to the aggregate number of particles of the material bulk. This can prompt surprising properties of nanoparticles, which are incompletely because of the surface of the material commanding over the mass properties. At this scale, the surface-to-volume proportions of materials turn out to be extensive and their electronic vitality states get to be discrete, prompting special electronic, optical, attractive and mechanical properties of the nanomaterials. When all is said in done, as the span of inorganic and natural materials diminishes towards the nanoscale, their optical and electronic properties to a great extent fluctuates from the mass material at the nuclear/atomic levels and is size and shape subordinate. In this way, the crystallographic surface structure and huge surface to volume proportion make the nanoparticles showed amazing properties. In addition, the expanded reactant action because of morphologies with exceptionally dynamic features and the customizing of its amalgamation according to the necessity makes the nanoparticles an appealing instrument to tackle different innovative issues (Gupta and Gupta 2005; Jamieson et al. 2007).

In the field of pharmaceutical, nanoparticles are being investigated broadly due to their size dependant concoction and physical properties. The span of nanoparticles is like that of most organic particles and structures. This makes them an intriguing contender for application in both *in vivo* and *in vitro* biomedical exploration. The consequence of their combination in the field of pharmaceutical has prompted their application basically in focused on medication conveyance, imaging, detecting and

counterfeit inserts. Another intriguing parkway for their investigation in medication is their utilization as antimicrobials to target exceedingly pathogenic and drug safe organisms. Yet, for the use of nanoparticles in science, biocompatibility is a much fancied attribute. Biocompatibility is the materials capacity to perform medicinally without effort of undesired nearby or systemic impacts (Samia et al. 2006).

5.1.4 Biomedical Nanotechnology

Nanotechnology offers new answers for the change of biosystems and gives a wide mechanical stage to applications in a few regions like bioprocessing in the industry and atomic solution like the location and treatment of diseases, body part substitution and regenerative pharmaceutical, nanoscale surgery, blend and focused on conveyance of medications (Bugunia-Kubik and Susisaga 2002; Schmidt and Montemagno 2002). Researching the well being impact of nanostructures in nature contamination by nanoparticles (Keanea et al. 2002), eco-toxicology (Moore 2002; Borm et al. 2006), enhancing sustenance and horticultural frameworks, improving rural yield, new nourishment items, nourishment preservation (Chen 2002) and enhancing human execution like upgrading sensorial limit, uniting cerebrum and psyche, incorporating neural frameworks with nanoelectronics and nanostructured materials. Polymers have additionally been utilized to grow new conveyance strategies for performing helpful capacities *in vivo* and to build nanostructured platforms for medication conveyance with around 97 % porosity. The nanoparticles were utilized for DNA conveyance into cells (Zhu et al. 2002; Quintana et al. 2002). Biocompatible inserts to supplant harmed or worn body parts and tissue building at the nanoscale (Mcintire et al. 1998) to make bioartificial organs. Nanotechnology guarantees to lessen genotyping by two requests of greatness, permitting relationship between hereditary varieties and maladies to be uncovered (Galvin 2002). It has been reported that medicinally valuable angiosperms have the greatest potential for synthesis of metallic nanoparticles with respect to quality and quantity (Song and Kim 2009). Biosynthesized Ag NPs are used in the label-free colorimetric assay to detect enzymatic reactions, (Wei et al. 2008), surface plasmon resonance studies (Turney et al. 2004; Kundu et al. 2004), antimicrobial materials (Duran et al. 2005), anti-viral and anti-HIV studies (Elechiguerra et al. 2005). Ag NPs have been found to have applications in various fields. Insecticide applications, although highly efficacious against the target species vector control, is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity (Liu et al. 2006). Biosynthesis approaches that have advantages over conventional methods involving chemical agents associated with environmental toxicity and eco-friendly bio-organisms contain proteins, which act as both reducing and capping agents forming stable and Shape-controlled TiO_2 NPs. This method of biological TiO_2 NPs production provides rates of synthesis faster or comparable to those of chemical methods and can potentially be used in various human contacting areas such as cosmetics, foods and sunscreen products applications (Quadros and Marr 2010).

5.1.5 Green Synthesis of Nanoparticles

The methods for getting nanoparticles utilizing normally happening reagents, for example, vitamins, sugars, plant concentrates, biodegradable polymers, and microorganisms as reductants and topping operators could be viewed as appealing for nanotechnology. Plant parts, for example, leaf, root, latex, seed and stem are being utilized for metal nanoparticle synthesis. Greener amalgamation of nanoparticles gives progression over different systems as it is straightforward, savvy and generally reproducible and frequently brings about more steady materials (Kalaiarasi et al. 2010). Microorganisms can likewise be used to create nanoparticles, however the rate of combination is moderate and just predetermined number of sizes and shapes are managable contrasted with courses including plant based materials. At present, organisms are increasing overall ubiquity as nano-production lines for the green combination of nanoparticles (Dhillon et al. 2012). By and large, organic materials give a domain amicable or greener compound system to deliver important materials in light of the fact that the biomaterial based courses kill the need to utilize unforgiving or lethal chemicals (Parsons et al. 2007). Synthesis of Ag NPs using the aqueous leaf extract of *Ocimum sanctum* (Brahmachari et al. 2014), and synthesis of Ag and Au NPs by the reduction of aqueous leaf extract of *Aerva lanata* (Joseph and Mathew 2014). Pd NPs were synthesized using a leaf extract of *Cinnamomum camphora* (Yang et al. 2010), bark extract of *Cinnamomum zeylanicum*, and tuber extract of *Curcuma longa* (Sathishkumar et al. 2009a, b), and peel extract of *Musa paradisiaca* and *Annona squamosa* (Bankar et al. 2010; Roopan et al. 2012). The biosynthesized TiO_2 NPs using the leaf aqueous extract of *Nyctanthes arbor-tristis* (Sundrarajan and Gowri 2011), *Eclipta prostrata* (Rajakumar et al. 2012), *Catharanthus roseus* (Velayutham et al. 2012), *Psidium guajava* (Santhoshkumar et al. 2014) and the flower aqueous extract of *Calotropis gigantea* (Marimuthu et al. 2013). Synthesis of ZnO NPs from fruit of *Citrus aurantifolia* (Ramesh et al. 2014), and root extract of *Polygala tenuifolia* (Nagajyothi et al. 2015). Syntheses of Cu NPs using Ficus religiosa leaf extract was reported (Sankar et al. 2014) (Fig. 5.1).

5.2 Metal Nanoparticles (MNPs)

The advances in the field of biotechnology and nanotechnology owes to the huge change in human life. Lately, an expanding rate of nanomaterial is developing and making headway in distinctive fields. Not at all like the mass partners, had nanoparticulate materials showed extremely fascinating electrical, optical, attractive and compound properties (Sahoo et al. 2009). Metal nanoparticles are generally connected in like manner items like cleansers, beatufiers, toothpaste, shampoos and medicines (Song et al. 2009).

Normally spurred investigational practice for the biosynthesis of metal nanoparticles is currently settled as a developing zone of nanoscience innovative work. As

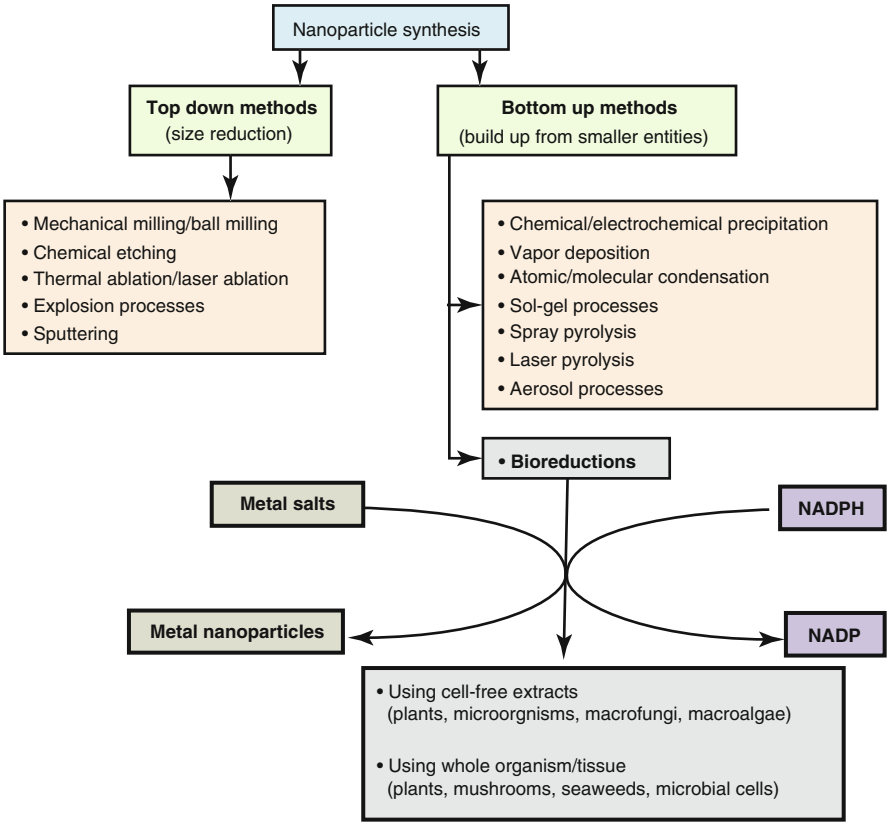

Fig. 5.1 Various approaches for making nanoparticles and cofactor dependent bioreduction

nature makes ideal utilization of materials and space, numerous inorganic materials are delivered in organic frameworks (Dwivedi and Gopal 2010). Plant mediated green synthesis of different MNPs has emerged as one of the options for implementation of green chemistry principles, and successfully made an important contribution towards green nanotechnology (Das and Brar 2013).

For the most part, MNPs can be arranged and settled by physical and chemical reduction, for example, synthetic diminishment, electrochemical procedures, and photochemical lessening are broadly utilized (Chen et al. 2001; Frattini et al. 2005). Studies have demonstrated that the size, morphology, and properties (substance and physical) of the MNPs are emphatically impacted by the trial conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of a stabilizing agent with MNPs (Knoll and Keilmann 1999; Sengupta et al. 2005). The biosynthesis of Ag NPs using the leaf extracts of *Manilkara zapota* (Rajakumar and Rahuman 2011), *Mimosa pudica* (Marimuthu et al. 2011) and *Nelumbo nucifera* (Santhoshkumar et al. 2011).

5.2.1 Silver Nanoparticles (Ag NPs)

Silver has long been perceived as having an inhibitory impact toward numerous bacterial strains and microorganisms regularly show in therapeutic and modern procedures (Jiang et al. 2004). The most broadly utilized and known uses of Ag and Ag NPs are in the restorative business. These incorporate topical balms and creams containing Ag to forestall disease of smolders and open injuries (Becker 1999). Other broadly utilized applications are therapeutic gadgets and inserts arranged with silver-impregnated polymers (Silver 2003). The employments of silver particle and additionally Ag NPs can be misused in drug for blaze treatment, dental materials, covering stainless steel materials, material fabrics, water treatment, sunscreen salves, and so on and had low harmfulness to human cells, high warm soundness and low unpredictability (Duran et al. 2007).

In creating nanoparticles utilizing plant concentrates, the concentrate is blended with an answer of the metal salt at room temperature. The way of the plant concentrates, its fixation, the amassing of the metal salt, pH, temperature and contact time are assumed real part for the rate of generation of the nanoparticles, their amount and different attributes (Dwivedi and Gopal 2010). Huang et al. (2007) exhibited the possibility of utilizing sun dried *Cinnamon camphora* leaf for the combination of the nano-sized metal Ag at surrounding conditions. The synthesis Ag NPs utilizing the parts like leaves, seeds of *Coleus amboinicus* (Narayanan and Sakthivel 2008; Subramanian 2012), *Calotropis gigantea* (Baskaralingam et al. 2012), *Musa paradisiaca* (Bankar et al. 2010a, b), *Ocimum sanctum* (Singhal et al. 2011), *Mentha piperita* (MubarakAli et al. 2011), *Prosopis chilensis* (Kandasamy et al. 2013), *Syzygium cumini* (Banerjee and Narendhirakannan 2011), *Sida acuta* (Veerakumar et al. 2013), *Piper nigrum* (Paulkumar et al. 2014), *Melia azedarach* (Sukirtha et al. 2012), *Moringa oleifera* (Prasad and Elumalai 2011), *Nelumbo nucifera* (Santhoshkumar et al. 2011), *Catharanthus roseus* (Ponarulselvam et al. 2012), *Ocimum sanctum* (Singhal et al. 2011), *Argemone mexicana* (Singh et al. 2010), *Ficus benghalensis* (Saxena et al. 2012), *Cassia auriculata* (Kumar et al. 2011), *Cinnamon zeylanicum* (Sathishkumar et al. 2009b), *Euphorbia hirta* (Elumalai et al. 2010), *Ficus racemosa* (Velayutham et al. 2013) and *Manilkara zapota* (Rajakumar and Rahuman 2012) had antibacterial, antifungal, cancer prevention agent, cytotoxicity, plasmodial pathogens, anticancer, antidiabetic, larvicidal and acaricidal activities.

5.2.2 Gold Nanoparticles (Au NPs)

Gold nanoparticles (Au NPs) have a developing part in restorative biotechnology (Song et al. 2009; Willner et al. 2006). Generation of nanoparticles can be accomplished for the most part through chemical, physical, and biological methods. Biological methods for nanoparticle synthesis using plants or plant extracts have

been recommended as would be prudent eco-accommodating distinct options for substance and physical strategies. Au NPs are discovered to be valuable in numerous applications, for example, biomedicine, catalysis and biosensing, electronic and attractive gadgets. Albeit existing chemical and physical methods have effectively delivered all around characterized nanoparticles.

Biosynthesis of Au NPs with the assistance of restorative plants have come into the spotlight in nanobiotechnology because of the developing need to create environment neighborly considerate innovations. Plants are an incredible wellspring of optional metabolites, and have been discovered to be savvy and eco-accommodating for the vast scale synthesis of nanoparticles (Sastry et al. 2003). Au NPs are most likely the most appealing individual from MNPs because of their entrancing properties and potential applications in nonlinear optics, catalysis, gadgets and different spaces of high innovation and medication (Wu and Yang 2011; Sreeja et al. 2009; Haruta 2003). Au NPs with natural base are fascinating on the grounds that they display the best similarity with biomolecules (Deshpande et al. 2010). Biosynthesis of Au NPs using leaf extracts of *Chenopodium album* (Dwivedi and Gopal 2010), *Sorbus aucuparia* (Dubey et al. 2010), *Hibiscus rosasinensis* (Philip 2010a), *Mangifera indica* (Philip 2010b), *Syzygium aromaticum* (Deshpande et al. 2010), *Anacardium occidentale* (Sheny et al. 2011), *Murraya koenigii* (Philip et al. 2011), *Psidium guajava* (Raghunandan et al. 2009), *Anthocepholus cadamba* (Kumar et al. 2013), *Punica granatum* (Ganeshkumar et al. 2013), *Dysosma pleiantha* (Karuppaiya et al. 2013), *Terminalia chebula* (Edison and Sethuraman 2012), *Trianthema decandra* (Geethalakshmi and Sarada 2013), *Allium cepa* (Parida et al. 2011), *Chrysopogon zizanioides* (Arunachalam and Annamalai 2013) and *Phoenix dactylifera* (Zayed and Eisa 2014) had antimicrobial, anticancer action, hostile to harmful, cell reinforcement against metastatic, larvicidal and have been accounted for as of late.

5.2.3 Palladium Nanoparticles (Pd NPs)

Palladium nanoparticles (Pd NPs) are interest of their properties and for hydrogen. Nanoparticles of palladium and palladium containing intermetallic have extraordinary applications like sensors (Fritsch et al. 2003). Pd NPs were through an extensive variety of wet including, sonochemical, electrochemical as well as polyol (Wu et al. 2001; Korovchenko et al. 2005; Chen et al. 2001). Furthermore the Pd (II) by PEG (Luo et al. 2005) or ascorbic and sonochemical of Pd $(NO_3)_2$ (Sun and Luo 2005) were for the Pd NPs.

As of late, the advances in ultrafine Pd NPs have increased incredible significance because of their application both in heterogeneous and homogeneous catalysis, because of their high surface-to-volume proportion and their high surface vitality (Narayanan and El-Sayed 2005). The standard engineered techniques for creating Pd NPs include synthetic methodologies for producing Pd NPs involve

chemical reduction decrease of Pd (II) by alcohol (Teranishi and Miyake 1998), $NaBH_4$/ascorbic acid (Jana et al. 2000), dinitrogen tetrahydride (N_2H_4) (Yonezawa et al. 2001), polyethylene glycol (PEG) (Luo et al. 2005), potassium isocyanoacetate ($CNCH_2COOK$) (Wang et al. 2004), ascorbic acid (Sun et al. 2007) and reduction of Pd(OAc)2 by dimethylamine-borane in supercritical carbon dioxide (Kameo et al. 2003). By and by, the majority of these procedures were performed in the vicinity of different stabilizers to keep the arrangement of undesired agglomerates or the totals of Pd NPs. Furthermore, there are few reports concerning of Pd NPs by plant concentrate or biomass, where the biomass was normally found to go about as both and stabilizer. The achievement was the creation of Pd NPs utilizing espresso and tea concentrate and the work offered moderately rare data with respect to Pd NPs (Nadagouda and Varma 2008).

Pd NPs are having a broad application in heterogeneous and homogeneous catalysis because of their high surface to volume proportion (Chen et al. 2010; Gopidas et al. 2003). Surface plasma reverberation (SPR) is another imperative element in the Pd NPs blend which is valuable in detecting, chemo-optical transducers and plasmonic wave guiding (TobisÏka et al. 2001; Chen et al. 2007; West et al. 2010). All in all, sonochemical (Nemamcha et al. 2006), electrochemical (Cha et al. 2007) and polyol (Xiong et al. 2005) have been explored for the structure controlled synthesis of MNPs. The testing errands in the Pd NPs union are size control and repress the agglomeration amid combination and also stockpiling (Nemamcha et al. 2006; Nguyen et al. 2010). Pd NPs was successfully blended utilizing leaf of *Diopyros kaki* (Song et al. 2010), *Cinnamom camphora* (Yang et al. 2010), bark of *Cinnamom zeylanicum*, tubers of *Curcuma longa* (Sathishkumar et al. 2009a, b) and *Musa paradisiaca* (Bankar et al. 2010a, b) concentrates, which went about as decreasing and in addition balancing out operators and had antimicrobial movement and anticancer action. Nonetheless, the union of Pd NPs utilizing plant has not settled as much with respect to Ag NPs and Au NPs.

5.2.4 Copper Nanoparticles (Cu NPs)

Copper nanoparticles (Cu NPs) have more intrigue contrasted with different NPs union in light of their valuable properties achievable at considerably less cost than silver and gold (Han et al. 2006). Cu NPs are in the range of nanotechnology and nanomedicine for most recent 10 years on account of their fabulous reactant, optical, electrical and antifungal/antibacterial applications (Ponce and Klabunde 2005; Huang et al. 2008). Polyol technique reported by Park et al. (2007) and integrated very monodispersive Cu NPs in air environment. Biosynthesis of Cu NPs was accounted for by Valodkar et al. (2011) plant leaf extracts of *Euphorbia nivulia* demonstrated their organic impacts on tumor cells. Cu NPs were naturally orchestrated utilizing *Magnolia kobus* leaf extricate as lessening specialists and their antibacterial movement was assessed against *E. coli* (Lee et al. 2013). Amalgamations of Cu NPs

utilizing Syzygium aromaticum fluid concentrate, the biomolecules introduce in the biomass decrease the metal particles and as well as settle the NPs by keeping them from being oxidized after the arrangement (Subhankari and Nayak 2013).

5.3 Metal Oxide Nanoparticles (MONPs)

Metal oxides play a very important role in many areas of chemistry, physics and materials science. The metal elements are able to form a large diversity of oxide compounds. Oxide nanoparticles showed remarkable physical and substance properties because of their constrained size and high thickness of the corner or edge surface sites (Henrich and Cox 1984; Kung 1989; Rodríguez and Fernández-García 2007). MONPs gives proof of the way that metal oxides incorporate numerous and various sorts of NPs with vast contrasts in synthetic piece and conduct; nanoparticles of Titanium dioxide (TiO2), Zinc oxide (ZnO), Copper oxide (CuO) and Cerium oxide (CeO_2) involve a percentage of the more regular samples. MONPs utilized for diverse applications, for example, photonics, energy conversion, stockpiling, catalysis, biomedical applications, social insurance items and self cleaning surfaces (Sharma 2009; Pinna and Niederberger 2008; Nowack and Bucheli 2007). Current chemical protocols in vogue for the synthesis of magnetic iron oxides incorporate sol-gel, forced hydrolysis, sonochemical and electrochemical systems (Fernández-García et al. 2004).

5.3.1 Titanium Dioxide Nanoparticles (TiO_2 NPs)

TiO_2 NPs have made another methodology for astounding applications as an alluring multi-useful material. TiO_2 NPs have exceptional properties, like higher solidness, durable, sheltered and expansive range antibiosis (Roessler et al. 2002; Cai et al. 2006; Fu et al. 2005; Bae et al. 2003). TiO_2 NPs have been particularly the focal point of consideration for their activities (Zhang et al. 2005; Rajagopal et al. 2006; Uddin et al. 2007; Wang et al. 2008). TiO_2 NPs utilized as a part of numerous fields, like self-cleaning against bacterial, and UV securing specialists (Han and Yu 2006) and air purifier (Li et al. 2005; Cermenati et al. 1997), gas sensors and highly efficient solar cell (Park and Kim 2005; Weibel et al. 2006; Verran et al. 2007). The photoactivity property is firmly identified with the structure, miniaturized scale structure and the powder (Weibel et al. 2006; Park and Kim 2005; Verran et al. 2007).

TiO_2 by light with more vitality contrasted with its band crevices produces electron gap combines that affect redox response at the surface of TiO_2. Various highly active oxygen species can oxidize organic compounds of cell to carbon dioxide (CO_2) and water (H_2O). Accordingly, TiO_2 can decompose common

organic matters in the air such as odor molecules, bacteria and viruses (Tan et al. 1996; Fu et al. 2005). Fu et al. (2005) have connected the sol-gel technique to create TiO_2 NPs in its anatase structure and the molecule size is accounted for to be delicate to arrangement pH and the rate of expansion of isopropoxide. TiO_2 NPs have been utilized to study the antibacterial activity of methicillin-resistant *Staphylococcus aureus* (Daoud and Xin 2004; Daoud et al. 2005), self-cleaning (Bozzi et al. 2005), UV-protection (Han and Yu 2006), hydrophilic (Sawada et al. 2003) or ultra-hydrophobic properties (Rios et al. 2008), dye degradation in textile effluent (Mahmoodi et al. 2006) and as a nano-catalyst for cross-linking cellulose with poly carboxylic acids (Wang and Chen 2005; Nazari et al. 2009a, b; Chen and Wang 2006).

The environmental fate and behaviour of TiO_2 NPs are a rapidly expanding area of research and used in a broad range of products as food colorant, sunscreen and cosmetics. Nanometer sized TiO_2 NPs were synthesized by inert gas condensation and co-condensation techniques. Both techniques were based on the evaporation of a metal into an inert atmosphere with subsequent cooling for the nucleation and growth of the nanoparticles. Physical and chemical procedures have been used for the synthesis of large quantities of nanoparticles exposed to short time period. However, the colloidal nanoparticles formed by electrostatic collaborations while in arrangement, upon extraction in powder form, the particles develop and lose their trademark properties. Combination of the nanoparticle is controlled by capping the nanoparticles by organic or inorganic molecules which captures their conglomeration in a grid of glass (Liu and Risbud 1990) and natural polymers (Kane et al. 1996).

Visible light activated TiO_2 NPs showed that a fundamentally higher proportion of all tested pathogens including *Staphylococcus aureus*, *Shigella flexneri* and *Acinetobacter baumannii* were killed by the nanoparticle with higher bacterial interaction property (Cheng et al. 2009). Reactive oxygen species, such as ^{-}OH, O_2^{-}, and H_2O_2 generated in the light irradiated TiO_2 surfaces were shown to operate in show to attack polyunsaturated phospholipids in bacteria (Maness et al. 1999). Antibacterial activities of apatite-coated TiO_2 against *S. aureus*, *E. coli*, methicillin-resistant *S. aureus* and *Micrococcus luteus* were investigated and suggested for its potential use in reducing the risk of microorganism transmission in textile applications (Kangwansupamonkon et al. 2009). The synthesized TiO_2 NPs using *Catharanthus roseus* extracts functional as effective, reducing and stabilizing agents and reported significant parasitic activity against *Hippobosca maculata* and *Bovicola ovis* (Velayutham et al. 2012). Sundrarajan and Gowri (2011) reported that the facile and eco-friendly method for the synthesis of TiO_2 NPs from titanium isopropoxide solution using *Nyctanthes arbortristis* leaves extract and reported the nanoparticles in biomedical and nanotechnology applications in the absence of adverse side effects. The synthesized TiO_2 NPs utilizing *Lactobacillus* sp. furthermore, *Sachharomyces cerevisae* had hostile to bacterial, against contagious properties, useful applications in photovoltaic cells, optical, biological sensors, conductive materials and coating formulations (Jha et al. 2009). The antifungal

activity of TiO_2 NPs synthesized using airborne fungus, *Penicillium expansum* was accounted for (Snowdon 1990). TiO_2 NPs are utilized to provide whiteness and opacity to items, for example, paints, plastics, papers, inks, nourishment colorants, and toothpastes (Cai et al. 2006). TiO_2 NPs is also used in cosmetic and likewise utilized as a part of corrective and healthy skin items, especially in sun blocks cream (Dankovic et al. 2007).

5.3.2 Zinc Oxide Nanoparticles (ZnO NPs)

A mixture of fabricated metal oxide nanoparticles (NPs) are being produced and consolidated into items where the are one of a kind synergist limit, optoelectronic properties, antimicrobial action and different attributes make them alluring for a wide scope of utilizations (Oskam 2006). Zinc oxide (ZnO) is generally utilized MONPs that has a wurtzite crystal structure that adds to its remarkable optoelectric properties (Wang et al. 2004). ZnO nanopowder is right now utilized as a part of items including plastics, earthenware production, glass, concrete, elastic, oils, paints, source of Zn nutrient), batteries, fire retardants, etc (Mitchnick et al. 1999). In addition, ZnO NPs are common constituents of personal care products including cosmetics and sunscreens because of their excellent UV retention and reflective properties (Cross et al. 2007). The worldwide creation of NPs for sunscreen items alone was assessed to be roughly 1000 t amid 2003/3004, comprising basically of ZnO particles (Borm et al. 2006).

ZnO is a potential of ultra violet absorbance, wide chemistry, piezoelectricity and luminescence at high temperatures, it has entered into industry, and now is one of the critical building blocks in today's modern society. It is found in paints, cosmetics, plastic and rubber manufacturing, electronics and pharmaceuticals. More recently, it has again increased huge enthusiasm for its semiconducting properties (Look 2001) and utilized for nano ZnO impregnated as a part of cotton materials demonstrated brilliant antibacterial action against *Staphylococcus aureus* and *Klebsiella pneumoniae* and promising security against UV radiation (Wiegand et al. 2013). ZnO is right now being explored as antibacterial operators in both microscale and nanoscale plans (Nair et al. 2008). It has been proposed that the principle of reactive oxygen species (ROS) produced on the surface of the particles, zinc particle discharge, layer brokenness, and nanoparticles disguise are the primary driver of cell swelling (Nair et al. 2008). *Parthenium hysterophorus* interceded ZnO NPs were incorporated and ended up being effective antifungal agents (Rajiv et al. 2013). The quick natural combination of ZnO NPs utilizing leaf extricate of *Calotropis gigantea* gave an effective course for amalgamation of nanoparticles (Vidya et al. 2013). Exceptionally steady and circular ZnO NPs were created by utilizing zinc nitrate and *Aloe barbadensis* (*Aloe vera*) leaf extract (Sangeetha et al. 2011). The aqueous leaf extract of *Corriandrum sativum* synthesized ZnO NPs using zinc acetic acid derivation and sodium hydroxide as a surrogate for Substance strategy (Gnanasangeetha and Thambavani 2013).

5.4 Biomedical Utilizations of Plant Synthesized MNPs and MONPs

5.4.1 Antimicrobial Activity

Plant mediated green synthesis of nanoparticles is an eco-friendly and efficacious approach effective methodology which finds tremendous application in the field of solution. Nanoparticles were ended up being the most productive as they have great antimicrobial (Jayesh et al. 2008; Anima and Saravanan 2009; Karunakar et al. 2013), mitigating (Tsai et al. 2007; Nadworny et al. 2008; Chaloupka et al. 2010), against plasmodial (Panneerselvam et al. 2011; Ponarulselvam et al. 2012), hostile to tumor (Saraniya Devi et al. 2013) and hostile to oxidant exercises (Joyita and Narendhirakannan 2011; Kumar et al. 2012; Edhaya Naveena and Prakash 2013). Nanoparticles connect to the cell surface of microbe's reasons basic changes and harm aggravating the imperative cell capacities lastly prompting cell demise (Li et al. 2010). The antibacterial activity of plant synthesized Ag NPs utilizing watery leaves concentrate of *Ficus benghalensis* for the control of *Escherichia coli* (Saxena et al. 2012). Antimicrobial action of the saponin disengaged from *Trianthema decandra*, combined Au NPs and Ag NPs utilizing microscopic organisms, *Staphylococcus aureus*, *Staphylococcus faecalis*, *Enterococcus faecalis*, *E. coli*, *Pseudomonas aeruginosa*, *Pseudomonas vulgaris*, *Bacillus subtilis*, *Yersinia enterocolitica*, *Klebsiella pneumoniae* and organism, *Candida albicans* (Geethalakshmi and Sarada 2013). The *in vitro* antimicrobial action of the combined Ag NPs utilizing *Rhinacanthus nasutus* leaf concentrate was powerful against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *K. pneumonia*, *E. coli*, *Aspergillus niger* and *Aspergillus flavus* utilizing a disc diffusion method (Pasupuleti et al. 2013). Das et al. (2013) reported the synthesized Ag NPs using the aqueous leaves extract of *Sesbania grandiflora*, and it has been assessed to study *in vitro* antibacterial activity against selected human pathogens, *Salmonella enterica* and *Staphylococcus aureus*. Adams et al. (2014) reported the antimicrobial capacity of synthesized Pd NPs from by pyrolysis method against *E. coli* and *S. aureus*. Biogenic Cu NPs nanoparticles synthesis using *Tabernaemontana divaricate* leaf extract and its antibacterial activity against urinary tract pathogen of *E.coli* (Sivaraj et al. 2014). Synthesis of Cu NPs uses *Piper betle* leaf extract against phytopathogens *Ralstonia solanacearum* and *Xanthomonas axonopodis* using well diffusion method (Praburaman et al. 2015). The antibacterial movement of the synthesized TiO_2 NPs using *Aeromonas hydrophila* bacteria was effective against *E. coli*, *P. aeruginosa*, *S. aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis* using a well diffusion method (Jayaseelan et al. 2013a, b). ZnO NPs were synthesized from *Parthenium hysterophorus* by inexpensive, eco-friendly and simple method and its antifungal activity was studied against *A. flavus* and *A. niger* (Rajiv et al. 2013).

5.4.2 Antioxidant Activity

Antioxidant therapy has gained an enormous importance. The application of nanotechnology to healthcare holds great promise in therapeutic field in zones, for example, imaging, speedier conclusion, drug conveyance and tissue recovery, and the advancement of new therapeutics. To be sure, various results of nanometric measurements are being assessed in clinical trials (Zhang et al. 2008). The stem bark concentrates of *Shorea roxburghii* utilized as a proficient green decreasing specialists for the generation of Ag NPs and its applications in the aversion of free radical related maladies (Subramanian et al. 2013). Combination of Ag NPs by using the fluid leaf concentrates of *Chenopodium murale* indicated antimicrobial action for *S. aureus* and cell reinforcement exercises for business application (Abdel-Aziz et al. 2014). A basic green synthesis for the preparation of Ag NPs using leaf extract of *Cleistanthus collinus* as a potential phyto reducer and the *in vitro* antioxidant activity of Ag NPs showed a significant effect on scavenging of free radicals (Kanipandian et al. 2014). The effect of the phytochemicals presents in *Memecylon umbellatum*, including saponins, phenolic compounds, phytosterols, and quinones, on the formation of stable Ag NPs and Au NPs was investigated by FTIR spectroscopy (Arunachalam et al. 2013). *Terminalia arjuna* bark extract was used to reduce Cu NPs under microwave irradiation method and showed very good antioxidant property (Yallappa et al. 2013). *Gardenia jasminoides* was used for the bioreduction of palladium chloride into the formation of Pd NPs and identified as good antioxidant activity (Jia et al. 2009). The synthesis of Pd NPs using gum of *Anogeissus latifolia* plant showed superior antioxidant at a much lower nanoparticle dose (Kora and Rastogi 2015). The efficacies of antioxidant activities of aqueous leaf extract of *Psidium guajava* mediated biosynthesis of TiO_2 NPs when compared with ascorbic acid (Santhoshkumar et al. 2014). A flavonoid rich extract of *Teucrium polium* synthesized ZnO NPs, which was used as physical sunscreen, for protection in a wide range of UV radiation and possessed good anti-inflammatory and anti-oxidant activities of flavonoids (Mehdi et al. 2013).

5.4.3 Anticancer Activity

Tumor is one of most essential scourges of humankind and in charge of significant mortality, more than ten million individuals are determined to have the malady every year, it was produced through different cell physiological frameworks, cell flagging and apoptosis. With the predominance and the development of different medication resistance, nonspecific systemic circulation of antitumor operators, insufficient medication fixations coming to the tumor site, horrendous cytotoxicity and restricted capacity to screen helpful reactions due to these difficulties disease

got to be hopeless. To defeat this issue, it is important to create and outline new methodologies, apparatuses and drugs for the analysis and treatment of growth (Acharya and Sahoo 2010). Union of Ag NPs by using the fluid leaf concentrates of Iresine herbstii demonstrated cancer prevention agent exercises of the combined NPs and their cytotoxicity toward the HeLa cervical growth cell line (Dipankar and Murugan 2012). Green amalgamation of Ag NPs utilizing the leaf concentrate of *Podophyllum hexandrum* was assessed for its anticancer potential against HeLa cell line under *in vitro* condition (Jeyaraj et al. 2013). Sukirtha et al. (2012) reported the incorporated Ag NPs utilizing leaf concentrate of *Melia azedarach* and reported compelling against HeLa cervical disease cell line. Jacob et al. (2012) reported the blend of Ag NPs utilizing *P. longum* leaf remove and demonstrated noteworthy cytotoxic impact on HEp-2 tumor cells. Parida et al. (2011) reported the combination of Au NPs intervened by a concentrate of *Allium cepa* and it was disguised by MCF-7 breast malignancy cells through endocytosis.

A straightforward organic technique for the blend of Ag NPs and Au NPs utilizing *Chrysopogon zizanioides*, and blended NPs can have clinical use as antibacterial, cancer prevention agent, and additionally cytotoxic operators and can be utilized for biomedical applications (Arunachalam and Annamalai 2013). Cytotoxicity against *in vitro* HeLa, HBL 100 cell lines and *in vivo* DAL cell line in mice model was concentrated on utilizing biosynthesis of stable Ag NPs from the leaves aqueous extract of *M. azedarach* (Sukirtha et al. 2012). The poisonous quality impacts of silver (nAg) and zinc oxide (nZnO) designed nanoparticles (ENPs) utilizing *Spirodela punctuta* were examined to explore the potential dangers postured by these ENPs towards higher amphibian plants (Thwala et al. 2013).

Apoptosis is incited by extracellular or intracellular signs, which trigger onset of flagging course with trademark biochemical and cytological marks, including atomic buildup and DNA discontinuity (Gopinath et al. 2010). Biomedical capability of Ag NPs combined from calli cells of *Citrullus colocynthis* was described by utilizing Fourier change infrared spectroscopy (FTIR), Nuclear power magnifying instrument (AFM) and its poisonous quality on HEp2 phone line was evaluated utilizing 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, a yellow tetrazole (MTT) test, caspase-3 measure, lactate dehydrogenase spillage examine and DNA discontinuity examine (Satyavani et al. 2011). The organic properties of a novel Ag NPs, combined from an aqueous leaf extract of *Albizia adianthifolia* were researched on A549 cell line (human lung carcinoma) was very much described by MTT examine, cell oxidative status (lipid peroxidation and glutathione (GSH) levels), ATP fixation, caspase-3/ -7, -8 and -9 exercises and DNA discontinuity were decided (Govender et al. 2013). Au NPs have as of late been explored as for biocompatibility as indicated by their collaborations with human breast epithelial MCF-7 cells was surveyed by cytotoxicity by MTT assay and caspase 3, 9, Bax and Bcl expression by real-time PCR assays examines (Selim and Hendi 2012). The cytotoxic impacts of ZnO NPs in hepatocellular carcinoma Hep-G2 cells were shown with stream cytometry investigation and lactate dehydrogenase discharge measures uncover that the method of cell demise fundamental the impacts of the nanoparticle was a mix of apoptotic and necrotic cell passing (Tadinada et al. 2013) (Fig. 5.2a, b).

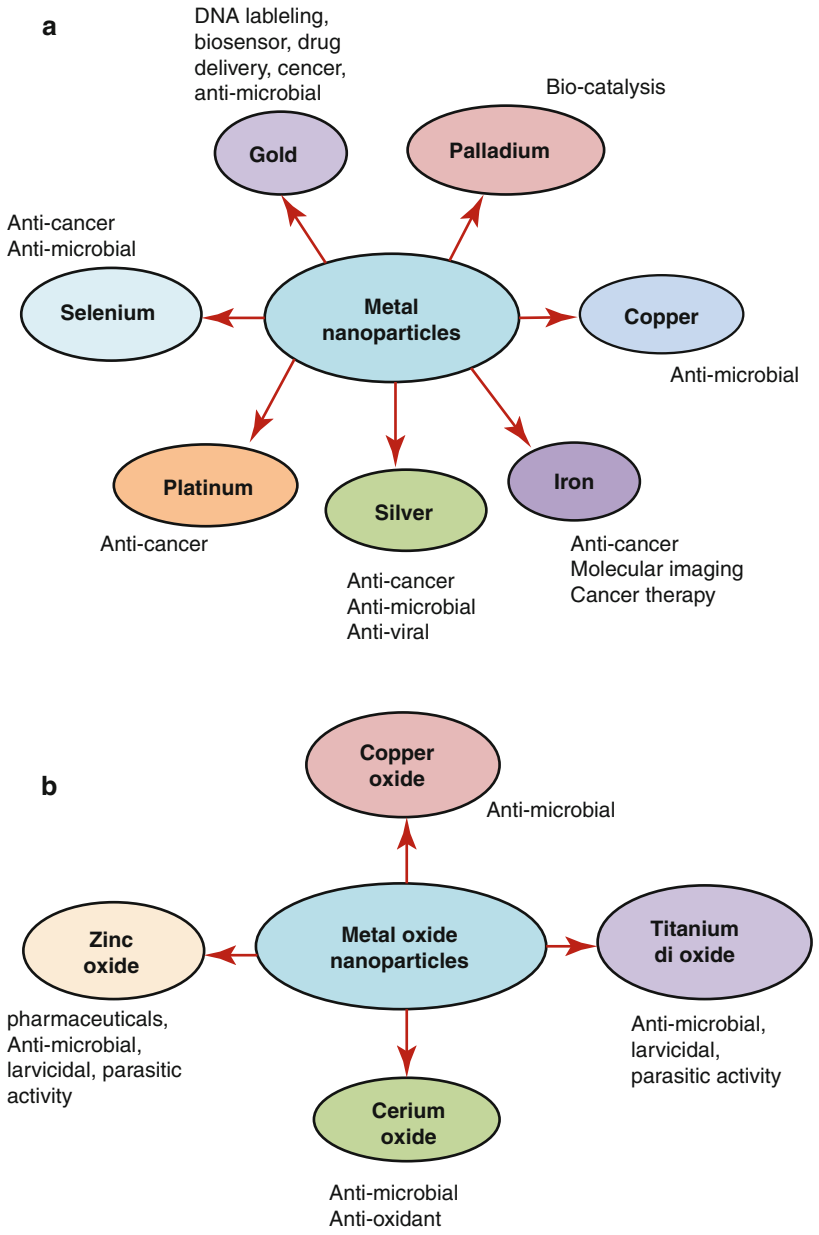

Fig. 5.2 Biomedical applications of (**a**) metal nanoparticles and (**b**) metal-oxide nanoparticles

5.5 Conclusion

The green synthesis approach was utilized as the reducing and capping agent for producing functionally stable and crystalline Ag NPs, Au NPs, Pd NPs, Cu NPs, TiO_2 NPs and ZnO NPs. Ag NPs, Au NPs, Pd NPs, Cu NPs, TiO_2NPs and ZnO NPs might be useful for the development of newer and more potent antimicrobial, antioxidants and anticancer agents.

The synthesized Au NPs, Pd NPs and ZnO NPs showed low toxicity were selected for the Caspase -3, -8, -9 assays. The results of this study the Pd NPs and ZnO NPs exhibit more significant activity against caspase-3 and down regulate caspase-9, which might indicate an activation of caspase-9 in Hep-G2 cells than Au NPs. These results suggest that Pd NPs and ZnO NPs can potentially change apoptotic protein expression and trigger apoptosis in mitochondria-dependent pathways in Hep-G2 cells. Apoptotic effect of Pd NPs and ZnO NPs was studied using DNA fragmentation assay. The data represented in our study contribute to a novel and unexplored area of nanomaterial as alternative medicine. Therefore, further studies are needed to fully characterize the toxicity and the mechanisms involved with the antimicrobial and antioxidant activity of these NPs. This chapter can be helpful in the utilization of an environment friendly solvent and reducing agent in the synthesis of functionally stable and crystalline noble nanomaterial, for modern industrial, bio-medical and other process green applications.

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Chapter 6
Microbial Nanoparticles as Mosquito Control Agents

C. Balasubramanian and A. Najitha Banu

6.1 Introduction

One of the first and most natural question asked starting to deal with nanoparticles is "Why are nanoparticles so interesting? Why work with these extremely small structures that are challenging to handle and synthesize especially when compared with their macroscopic counterparts"? The answer lies in the unique properties possessed by these nanoparticles. Norio Taniguchi first defined the term Nanotechnology in 1970. The term nano is adapted from the Greek word meaning "dwarf". When used as a prefix it implies 10^{-9}.

Throughout history, silver and its compounds have been used extensively for many applications as a result of their useful properties. It is believed that silver was known and used longer than what is recorded in history. Archeological evidence suggests that civilizations have been using silver since at least 3000 B.C. Ancient Egyptians and Persians used silver vessels to keep their water clean and safe. Romans and Greeks knew its powerful bactericidal effect and used it for healing wounds. During World War I, silver compounds were used to prevent wound infection before the emergence of antibiotics. In the American Old West, pioneers traveling along Oregon trails used to toss silver coins into their water storage barrels to keep their water fresh (Russell and Russell 1995; Wijnhoven et al. 2009; Information and History 2010; History of Silver 2010). During the nineteenth century, beyond home remedies, silver was applied in practical medicine such as eye treatment and the treatment of skin ulcers (Foot Defense 2010). The US Food and Drug Administration approved silver solutions in the 1920s to be used as antibacterial agents (Wikipedia 2010).

C. Balasubramanian • A.N. Banu (✉)
Postgraduate and Research Department of Zoology and Microbiology, Thiagarajar College (Autonomous), Madurai, Tamil Nadu 625009, India
e-mail: najirila2010@gmail.com

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Nanosilver is not a new discovery; it has been known for over 100 years (USFDA 2010). Previously, nanosilver or suspensions of nanosilver were referred to as colloidal silver. To produce colloidal silver, a positive electrical current is applied through pure silver bars suspended in water resulting in colloidal silver particles with a size range of 15–500 nm (Lindemann 1997). Before the invention of penicillin in 1928, colloidal silver had been used to treat many infections and illnesses (Nano Health Solutions 2010). By converting bulk silver into nanosized silver, its effectiveness for controlling bacteria and viruses was increased multifold, primarily because of the nanomaterials' extremely large surface area when compared to bulk silver, thus resulting in increased contact with bacteria and fungi. Nanosilver, when in contact with bacteria and fungus, adversely affects the cellular metabolism of the electron transfer systems, and the transport of substrate in the microbial cell membrane. Nanosilver also inhibits multiplication and growth of those bacteria and fungi which caused infection, odor, itchiness and sores (Nanotech Plc 2010).

6.2 Synthesis of Silver Nanoparticle

The top-down or bottom-up approaches are commonly used to synthesize silver nanoparticles; typically, the bottom-up approaches involve wet chemistry techniques. It has to be mentioned that there is plenty of overlap between all the previously mentioned categories. For instance, using microbes to synthesize nanosilver is a conventional/green/bottom-up synthesis.

The synthesis methods in the early 1980s described the reduction of metal ion as a two-step procedure; in first step very small particles were synthesized, which were then enlarged to several nanometers. The difference remained in the use of the reducing agent for the synthesis where in the former step a stronger reducing agent was used and in latter step a weaker reducing agent was used (Sintubin et al. 2009). Chemical methods were used for the size-dependent synthesis of silver nanoparticles, a controlled process mediated by the addition of specific reducing agents at raised temperatures and various pH. Silver nanoparticles were also synthesized through an array of methods such as spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis. The biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increasing attention due to a growing need to develop environmentally benign technologies in material synthesis (Kalishwaralal et al. 2008).

Silver nitrate ($AgNO_3$) is the most widely used silver ion precursor for the production of nanosilver. As a result of its low cost and chemical stability compared to the other available silver salts. The use of silver nitrate makes it likely that nitrate (NO_3^-) will be the dominant anion associated with the silver nanomaterial synthesis processes. The reducing agents can refer to any chemical agents, plant derivatives, biological agents or irradiation methods that provide free electrons to reduce silver ions and form silver nanoparticles. For the production of silver nanoparticles, vari-

ous reducing agents are reported such as H_2 gas (Evanoff and Chumanov 2004), sodium borohydride (Lee and Meisel 1982), hydrazine (Kim et al. 2007), ethylene glycol (Iyer et al. 2007), Tollen's reagent (Fernandez et al. 2008), ascorbic acid (Kashiwagi et al. 2006) and aliphatic amines (Rao and Trivedi 2006). Depending on the strength of the reducing agents, the particle size can be controlled.

6.3 Characterization of Silver Nanoparticles

Characterization of nanoparticles is important to understand and control nanoparticles synthesis and applications. Characterization is performed using a variety of different techniques such as transmission and scanning electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), and UV–Visual spectroscopy (Sun et al. 2000; Yeo et al. 2003; Hutter and Fendler 2004; Chimentao et al. 2004; He et al. 2004; Zhang et al. 2004, 2006; Choi et al. 2007; Yoosaf et al. 2007; Vilchis-Nestor et al. 2008). These techniques are used for determination of different parameters such as particle size, shape, crystallinity, fractal dimensions, pore size and surface area. Moreover, orientation, intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials could be determined by these techniques. For instance, the morphology and particle size could be determined by TEM, SEM and AFM. The advantage of AFM over traditional microscopes such as SEM and TEM is that AFM measures three-dimensional images so that particle height and volume can be calculated. Furthermore, dynamic light scattering is used for determination of particles size distribution. Moreover, X-ray diffraction is used for the determination of crystallinity, while UV–Vis spectroscopy is used to confirm sample formation by showing the Plasmon resonance.

Saifuddin et al. (2009) studied the development of rapid and reliable for the synthesis of nanosized materials is of great importance in the field of nanotechnology. Synthesis of silver nanoparticles using microorganism have been reported, but the process is rather slow. A novel combinatorial synthesis approach which is rapid, simple and "green" for the synthesis of metallic nanostructure of noble metals such as silver (Ag), by using a combination of culture supernant of *Bacillus subtilis* and Microwave (MW) irradiation in water in absence of a surfactant or soft template. It was found that exposure of culture supernatant of *B. subtilis* and microwave irradiation to silver ion lead to the formation of silver nanoparticles. The silver nanoparticles were in the range of 5–60 nm in dimension. The nanoparticles were examined using UV-Visible spectroscopy and Transmission Electron Microscopy (TEM) analyses. The formation of nanoparticles by this method is extremely rapid, requires no toxic chemicals and the nanoparticles are stable for several months. The main conclusion is that the bio-reduction method to produce nanoparticles is a good alternative to the electrochemical methods.

6.4 Mechanism Behind the Synthesis of Silver Nanoparticles

The mechanism behind the extracellular synthesis of nanoparticles using microbes is not fully known. But it is considered that the enzymes like nitrate reductase secreted by microbes help in the bioreduction of metal ions to metal nanoparticles (Duran et al. 2005).

Anil Kumar et al. (2007) and Kalimuthu et al. (2008) reported that all the organisms are not found to be competent for the synthesis of silver nanoparticles. Those organisms which contain the "Silver resistance machinery" can synthesize silver nanoparticles provided that the concentration of the silver ions does not cross the "threshold limit". The resistance mechanism differs with organisms. Extracts from bio-organisms may act both as reducing and capping agents in AgNPs synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides and vitamins is environmentally benign, yet chemically complex. But, the mechanism which is widely accepted for the synthesis of silver nanoparticles is the presence of enzyme "Nitrate reductase". The reduction mediated by the presence of the enzyme in the organism has been found to be responsible for the synthesis. The use of a specific enzyme a-NADPH- dependent nitrate reductase in the *in-vitro* synthesis of nanoparticles is important because this would do away with the downstream processing required for the use of these nanoparticles in homogeneous catalysis and other applications such as non- linear optics. During the catalysis, nitrate is converted to nitrite, and an electron will be shuttled to the incoming silver ions. This has been excellently described in the organism *Bacillus licheniformis*. It is known to secrete the co-factor NADH and NADH-dependent enzymes, especially nitrate reductase, that might be responsible for the bioreduction of Ag^+ to Ag^0 and the subsequent formation of silver nanoparticles (Kalimuthu et al. 2008).

Although all these speculation, direct evidence was provided by Anil Kumar et al. (2007) who directly used the purified nitrate reductase from the organism *Fusarium oxysporum* for the synthesis of silver nanoparticle in a test tube. Their reaction mixture contained only the enzyme nitrate reductase, silver nitrate and NADPH. Slowly, the reaction mixture turned brown with all the characteristics of silver nanoparticles. This is the first direct evidence for the involvement of nitrate reductase for the synthesis of silver nanoparticles (Fig. 6.1).

6.5 Properties, Biological Applications and Important Nanoparticles

The high aspect ratio and resultant special properties exhibited by matter at nanoscale has been a great attraction for development and study of nanoparticles from every possible material. In order to study and exploit the enormous potential provided by "nanoscale", every possible building block for development of

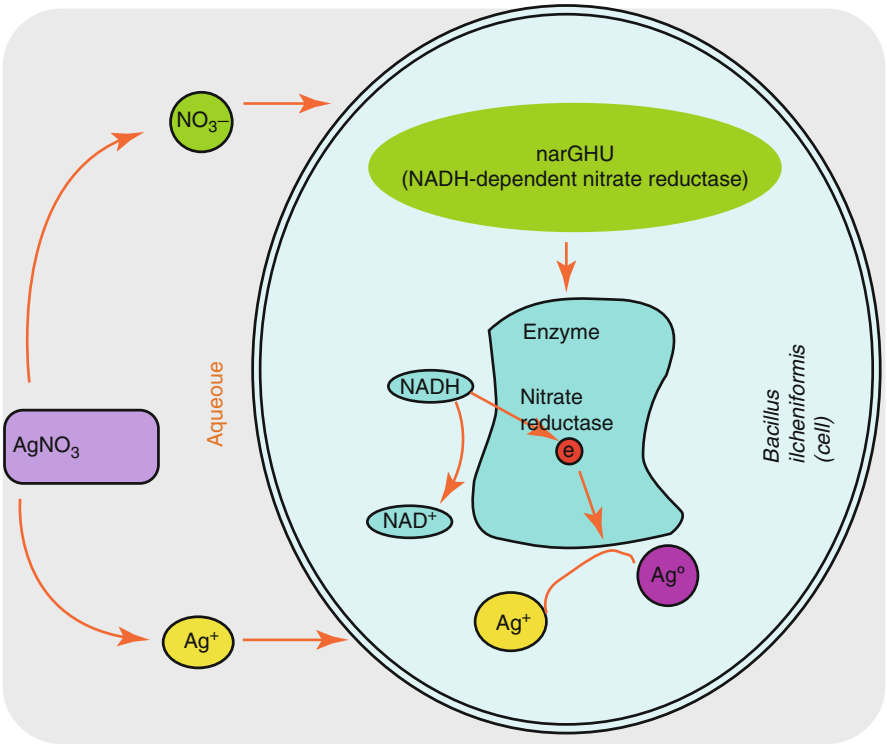

Fig. 6.1 Possible mechanism for silver nanoparticles synthesis using *B. licheniformis*

nanoparticles is being explored. The design and synthesis and surface modification of nanomaterials with novel properties has become an exciting area of research. Considering that primary limit is only the size range of 1-100 nm, there is virtually no limit of the possible ways, for fabrication of nanoparticles. Indeed, almost limitless types of nanoparticles of different shape, size and surface properties are being developed from a range of materials of inorganic, organic, biological or hybrid nature. Availability of new methods of fabrication and tools for characterization and manipulation has resulted in a variety of innovative application of nanoparticles (Rao and Cheetham 2001).

In decade over the nano materials research which was initially restricted to materials science has seen cross-disciplinary expansions to almost every field of science including biology and medicine. The novel properties of nanoparticles hold enormous potential for applications in both basic and applied area of research in Biology. Addressing different problems in biology using the nanoparticles has been an active area of research. This merger of nanomaterials research with Biotechnology has given birth to a new discipline called Nanobiotechnology, in which innumerable types of nanoparticles are being constantly developed and investigated for better understanding of biological system as well as development

of new products and technologies (Niemeyer 2001; Roco 2003). Nanobiotechnology is now a burgeoning field, having influence in almost every aspect in biomedical research. Because of the diversity of the field it is almost impossible to review different aspects of every type of nanoparticle. So let us examine few most important nanoparticles which are being studied for applications in biology and medicine.

Silver nanoparticles have been used extensively as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications. It is important to note that despite of decades of use, the evidence of toxicity of silver is still not clear. Products made with AgNps have been approved by a range of accredited bodies, including the US FDA, US EPA, SIAA of Japan, Korea's Testing and Research Institute for Chemical Industry and FITI Testing and Research Institute (Deng et al. 2007; Bhattacharya and Mukherjee 2008). As anti-bacterial agents, AgNps were applied in a wide range of applications from disinfecting medical devices and home appliances to water treatment (Bosetti et al. 2002; Cho et al. 2005; Li et al. 2008). Moreover, this encouraged the textile industry to use AgNps in different textile fabrics. In this direction, silver nanocomposite fibers were prepared containing silver nanoparticles incorporated inside the fabric (Yeo et al. 2003). The cotton fibers containing AgNps exhibited high anti-bacterial activity against *Escherichia coli* (Yeo et al. 2003; Duran et al. 2007). Furthermore, the electrochemical properties of AgNps incorporated them in nanoscale sensors that can offer faster response times and lower detection limits.

Use of organisms to synthesize nanoparticles one of the primary processes in biomimetics (biological principles for materials formation) involves bioreduction. Initially bacteria were used to synthesize nanoparticles and this was later succeeded with the use of bacteria, fungi, actinomycetes and more recently plants.

6.6 Biological Synthesis of Silver Nanoparticles

In recent years, the development of efficient green chemistry methods employing natural reducing, capping, and stabilizing agents to prepare silver nanoparticles with desired morphology and size have become a major focus of researchers. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances (Ahmad et al. 2003a; Shankar et al. 2004; Ankamwar et al. 2005; Huang et al. 2007). The bioreduction of metal ions by combinations of biomolecules found in the extracts of certain organisms (e.g., enzymes/proteins, amino acids, polysaccharides, and vitamins) is environmentally benign, yet chemically complex. Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganisms and biological systems) (Sastry et al. 2003; Korbekandi et al. 2009; Iravani 2011).

6.7 Synthesis of Silver Nanoparticles by Bacteria

Synthesis of metal nanoparticles by using of microbial cells has emerged as a novel approach. Recently, the efforts directed towards the biosynthesis of nanomaterials, the interactions between microorganisms and metals have been well documented and the ability of microorganisms to extract and or accumulate metals is employed in commercial biotechnological processes such as bioleaching and bioremediation. Bacteria are well known to produce inorganic materials either intracellularly or extracellularly. Compare to the intra cellular synthesis extracellular biosynthesis is cheap and it requires simpler downstream processing. This favors large-scale production of silver nanoparticles to explore its potential applications. Because of this, many studies were focussed on extracellular methods for the synthesis of metal nanoparticles (Duran et al. 2005; Gericke and Pinches 2006).

Minaeian et al. (2008) reported the first time production of silver nanoparticles in enterobacteria. The silver nanoparticles were effectively produced, by *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*. They did not observe any extracellular biosynthesis activity from other micro organisms such as the *Staphylococcus aureus*, *B. subtilis*, *Lactobacillus acidophilus* and *Candida albicans* in conditions tested during investigation. Studies on reduction of Ag^+ ions to AgNPs by *Staphylococcus aureus* also high- light the potential of extracellular method of nanoparticle formation (Nanda and Saravanan 2009).

Jain et al. (2010) reported that the spore crystal mixture of *Bacillus thuringiensis* was used for the synthesis of silver nanoparticles which were characterized using UV-vis absorption spectroscopy, XRD and TEM, X-ray diffraction. The average particle size 15 nm and mixed (cubic and hexagonal) structure. Thus, the bacterial spore crystal mixture was used for the synthesis of nanoparticles. Further, these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogenic bacteria.

When the culture supernatant of *Bacillus megaterium* was treated with aqueous solutions of Ag^+ ions, within few minutes it formed silver nanoparticles (AgNPs) extracellularly (Saravanan et al. 2011). The culture supernatants of bacteria like *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, *Bacillus indicus*, *Bacillus thuringiensis*, *Bacillus strain* CS 11, etc., were also proven its property to form extracellular nanoparticles very effectively (Shivaji et al. 2011; Das et al. 2014; Najitha Banu et al. 2014).

6.8 Synthesis of Silver Nanoparticles by Fungi

Fungi are the most promising group of bioactive compounds producer among fungal species. The various microscopic filamentous fungi (Ascomycetes, *Fungi Imperfecti*, etc.) are the most frequent producers with about 6400 produced compounds (Berdy 2005). Siddhardha et al. (2012) reported that the fungi are the

promising source of secondary metabolites. They were screened seven species of fungi namely *Cladosporium spherospermum*, *Cladosporium oxysporum*, *Chaetomium indicum*, *Gilmaniella Subornata*, *Penicillium purpurogenum* for their ability to secondary metabolites. The crude extracts of fungi were evaluated for antimicrobial and larvicidal activity.

Extracellularly produced nanoparticles were stabilized by the proteins and reducing agents secreted by the fungus. A minimum of four high molecular weight proteins released by the fungal biomass have been found in association with nanoparticles. One of these was strain specific NADH-dependent reductase. However, emission band produced by fluorescence spectra indicate the native form of these proteins present in the solution as well as bound to the surface of nanoparticles (Macdonald and Smith 1996; Kumar and McLendon 1997).

Silver nanoparticles (5–50 nm) could be synthesized extracellularly using *F. oxysporum*, with no evidence of flocculation of the particles even a month after the reaction (Ahmad et al. 2003a). The long-term stability of the nanoparticle solution might be due to the stabilization of the silver particles by proteins. The morphology of nanoparticles was highly variable, with generally spherical and occasionally triangular shapes observed in the micrographs. Silver nanoparticles have been reported to interact strongly with proteins including cytochrome c (Cc). This protein could be self-assembled on citrate-reduced silver colloid surface (Macdonald and Smith 1996). Interestingly, adsorption of (Cc)-coated colloidal Au nanoparticles onto aggregated colloidal Ag resulted Ag: Cc: Au nanoparticle conjugates (Keating et al. 1998).

In UV-vis spectra from the reaction mixture after 72 h, the presence of an absorption band at 270 nm might be due to electronic excitations in tryptophan and tyrosine residues in the proteins. In *F. oxysporum*, the bioreduction of silver ions was attributed to an enzymatic process involving NADH-dependent reductase (Ahmad et al. 2003b). The exposure of silver ions to *F. oxysporum*, resulted in release of nitrate reductase and subsequent formation of highly stable silver nanoparticles in solution (Anil Kumar et al. 2007). The secreted enzyme was found to be dependent on NADH cofactor. They mentioned high stability of nanoparticles in solution was due to capping of particles by release of capping proteins by *F. oxysporum*. Stability of the capping protein was found to be pH dependent. At higher pH values (>12), the nanoparticles in solution remained stable, while they aggregated at lower pH values (<2) as the protein was denatured.

Anil Kumar et al. (2007) have demonstrated enzymatic synthesis of silver nanoparticles with different chemical compositions, sizes and morphologies, using-NADPH-dependent nitrate reductase purified from *F. oxysporum* and phytochelatin, *in vitro*. Silver ions were reduced in the presence of nitrate reductase, leading to formation of a stable silver hydrosol 10–25 nm in diameter and stabilized by the capping peptide. Use of a specific enzyme in *in vitro* synthesis of nanoparticles showed interesting advantages. This would eliminate the downstream processing required for the use of these nanoparticles in homogeneous catalysis and other applications such as non-linear optics. The biggest advantage of this protocol based on purified enzyme was the development of a new approach for green synthesis of nanomaterials over a range of chemical compositions and shapes without possible aggregation.

Ingle et al. (2008) assessed the potential ability of *Fusarium acuminatum* Ell. and Ev. (USM-3793) cell extracts in biosynthesis of silver nanoparticles produced within 15–20 min and were spherical with a broad size distribution in the range of 5–40 nm with the average diameter of 13 nm. A nitrate-dependent reductase enzyme might act as the reducing agent. The white rot fungus, *Phanerochaete chrysosporium*, also reduced silver ions to form nano-silver particles (Vigneshwaran et al. 2006). The most dominant morphology was pyramidal shape, in different sizes, but hexagonal structures were also observed. Possible involvement of proteins in synthesizing silver nanoparticles was observed in *Plectonema boryanum* UTEX 485 (a filamentous cyanobacterium) (Lengke et al. 2007). Stable silver nanoparticles could be achieved by using *Aspergillus flavus*. These nanoparticles were found to be stable in water for more than 3 months with no significant aggregation because of surface binding of stabilizing materials secreted by the fungus (Vigneshwaran et al. 2007).

Bhainsa and D'Souza (2006) reported that the extracellular biosynthesis of silver nanoparticles using *Aspergillus fumigates* (an ubiquitous saprophytic mold) has also been investigated. The resulted TEM micrograph showed well-dispersed silver nanoparticles (5–25 nm) with variable shapes. Most of them were spherical in nature with some others having occasionally triangular shapes. Compared to intracellular biosynthesis of nanoparticles; extracellular synthesis could be developed as a simple and cheap method because of uncomplicated downstream processing and handling of biomasses.

The extracellular filtrate of *Cladosporium cladosporioides* biomass was used to synthesize silver nanoparticles (Balaji et al. 2009). It was suggested that proteins, organic acids and polysaccharides released by *C. cladosporioides* were responsible for formation of spherical crystalline silver nanoparticles. Kathiresan et al. (2009) have shown that when the culture filtrate of *Pencillium fellutanum* was incubated with silver ions and maintained under dark conditions, spherical silver nanoparticles could be produced. They also changed crucial factors such as pH, incubation time, temperature, silver nitrate concentration and sodium chloride to achieve the maximum nanoparticle production. The highest optical density at 430 nm was found at 24 h after the start of incubation time, 1 mM concentration of silver nitrate, pH 6.0, 5 °C temperature and 0.3 % sodium chloride. Fungi of *Penicillium* genus were used for green synthesis of silver nanoparticles (Sadowski et al. 2008). *Penicillium* sp. J3 isolated from soil was able to produce silver nanoparticles (Maliszewska et al. 2009). The bioreduction of silver ions occurred on the surface of the cells and proteins might have critical role in formation and stabilization of the synthesized nanoparticles.

Sanghi and Verma (2009) have investigated the ability of *Coriolus versicolor* in formation of monodisperse spherical silver nanoparticles. Under alkaline conditions (pH 10) the time taken for production of silver nanoparticles was reduced compared to the normal pH from 72–1 h. It was indicated that alkaline conditions might be involved in bioreduction of silver ions, water hydrolysis and interaction with protein functionalities. Findings of this study have shown that glucose was necessary for the reduction of silver nanoparticles, and S-H of the protein played an important role in

the bioreduction. Extracellular synthesis of silver nanoparticles was reported using fungus like *Aspergillus niger*, *Chrysosporium tropicum*, *Pencillium sp*, *A. niger* 2587 etc., (Soni and Prakash 2012, 2013; Dhanasekaran and Thangaraj 2013).

6.9 Silver Nanoparticles Used as a Mosquitocidal Agent

Sap-Iam et al. (2010) investigated the pest control of mosquito *Ae. aegypti* by means of larvicidal is still necessity in order to diminish the vector of some life-threaten diseases. In this study, Polymethacrylate (PMA)-stabilize silver nanoparticles were synthesized by UV irradiation, characterized by surface Plasmon Resonance (SPR), Transmission Electron Microscopy (TEM) and zeta potential measurement and evaluated for their larvicidal activity toward *A. aegypti* larvae. Through the processes of characterization and larvicidal assay, silver nanoparticles were concentration-dependent and supposed to arise from the penetration of the nanoparticles into the larval membrane. The PMA-capped silver nanoparticles at a concentration of 5 ppm exhibited less than 10 % survival of larvae within 3 h exposure time. The study suggests that the silver nanoparticles synthesized by UV- irradiation can be employed in biocontrol of pest including mosquito larvae. Soni and Prakash (2012) reported that the fungus, *A. niger* synthesized gold nanoparticles (AuNPs) was more and rapid and environmentally friendly approach for mosquito control than current approach. This could potentially lead to a new vector control strategy.

Sareen Sarah et al. (2012) studied the larvicidal potential of *Hibiscus rosasinensis* synthesized silver nanoparticles adjacent to *Aedes albopictus*. They are revealed the significant larvicidal activity. This method is considered as an innovative alternative approach using green nanochemistry technique to control vector parasites and is the first report on mosquito larvicidal activity of *H. rosasinensis* leaf mediated synthesized silver nanoparticles.

Soni and Prakash (2012) have investigated the entomopathogenic fungus *Chrysosporium tropicum* synthesized silver and gold nanoparticles against the *Culex quinquefasciatus* and *Anopheles stephensi* larvae and they are reported the all larval stages of *Cx. quinquefasciatus* were found more susceptible to the synthesized silver nanoparticles. Whereas, the larvae of *An. stephensi* were found more susceptible to larvicide synthesized with gold nanoparticles.

Dhanasekaran and Thangaraj (2013) studied the pathogenicity and synthesis efficiency of ten selected microbial (bacterial and fungal) isolates, among the ten isolates four isolates such as *A. bisporus*, *E. coli*, *Pencillium sp. and Vibrio sp*. showed 100 % mortality was observed at 24 h of post treatment.

Soni and Prakash (2013) have synthesized the silver nanoparticles (AgNPs) by using the soil fungus *A. niger* 2587. They are tested the efficacy at different concentration against larvae and pupae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The larvae of *Cx. quinquefasciatus* have shown the 100 % mortality to the synthesized AgNPs after 1 h of exposure, while the larvae of *An. stephensi* and *Ae. aegypti* were found less susceptible to the synthesized AgNPs.

Najitha Banu et al. (2014) reported that the entomopathogenic bacteria, *B. thuringiensis* (Bt) synthesized silver nanoparticles against dengue vector, *A. aegypti*. The mortality rendered by Bt-AgNPs was comparatively higher than that of the control against third instar larvae of *A. aegypti* (LC_{50} 0.10 ppm and LC_{90} 0.39 ppm) in all the tested concentrations, viz. 0.03, 0.06, 0.09, 0.12, and 0.15 ppm.

The nanoparticles encoded by secondary metabolite of bacteria and entomopathogenic fungi are a novel tool to havoc the larval population from the nuisance where vector species cause endemic diseases. This approach has been successfully adopted in vector control programme in future and also thus the reduction of xenobiotic chemicals loads in the environment directly (Najitha Banu and Balasubramanian 2014a, b)

6.10 Role of Dissolved Silver (Ag^+) in Toxicity

Silver nanoparticles release silver ions (Ag^+) in the aqueous state (Kim et al. 2009), it is necessary to distinguish between the toxic effects of Ag NP and dissolved Ag^+ (Johnston et al. 2010). Results from recent studies appear ambiguous. Some investigators suggested that Ag NP might act as a "Trojan horse", bypassing typical barriers and then releasing Ag^+ ions that damage cell machinery (Lubick 2008). This hypothesis of similar biological response of AgNPs and Ag^+ ions was further supported by some other investigators (Foldbjerg et al. 2009; Laban et al. 2009). Alternatively, a combination of both may be responsible, as the release of ions would be expected to be greater for smaller particles. Navarro et al. (2008) examined the rate of photosynthesis in algae exposed to Ag NP or Ag^+ in the presence and absence of cysteine (a chelator of free Ag^+ ions). This study showed that AgNPs were more toxic than Ag^+ ions. Interestingly, a higher concentration of cysteine was required to eliminate Ag^+ ion toxicity. These findings suggest that interactions between algae and nanoparticles may enhance the release of Ag^+ ions, i.e., nanoparticles acted as an effective delivery vehicle for Ag^+ ions. Kawata et al. (2009) also suggest that both "nanosized particle of Ag" as well as "ionic Ag^+" contribute to the toxic effects of AgNP.

Chae et al. (2009) further elaborated that these two silver forms have distinguishable toxicological fingerprints. While AgNP led to cellular and DNA damage, as well as carcinogenic and oxidative stresses, genes related with metal detoxification/metabolism regulation and radical scavenging action were also induced. In contrast, the Ag^+ led to an induction of inflammatory response and metallic detoxification processes, but resulted in a lower overall stress response when compared to AgNP. In contrast, Kim et al. (2009) suggested that AgNP induced toxicity independent of free Ag^+ ions. Asharani et al. (2008) compared Ag^+ ions and AgNP on the prevalence of phenotypic defects in zebrafish. None of the phenotypic defects observed in AgNP treatment were observed in Ag^+ ion treated embryos. These preliminary studies appear to indicate that AgNP-mediated toxicity is independent of Ag^+ ions.

The nanoparticles were detected in the brain indicating that silver nanoparticles have the ability to penetrate blood brain as observed in *Danio rerio* (Kashiwada

2006; Asharani et al. 2008; Fent et al. 2010), mice and rat model (Kiruba Daniel et al. 2010). It was suggested that the nanoparticles could enter the cells through many routes, some of which include diffusion or endocytosis through the skin of embryos. Both nanocopper and nanosilver exposures increased metal content associated with gill tissue, through silver concentrations were much higher following, nanosilver exposures suggesting that inact silver nanoparticles are associated with the gill (Kashiwada 2006; Asharani et al. 2008; Fent et al. 2010; Bai Wei et al. 2010; Zhu et al. 2008; Wei Bai et al. 2010).

Asharani et al. (2008) reported that the AgNPs treated embryos showed a normal cardiac morphology with atria and ventricle differentiated normally with proper orientation with time. Only at higher concentrations of AgNPs resulted in significant growth retardation, which could be due to delay of inhibition of cell division.

Kiruba Daniel et al. (2011) reported that *Ocimum tenuiflorum* synthesized silver nanoparticles against Zebra fish (*Danio rerio*) model. They were confirmed, there was no toxic effect occur at the concentration of 160 µg, but it could penetrate all tissues including the brain through life time protection can be given to healthy young ones.

Comparative toxicity of several metal oxide nanoparticles aqueous suspensions to *D. rerio* development stage was reported earlier. The embryo toxicity test revealed that nano ZnO killed *D. rerio* embryos (50 and 100 mg/L), retarded the embryo hatching (1–25 µg/L), reduced the body length of larvae, and caused tail. The embryo toxicity of nano-Cu at 0.01 and 0.05 mg/L showed no significant difference from $Cu2^{+}$ at the corresponding concentrations (0.006 and 0.03 mg/L), but 0.1 mg/L nano Cu had greater toxicity than 0.06 mg/L $Cu2^{+}$ (Fent et al. 2010). As nanoparticles concentration increased, the number of normally developed *D. rerio* decreased, while the number of dead *D. rerio* increased (Kerry et al. 2007; Zhu et al. 2008; Cristina Ispas et al. 2009; Bai Wei et al. 2010; Wei Bai et al. 2010). But the real time study transport and biocompatibility in early embryoning development in Zebra fish embryo single silver nanoparticles (5–46 nm) showed at 0.19 nm concentration no toxicity.

The major implication of this biological approach is simple and less time consuming. In addition to this the high yield, low toxicity, low cost, and its biocompatibility add to its value (Kalimuthu et al. 2010). An additional advantage is that the size of the nanoparticles synthesized can also be easily controlled by various controlling parameters like pH, temperature (Gurunathan et al. 2009), and the use of stabilizers to prevent aggregation is not required as the proteins in the system act as stabilizers (Kalishwaralal et al. 2010). Nanoparticles with smaller radius of curvature have higher catalytic activity; hence angular shapes are preferable due to their smaller radii of curvature compared to spherical particles of the same volume. Several research groups have successfully demonstrated the superior antimicrobial efficacy of AgNPs either as they are or in composites with polymer (Sondi and Salopek-Sondi 2004; Morones et al. 2005; Gogoi et al. 2006; Sanpui et al. 2011; Banerjee et al. 2011). In addition, another research group demonstrated that AgNPs have potential cytotoxicity against cancer (Gopinath et al. 2008; Rani et al. 2009; Sriram et al. 2010) and antiangiogenic property in microvascular endothelial cells (Gurunathan et al. 2009; Kalishwaralal et al. 2009).

Arora et al. (2008) have studied the interaction of synthesized AgNPs with HT-1080 (human fibrosarcoma) and A431 (human skin/carcinoma) cells in vitro. Results showed that a concentration of AgNPs was safe in the range from 1.56 to 6.25 μg ml^{-1}, and some effects appeared when concentrations >6.25 μg ml^{-1}.

Sangiliyandi et al. (2013) studied the cytotoxic effects of AgNPs on MDA-MB-231 breast cancer cells and its mechanism of cell death. They are developed a green method for synthesis of AgNPs using culture supernatant of *Bacillus funiculus*, and synthesized AgNPs were characterized by various analytical techniques such as UV-visible spectrophotometer, particle size analyzer, and transmission electron microscopy (TEM). The toxicity was evaluated using cell viability, metabolic activity, and oxidative stress. MDA-MB-231 breast cancer cells were treated with various concentrations of AgNPs (5–25 μg/mL) for 24 h. They were found that AgNPs inhibited the growth in a dose-dependent manner using MTT assay. AgNPs showed dose-dependent cytotoxicity against MDA-MB-231 cells through activation of the lactate dehydrogenase (LDH), caspase- 3, reactive oxygen species (ROS) generation, eventually leading to induction of apoptosis which was further confirmed through resulting nuclear fragmentation. For their results showed that AgNPs might be a potential alternative agent for human breast cancer therapy.

So that, the further investigation was required to find out the, most challenging aspects of microbial synthesis of nanomaterials is to identify the proteins/enzymes and their subsequent DNA fragment, which actually governs the biochemical pathway. This would lead to synthesis of respective nanomaterials in bulk quantity and thus a material was formulated and applies for field, patent and commercialization of the entomopathogenic microbes-AgNPs as bio-larvicides for vectors and agricultural crop pests.

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Chapter 7
Green Synthesized Silver Nanoparticles: A Potential New Insecticide for Mosquito Control

Marimuthu Govindarajan

7.1 Introduction

7.1.1 Mosquitos and Diseases

Mosquitoes (Family: Culicidae) are by far the most thoroughly researched insects. They probably have a much greater influence on human health and well-being throughout the world than any other arthropod, mainly because of their involvement in both transmitting a number of dreadful diseases (such as malaria, filariasis, dengue, Japanese encephalitis, Rift valley fever, Chikungunya and West Nile virus) and creating nuisance of great public health importance. Consequently, among the many species of blood-sucking insects, mosquitoes belonging to genera *Culex*, *Aedes*, and *Anopheles* are the most important arthropods from medical standpoint as they are responsible for nearly 10 % of all the sickness of human. Although, there are more than 3500 species all over the world. However, only fewer than 100 species under eight genera only are vectors of diseases. These vectors are organized in two of the three subfamilies, Anophelinae and Culicinae, while the third subfamily Toxorhynchitinae has members with mouthparts suited to feed on plant sap only. Mosquitoes are cosmopolitan in distribution and are found in all climatic zones and zoogeographical regions (Govindarajan et al. 2008a).

Culex tritaeniorhynchus (Giles) (Diptera: Culicidae) has emerged as an important vector of Japanese encephalitis (JE) virus in east, southeast, and south Asia (Govindarajan et al. 2008b). While *C. tritaeniorhynchus* is present throughout India, JE is endemic only in seven states (Andhra Pradesh, Assam, Bihar, Karnataka, Tamilnadu, Uttar Pradesh, and West Bengal). The change in the pattern of JE virus

M. Govindarajan
Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu 608 002, India
e-mail: drgovindzoo@yahoo.com

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transmission in some parts of India from epidemic to endemic is partly correlated with the establishment of *C. tritaeniorhynchus* in these areas. The ability of *C. tritaeniorhynchus* to transmit and spread JE across India has become a topic of concern. Understanding the role of *C. tritaeniorhynchus* as a zoonotic and epizootic vector of JE is of primary importance in the understanding the JE epidemiology. Differences in transmission and vector competence of *C. tritaeniorhynchus* for JE provide reasons for investigation of variations in the populations of this species. However, in spite of its epidemiological importance in JE transmission, few studies have been done on *C. tritaeniorhynchus*. Neither the evolutionary history nor the population dynamics of this species is well understood (Govindarajan et al. 2011a, b, c, d).

Malaria is one of the most common vector-borne diseases widespread in tropical and subtropical regions, including parts of the America, Asia, and Africa (WHO 2007). Worldwide, there were about 247 million malaria cases with 0.881 million deaths reported in 2006 (WHO 2008). Malaria is the world's most dreadful tropical disease. As reported recently, 406 million Indians were at risk of stable *Plasmodium falciparum* transmission in 2007 with an uncertainty point estimate of 101.5 million clinical cases (95 % CI 31.0–187.0 million cases) (Hay et al. 2010; Govindarajan et al. 2008c). In India, Malaria is still the most important cause of morbidity and mortality with approximately two to three million new cases arising every year (Govindarajan et al. 2008d). Rapid increases in population, limited funds, and know-how together with environmental change and an increase in the resistance of vectors and pathogens to insecticides and drugs and a shift in vector-control operations from long-term preventive measures to on-the-spot responses have led to an increase in vector transmitted diseases (Gubler 1998). Malaria causes 1.3 % loss in economic growth in Africa per year, and the long-term impact over a 15-year period is estimated at a 20 % loss in the gross national product (Zaim and Guillet 2002). *Anopheles subpictus* is known to transmit malaria in an isolated study of multiple host-feeding in field populations, and its specific role in transmitting malaria in Sri Lanka revealed that multiple blood feeding within the same gonotrophic cycle was attributed to a local "frequent feeding strategy" in this primarily zoophagic and endophilic malaria vector (Govindarajan 2009).

Aedes albopictus, the Asian tiger mosquito, is a vector of dengue haemorrhagic fever (DHF), and is capable of breeding in a wide range of container types and water holding habitats. In Thailand, *A. albopictus* has been found in forested habitats ranging in elevation from 450 to 1,800 m as well as in a variety of other habitats in rural and suburban areas (Scanlon and Esah 1965; Thavara et al. 1996). Ubiquitous breeding sites, such as tree holes, coconut shells, fruit peels, water jars, unused and discarded tires and boats holding water have been found to contain *A. albopictus* larvae. Because of the diverse breeding sites of *A. albopictus*, especially in the forested areas, they may be hard to reach to monitor larval populations. Detection and measuring mosquito abundance through their egg-laying activities using ovitraps is the most common surveillance or sampling method for this and some other *Aedes* mosquitoes (Service 1992; Govindarajan et al. 2008e). Yap et al. (1995) pointed out the importance of ovipostion site preferences in planning vector control programs against *Aedes* mosquitoes. However, information on oviposition attractants for *A.*

albopictus is rather limited at the present time. Sucharit et al. (1980) studied the oviposition behavior of *A. aegypti* and *A. albopictus* to be influenced by their own larval holding water or that of other species. They found that larval holding water of *A. albopictus* significantly increased oviposition by *A. aegypti*, but there was no oviposition attractancy for *A. albopictus*. Thavara et al. (1989) demonstrated that *A. albopictus* (*A. aegypti* absent) prefer to lay eggs in the field in containers with conditioned water that was left outside for a long period and with a stable flora together with the immature stages of this species.

7.1.2 Vector Control

The medical importance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, mortality, economic loss and social disruption such as malaria, lymphatic filariasis and viral diseases is well documented. Rapid increases in population, limited funds, and know-how together with environmental change and an increase in the resistance of vectors and pathogens to insecticides and drugs and a shift in vector-control operations from long-term preventive measures to on-the-spot responses have led to an increase in vector transmitted diseases (Govindarajan 2010a). The mosquito borne diseases remains endemic in more than 100 developing tropical countries and its control is a major goal for improved worldwide health. In the Indian scenario, almost the entire country is endemic to the mosquito-borne diseases due to favorable ecological conditions. Vector control is a global problem. It may be directed against the immature or adult stages of mosquitoes. Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings. It is known that larvicides play a vital role in controlling mosquitoes in their breeding sites. However, it is undoubtedly the best method of protecting the community against the diseases. In recent years, it has been realized that personal protection from biting mosquitoes and other haematophagous arthropods is the first line of defence against the infectious disease. Mosquito control has been mainly affected by use of conventional insecticides, but these have caused their own problems, such as adverse effects on the environment and the encouragement of pesticide resistance in some mosquitoes (Govindarajan 2011a, b).

7.1.3 Disadvantage of Chemical Control

The current mosquito control approach is based on synthetic insecticides. Even though they are effective, they created many problems like insecticide resistance (Govindarajan and Karuppannan 2011). Chemical control using synthetic insecticides had been favorable so far because of their speedy action and easy application.

The control of mosquito larvae worldwide depends primarily on continued applications of organophosphates, such as temephos and fenthion, and insect growth regulators, such as diflubenzuron and methoprene (Yang et al. 2002). Although effective, continued use of synthetic insecticides for mosquito control has disrupted natural biological control systems and also resulted in lower efficacy of such insecticides and development of resistance in the mosquito population, which are undesirable effects on nontarget organisms (Govindarajan et al. 2005). Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb ecological balance (Govindarajan 2010a). Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into the food chain, and thereby, the liver, kidney, etc., may be irreversibly damaged. They even result in mutation of genes and these changes become prominent only after a few generations (Ghosh and Chandra 2006). Chemical insecticides are very costly. In larval mosquito control, application of insecticides in ponds, wells, and other water bodies may cause health hazards to human and larvivorus fishes. Nowadays, mosquito coils containing synthetic pyrethroids and other organophosphorus compounds because so many side effects, such as breathing problem, eye irritation, headache, asthma, itching, and sneezing to the users. With the use mosquito repellent, people complained of ill health effect and sometimes required medical treatment. In addition, pests were becoming resistant to chemical treatments. Indoor residual spraying of insecticides stains the walls and leaves a long lasting unpleasant odor. There is an urgent need to develop new insecticides which are more environmentally safe and also biodegradable and target specific against mosquitoes (Govindarajan 2011c).

7.1.4 Advantage of Botanical Insecticides

Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages (Liu et al. 2012; Govindarajan 2011d). Recently there has been a concerted effort to promote the use of botanical pesticides (as possible alternative to synthetic chemical insecticides), which provide a pest specific, cost effective, easy to use, readily biodegradable and environment friendly method (Govindarajan 2010b). Therefore, an effort should be made to find alternative insecticides. Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest-managing agents. A number of plants and microbes have been reported as selective with little or no harmful effect on non-target organisms and the environment (Govindarajan and Sivakumar 2011). One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise

botanical blends of chemical compounds which act concertedly on both behavourial and physiological processes. Thus there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases. These well known drawbacks with synthetic insecticides shifted the mosquito control programme to use of eco-friendly, biodegradable and microbial plant compounds with mosquitocidal property (Govindarajan et al. 2006a, b).

Natural products of plant origin are generally preferred because of their less harmful nature to nontarget organisms and their innate biodegradability. Medicinal plants may be an alternative source of mosquito control agent because they have been reported to show several bioactivities such as insecticidal, antifungal, and nematicidal activities. It has been shown that the use of botanicals as mosquito control agents can be effectual in minimizing these adverse impacts due to their ecosafety, target specificity, negligible resistance, reduced number of applications, higher acceptability, and suitability for rural areas. Many researchers have reported that extracts from various plants can be used as effective and advantageous alternatives to synthetic insecticides or along with other insecticides under integrated vector control programs for the control of mosquitoes (Govindarajan et al. 2007).

7.1.5 Nanoparticles

Nanomaterials are defined as materials that have at least one dimension\100 nm (1 nm = 10^{-9} m) and they can be divided into two large groups: ultrafine nanosized particles not intentionally produced and engineered nanoparticles produced in a controlled, engineered way (Oberdorster et al. 2005). Nanotechnology is rapidly growing by producing nanoproducts and nanoparticles (NPs) that can have novel and size-related physico-chemical properties differing significantly from larger matter (Ju-Nam and Lead 2008). The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices (Lu et al. 2007). Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts (Sharma et al. 2009). Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behavior (Krutyakov et al. 2008). These properties make them of potential value in inks, microelectronics, and medical imaging. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity (Ahamed et al. 2010) that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value. To date,

nanosilver technologies have appeared in a variety of manufacturing processes and end products. Nanosilver can be used in a liquid form, such as a colloid (coating and spray) or contained within a shampoo (liquid) and can also appear embedded in a solid such as a polymer master batch or be suspended in a bar of soap (solid). Nanosilver can also be utilized either in the textile industry by incorporating it into the fiber (spun) or employed in filtration membranes of water purification systems. In many of these applications, the technological idea is to store silver ions and incorporate a time-release mechanism. This usually involves some form of moisture layer that the silver ions are transported through to create a long-term protective barrier against bacterial/fungal pathogens (Dallas et al. 2011).

7.1.6 Synthesis of Nanoparticle

7.1.6.1 Chemical Synthesis

Chemical synthesis of Ag NPs requires three components: precursor for Ag NPs usually silver nitrate ($AgNO_3$), reducing agents such as sodium borohydrate ($NaBH_4$) and capping agent like polyvinylpyrrolidone $(C_6H_9NO)n$. In chemical synthesis, there is single reducing and capping agent which allow synthesis of nanoparticles with defined shape and size which is major advantage of this method but use of hazardous chemicals, harsh reaction parameters such as high temperature, pressure and toxic by-product creates environmental concern (Brichkin et al. 2008). There are chances of adherence of toxic chemical residues on surface of nanoparticles. This restricts use of chemically synthesized Ag NPs in medicine and healthcare. Moreover, the Ag NPs produced by chemical methods tend to agglomerate or become insoluble in aqueous system therefore, their application in living system which is aqueous too raise concern about stability and safety (Mafune et al. 2001).

Currently, many methods have been reported for the synthesis of Ag NPs by using chemical, physical, and biological routes. Each method has advantages and disadvantages with common problems being costs, scalability, particle sizes and size distribution. Among the existing methods, the chemical methods have been mostly used for production of Ag-NPs. Chemical methods provide an easy way to synthesize Ag-NPs in solution. Monodisperse samples of silver nanocubes were synthesized in large quantities by reducing silver nitrate with ethylene glycol in the presence of polyvinylpyrrolidone (PVP) (Sun and Xia 2002), the so-called polyol process. In this case, ethylene glycol served as both reductant and solvent. It showed that the presence of PVP and its molar ratio relative to silver nitrate both played important roles in determining the geometric shape and size of the product. It suggested that it is possible to tune the size of silver nanocubes by controlling the experimental conditions. Spherical Ag NPs with a controllable size and high monodispersity were synthesized by using the polyol process and a modified precursor injection technique (Kim et al. 2006).

In the precursor injection method, the injection rate and reaction temperature were important factors for producing uniform-sized Ag NPs with a reduced size. Ag NPs with a size of 17 ± 2 nm were obtained at an injection rate of 2.5 ml s^{-1} and a reaction temperature of 100 °C. The injection of the precursor solution into a hot solution is an effective means to induce rapid nucleation in a short period of time, ensuring the fabrication of Ag NPs with a smaller size and a narrower size distribution. Nearly monodisperse Ag NPs have been prepared in a simple oleylamine-liquid paraffin system (Chen et al. 2007). It was shown that the formation process of Ag NPs could be divided into three stages: growth, incubation and Oatwald ripening stages. In this method, only three chemicals, including silver nitrate, oleylamine and liquid paraffin, are employed throughout the whole process. The higher boiling point of 300 °C of paraffin affords a broader range of reaction temperature and makes it possible to effectively control the size of Ag NPs by varying the heating temperature alone without changing the solvent. Otherwise, the size of the colloidal Ag-NPs could be regulated not only by changing the heating temperature, or the ripening time, but also by adjusting the ratio of oleylamine to the silver precursor.

7.1.6.2 Physical Synthesis

For a physical approach, the metallic NPs can be generally synthesized by evaporation–condensation, which could be carried out by using a tube furnace at atmospheric pressure. However, in the case of using a tube furnace at atmospheric pressure there are several drawbacks such as a large space of tube furnace, great consumption energy for raising the environmental temperature around the source material and a lot of time for achieving thermal stability. Therefore, various methods of synthesis of Ag NPs based on the physical approach have been developed. A thermal-decomposition method was developed to synthesize Ag NPs in powder form (Lee and Kang 2004). The Ag NPs were formed by decomposition of a Ag1+– oleate complex, which was prepared by a reaction with $AgNO_3$ and sodium oleate in a water solution, at high temperature of 290 °C. Average particle size of the Ag NPs was obtained of about 9.5 nm with a standard deviation of 0.7 nm. This indicates that the Ag NPs have a very narrow size distribution. Jung et al. (2006) reported an attempt to synthesize metal NPs via a small ceramic heater that has a local heating area. The small ceramic heater was used to evaporate source materials. The results showed that the geometric mean diameter, the geometric standard deviation and the total number concentration of NPs increase with heater surface temperature. The particle generation was very stable, because the temperature of the heater surface does not fluctuate with time. Spherical NPs without agglomeration were observed, even at high concentration with high heater surface temperature. The generated Ag NPs were pure silver, when air was used as a carrier gas. The geometric mean diameter and the geometric standard deviation of Ag NPs were in the range of 6.2–21.5 nm and 1.23–1.88 nm, respectively. Tien et al. (2008) used the arc discharge method to fabricate Ag NPs suspension in deionized water with no added

surfactants. In this synthesis, silver wires (Gredmann, 99.99 %, 1 mm in diameter) were submerged in deionized water and used as electrodes. The experimental results show that Ag NPs suspension fabricated by means of arc discharge method with no added surfactants contains metallic Ag NPs and ionic silver. With a silver rod consumption rate of 100 mg min^{-1}, yielding metallic Ag NPs of 10 nm in size and ionic silver obtained at concentrations of approximately 11 ppm and 19 ppm, respectively.

7.1.6.3 Biological Synthesis

The biological synthesis of Ag NPs, the reducing agent and the stabilizer are replaced by molecules produced by living organisms. These reducing and/or stabilizing compounds can be utilized from bacteria, fungi, yeasts, algae or plants (Sintubin et al. 2012). The formation of small, spherical, nearly monodispersed Ag NPs in the size range from 2 to 11 nm (average size of 4 ± 1.5 nm) was observed. The Ag NPs exhibit useful properties such as being hydrophilic, stable, and having a large surface area. This bacterially based method of synthesis is economical, simple, reproducible, and requires less energy when compared to chemical synthesis routes. In another study, the use of the fungus *Trichoderma viride* (*T. viride*) for the extracellular biosynthesis of Ag NPs from silver nitrate solution was reported (Fayaz et al. 2010). In this regard *T. viride* proves to be an important biological component for extracellular biosynthesis of stable Ag NPs. The morphology of Ag NPs is highly variable, with spherical and occasionally rod-like NPs observed on micrographs. The obtained diameter of Ag NPs was in the range from 5 to 40 nm. In another study, stable Ag NPs of 5–15 nm in size were synthesized by using an air borne bacteria (*Bacillus* sp.) and silver nitrate (Pugazhenthiran et al. 2009). The biogenic NPs were observed in the periplasmic space of the bacterial cells, which is between the outer and inner cell membranes. Also, the Ag NPs were produced by using the *Lactobcillus* spp. as reducing and capping agent. Sintubin et al. (2009) were carried with different *Lactobcillus* species to accumulate and subsequently reduce Ag+. The result showed that only the lactic acid bacterial were confirmed to have the ability to produce Ag0. In addition, both particle localization and distribution inside the cell were dependent on *Lactobcillus* species. The mean diameter of the biogenic Ag NPs produced by this method varied with the *Lactobacillus* spp. used. The smallest NPs were produced by *L. fermentum* and had a diameter of 11.2 nm. The recovery of silver and the reduction rate were pH dependent. On the other hand, Naik et al. (2002) have demonstrated the biosynthesis of biogenic Ag NPs using peptides selected by their ability to bind to the surface of silver particles. By the nature of peptide selection against metal particles, a 'memory effect' has been imparted to the selected peptides. The silver-binding clones were incubated in an aqueous solution of 0.1 mM silver nitrate for 24–48 h at room temperature. The silver particles synthesized by the silver-binding peptides showed the presence of silver particles 60–150 nm in size. In summary, the biological method provides a

wide range of resources for the synthesis of Ag NPs, and this method can be considered as an environmentally friendly approach and also as a low cost technique. The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions. In biological synthesis, the cell wall of the microorganisms pays a major role in the intracellular synthesis of NPs. The negatively charged cell wall interacts electrostatically with the positively charged metal ions and bioreduces the metal ions to NPs (Thakkar et al. 2010). When microorganisms are incubated with silver ions, extracellular Ag NPs can be generated as an intrinsic defense mechanism against the metal's toxicity. Other green syntheses of Ag NPs using plant exacts as reducing agents have been performed. This defense mechanism can be exploited as a method of NPs synthesis and has advantages over conventional chemical routes of synthesis. However, it is not easy to have a large quantity of Ag NPs by using biological synthesis.

7.1.6.4 Mosquitocidal Properties of Ag NPs

Nanotechnology is an exciting and powerful discipline of science; the altered properties of which have offered many new and profitable products and applications. Agriculture, food and medicine sector industries have been investing more in nanotechnology research. Plants or their extracts provide a biological synthesis route of several metallic nanoparticles which is more eco-friendly and allows a controlled synthesis with well-defined size and shape. The rapid drug delivery in the presence of a carrier is a recent development to treat patients with nanoparticles of certain metals. The engineered nanoparticles are more useful in increasing the crop production, although this issue is still in infancy. This is simply due to the unprecedented and unforeseen health hazard and environmental concern. The well-known metal ions such as zinc, iron and copper are essential constituents of several enzymes found in the human system even though the indiscriminate use of similar other metal nanoparticle in food and medicine without clinical trial is not advisable. These attempts to develop novel materials as mosquito larvicides are still necessary. With the progress of nano-technology, many laboratories around the world have investigated silver nanoparticle (AgNPs) production as the nanoparticle possesses more surface atoms than a microparticle, which greatly improves the particle's physical and chemical characteristics. Some physical or chemical methods that are currently available for silver nanoparticle production include mechanical smashing, a solid-phase reaction, freeze-drying, spread drying, and precipitation (co- and homo-precipitation). In general, these methods consume a lot of energy in order to maintain the high pressures and temperatures that are needed for them to work. In contrast, many bioprocesses occur under normal air pressure and temperature, resulting in vast energy savings. As a consequence, this type of procedure attracted the attention of microbiologists and chemists (Chen et al. 2003).

Green Ag NPs have been synthesized using various natural products like *Azadirachta indica* (Tripathi et al. 2009), *Glycine max* (Vivekanandhan et al. 2009), *Cinnamon zeylanicum* (Sathishkumar et al. 2009), and *Camellia sinensis* (Begum et al. 2009). Such studies could prove to have an enormous impact in the immediate future if plant tissue culture and downstream processing procedures were applied in order to synthesize metallic as well as oxide nanoparticles on industrial scale. Currently, there is limited knowledge about the possible adverse effects that Ag nanotechnologies can exert to aquatic organisms, but there could be a potential for increased exposure to both ionic Ag and Ag NPs because of the rapid development of commercialized nanoproducts. Ag NPs may be released into the environment from discharges at the point of production, fromerosion of engineered materials in household products (antibacterial coatings and silver-impregnated water filters), and from washing or disposal of silver-containing products (Benn and Westerhoff 2008). Elumalai et al. (2010) have reported that the aqueous extract of shade dried leaves of *Euphorbia hirta* was used for the synthesis of Ag NPs and their antibacterial activities. The larvicidal activity of synthesized Ag NPs utilizing aqueous extract from *Eclipta prostrata*, a member of the Asteraceae, has been investigated against fourth instar larvae of filariasis vector, *C. quinquefasciatus* and malaria vector, *A. subpictus* (Rajakumar and Abdul Rahuman 2011). The larvicidal activities of mycosynthesized Ag NPs against vectors *A. aegypti* and *A. stephensi* responsible for diseases of public health importance have been evaluated (Salunkhe et al. 2011).

The pediculocidal and larvicidal activities of synthesized silver nanoparticles using the aqueous leaf extract of *Tinospora cordifolia* have been reported against the human capitis and fourth- instar larvae of *A.subpictus* and *C. quinquefasciatus* (Jayaseelan et al. 2011). The larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex has been screened against *A. aegypti*, *A. stephensi*, and nontarget fish *Poecilia reticulata* (Patil et al. 2012b) Synthesis of silver nanoparticles was carried out using leaves of *Catharanthus roseus* and their antiplasmodial activities against *P. falciparum* (Ponarulselvam et al. 2012). Biolarvicidal and pupicidal potential of silver nanoparticles synthesized with *Euphorbia hirta* has been screened against the larvae of *A. stephensi* (Priyadarshini et al. 2012). The larvicidal activity of crude petroleum ether, ethyl acetate, and methanol extracts of the whole plants of *Phryma leptostachya* was assayed for its toxicity against the early fourth instar larvae of *C. pipiens* pallens. The larval mortality was observed after 24 h of exposure (Xiao et al. 2012). The hexane, ethyl acetate, and methanol extracts of *Aristolochia indica*, *Cassia angustifolia*, *Diospyros melanoxylon*, *Dolichos biflorus*,*Gymnema sylvestre Schult*, *Justicia procumbens*, *Mimosa pudica*, and *Zingiber zerumbet* were tested for the adulticidal, repellent, and larvicidal activity against *C. gelidus* and *C. quinquefasciatus* (Kamaraj and Rahuman 2010). The extracts of *Coccoloba mollis*, *Guettarda grazielae*, *Merremia aegyptia*, *Rourea doniana*, *Spermacoce verticillata*, and *Triplaris americana* were tested for larvicidal activity against *A. aegypti* (Oliveira et al. 2010). The early fourth instar larvae

of *C. quinquefasciatus*, reared in the laboratory, were used for larvicidal assay with water, hot water, acetone, chloroform, and methanol leaf, stem bark, and flower extracts of *Acacia arabica*, *Cedrus deodara*, *Hibiscus rosasinensis*, *Mangifera indica*, *Nerium indicum*, *Nicotiana tabacum*, *Pongamia pinnata*, and *Solanum nigrum* (Rahuman et al. 2009). The chloroform–methanol extract of the mature leaves of *Solanum villosum* was investigated to establish its biocontrol potentiality under laboratory condition against the larval forms of *A. subpictus* (Chowdhury et al. 2009).

Murugan et al. (2003) studied the interactive effect of botanicals (Neem, *Pongamia*) and *Leucas aspera*, *Bacillus sphaericus* against the larvae of *C. quinquefasciatus*. The aqueous extracts of seed kernels of *Pongamia glabra*, *Adenanthera pavonina*, and *Sapindus emarginatus* were found to exhibit effective ovicidal, larvicidal, and pupicidal activity on *A. aegypti* (Koodalingam et al. 2009). The ethanolic extracts of the orange peel (*C. sinensis*) was tested for the toxicity effect on the larvae of the yellow fever mosquito *A. aegypti* (Amusan et al. 2005); the petroleum ether extract showed larvicidal activity against the *A. aegypti*, *C. quinquefasciatus*, *A. dirus* and *Mansonia uniformis* (Komalamisra et al. 2005). Larvicidal activity of ethyl acetate, butanol, and petroleum ether extracts of five species of Euphorbiaceae plants, *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli*, were tested against the early fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* (Rahman et al. 2007). Hexane extract obtained from leaves of *Eucalyptus citriodora* was tested against larvae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* to assess its toxicity and growth-inhibiting activity (Singh et al. 2007).

Aqueous extracts of nine medicinal plants were bioassayed against larvae of *C. quinquefasciatus* and *A. aegypt* among these plants, the long pepper, *Piper retrofractum* Vahl (Piperaceae), showed the highest level of activity against mosquito larvae. Larvicidal activity of *P. retrofractum*, fresh fruits of this plant were extracted in water and the extracts made into powder and bioassayed against 3rd and 4th instar larvae of *C. quinquefasciatus* and *A. aegypti* in the laboratory (Chansang et al. 2005). Larvicidal potential of petroleum ether (Pee), carbon tetrachloride (Cte) and methanol extract (Mee) of *Artemisia annua*, *Chenopodium album* and *Sonchus oleraceus* was observed against malaria vector, *A. stephensi* (Sharma et al. 2006). Nanotechnology provides the cutting edge to engineer these properties of nanomaterials for need-based application in bioscience such as biomedicine, biosensor, etc. (Thevenot et al. 2001). Nanosilica was reported to have potential as a drug delivery vehicle for medical and veterinary treatments and as pesticides in agriculture, but information on the effect on mosquitoes is not available. The reported mode of action for insecticidal activity of nanosilica is through desiccation of insect cuticle by physicosorption of lipid and is also expected to cause damage in the cell membrane resulting in cell lysis and death of the insects (Tiwari and Behari 2009).

Plants and microbes are currently used for nanoparticle synthesis. The use of plants for synthesis of nanoparticles is rapid, low-cost, eco-friendly, and a

single-step method for biosynthesis process (Huang et al. 2007). Among the various known synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use (Kumar and Yadav 2009). It has been reported that medicinally valuable angiosperms have the greatest potential for synthesis of metallic nanoparticles with respect to quality and quantity (Song and Kim 2009). Biosynthesized Ag NPs are used in label-free colorimetric assay to detect enzymatic reactions (Wei et al. 2008), surface plasmon resonance studies (Turney et al. 2004; Kundu et al. 2004), antimicrobial materials (Duran et al. 2005), antiviral, and anti-HIV studies (Elechiguerra et al. 2005). The silver and gold nanoparticles synthesized with *Chrysosporium tropicum* have been tested as a larvicide against the *A. aegypti* larvae (Soni and Prakash 2012). They found that the silver nanoparticles were more effective against the mosquito larval stages than the gold nanoparticles. The silver nanoparticles synthesized with *Nelumbo nucifera* leaf extract have been tested against the malaria and filariasis vectors (Santhoshkumar et al. 2011). The efficacies of synthesized silver nanoparticles using the aqueous leaf extract of *Mimosa pudica* have been evaluated against the larvae of *A. subpictus*, *C. quinquefasciatus*, and *Rhipicephalus microplus* (Marimuthu et al. 2010).

The larvicidal and repellent properties of essential oils is from various parts of four plant species *C. citratus*, *C. zeylanicum*, *Rosmarinus officinalis*, and *Z. officinale* against *C. tritaeniorhynchus* and *A.subpictus* (Govindarajan 2011c). The larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis*, with three different solvents like methanol, benzene, and acetone, were tested against the early second, third, and fourth instar larvae of *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* (Govindarajan 2010c). The leaf extract of *Acalypha indica* with different solvents—benzene, chloroform, ethyl acetate, and methanol—has been tested for larvicidal-ovicidal activity and oviposition attractancy against *A. stephensi* (Govindarajan et al. 2008c). Nanoparticles, generally considered as particles with sizes of up to 100 nm, exhibit completely new or improved properties compared to the larger particles of the bulk material that they are composed of, based on specific characteristics such as size, distribution, and morphology (Willem and van den Wildenberg 2005). Anti-fungal, antiinflammatory, and antiviral activities of silver nanoparticles were reported (Kim et al. 2009; Nadworny et al. 2008). However, the silica nanoparticles have been tested against the larvae and pupae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* (Barik et al. 2012). Mosquitocidal properties of the Ag-NPs are summarized in Table 7.1. As far as our literature survey could ascertain, no information was available on the larvicidal activity of the experimental plant species given here against *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*. Therefore, the aim of this study was to investigate the mosquito larvicidal activity of aqueous crude extract and Ag NPs from *Feronia elephantum*, *Heliotropium indicum* and *Sida acuta*. This is the first report on the mosquito larvicidal activity of selected plant against the target mosquitoes.

Table 7.1 Mosquitocidal efficacy of silver nanoparticles

Plant name	Family	Particle size (nm)	Shape	Target species	Reference
Annona squamosa	Annonaceae	20	Spherical	*A. aegypti*, *A. stephensi*, *C. quinquefasciatus*	Arjunan et al. (2012)
Parthenium hysterophorus	Asteraceae	50	Spherical	*C. quinquefasciatus*	Mondal et al. (2014)
Nerium oleander	Apocynaceae	20–35	Spherical	*A. stephensi*	Roni et al. (2013)
Leucas aspera	Lamiaceae	0.5	Clustered and irregular	*A. aegypti*	Suganya et al. (2014)
Rhizophora mucronata	Rhizophoraceae	420	Spherical	*C. quinquefasciatus*	Gnanadesigan et al. (2011)
Cadaba indica	Capparaceae	30–60	Spherical	*A.stephensi*, *C. quinquefasciatus*	Kalimuthu et al. (2013)
Murraya koenigii	Rutaceae	20–35	Spherical	*A. stephensi*, *A. aegypti*	Suganya et al. (2013)
Pedilanthus tithymaloides	Euphorbiaceae	15–30	Spherical and crystalline nature	*A. aegypti*	Sundaravadivelan et al. (2013)
Trichoderma harzianum	Hypocreaceae	10–20	Crystalline nature	*A. aegypti*	Sundaravadivelan and Nalini Padmanabhan (2014)
Anthocepholus cadamba	Rubiaceae	20–50	Crystalline nature	*C. quinquefasciatus*	Naresh kumar et al. (2013)
Eclipta prostrata	Asteraceae	35–60	triangles, pentagons, and hexagons	*C. quinquefasciatus*, *A. subpictus*	Rajakumar and Abdul Rahuman (2011)
Solanum nigrum	Solanaceae	50–100	Spherical to polyhedral in shape	*C. quinquefasciatus*, *A. stephensi*	Rawani et al. (2013)
Ficus racemosa	Moraceae	250–60	Cylindrical, uniform, Rod shaped	*C. quinquefasciatus*, *C. gelidus*	Velayutham et al. (2013)

(continued)

Table 7.1 (continued)

Plant name	Family	Particle size (nm)	Shape	Target species	Reference
Pithecellobium dulce	Fabaceae	300–800	Crystallite	*C. quinquefasciatus*	Raman et al. (2012)
Nelumbo nucifera	Nelumbonaceae	25–80	Roughly circular	*A.subpictus*, *C. quinquefasciatus*	Santhoshkuma et al. (2011)
Mimosa pudica	Mimosaceae	25–50	Cubic	*A. subpictus*, *C. quinquefasciatus*	Marimuthu et al. (2011)
Tinospora cordifolia	Menispermaceae	50–100	Spherical	*A. subpictus*, *C. quinquefasciatus*	Jayaseelan et al. 2011)
Plumeria rubra	Apocynaceae	32–220	Spherical	*A. aegypti*, *A. stephensi*	Chandrashekhar et al. (2012)
Euphorbia hirta	Euphorbiaceae	30–60	Cubic	*A. stephensi*	Priyadarshini et al. (2012)
Pedilanthus tithymaloides	Euphorbiaceae	15–30	Spherical	*A. aegypti*	Sundaravadivelan et al. (2013)
Vinca rosea	Apocynaceae	25–47	Spherical	*A. stephensi*, *C. quinquefasciatus*	Subarani et al. (2013)
Feronia elephantum	Rutaceae	20–60	Spherical	*A. stephensi*, *A. aegypti*, *C.quinquefasciatus*	Veerakumar et al. (2014a)
Feronia elephantum	Rutaceae	20–60	Spherical	*A. stephensi*, *A. aegypti*, *C.quinquefasciatus*	Veerakumar and Govindarajan (2014)
Heliotropium indicum	Boraginaceae	20–60	Spherical	*A. stephensi*, *A. aegypti*, *C.quinquefasciatus*	Veerakumar et al. (2014b)
Sida acuta	Malvaceae	20–60	Spherical	*C. quinquefasciatus*, *A. stephensi*, *A. aegypti*	Veerakumar et al. (2013)

7.2 Materials and Methods

7.2.1 Collection of Materials

Fresh leaves of *F. elephantum*, *H. Indicum* and *S. acuta*, (Figs. 7.1, 7.2, and 7.3) were collected from Tamil Nadu, India and the taxonomic identification was made by Dr. V. Vengatesalu, Professor, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The voucher specimens were numbered and kept in our research laboratory for further reference. Silver nitrate was obtained from Qualigens Fine Chemicals, Mumbai, India.

Fig. 7.1 *Feronia elephantum* Correa (Family: Rutaceae). Common names: Wood Apple, Elephant Apple, Monkey Fruit, Curd Fruit; Plant: Erect slow growing tree; Leaves: Deciduous, alternate, 7.5–12.5 cm long, dark-green, leathery, dotted with oil glands and slightly lemon-scented when crushed; Flower: Dull-red or greenish flowers 1.25 cm wide, borne in small, loose, terminal or lateral panicles. Usually bisexual; Fruit: Round to oval, 5–12.5 cm wide, with a hard, woody, grayish-white, scurfy rind about 6 mm thick; The pulp is brown, mealy, odorous, resinous, astringent, acid or sweetish, with numerous small, white seeds scattered through it. The wood-apple is native and common in the wild in dry plains of India. The rind must be cracked with a hammer. The fruit shell is fashioned into snuffboxes and other small containers. The fruit is much used in India as a liver and cardiac tonic, and, when unripe, as an astringent means of halting diarrhea and dysentery and effective treatment for hiccough, sore throat and diseases of the gums. The pulp is poulticed onto bites and stings of venomous insects, as is the powdered rind. Juice of young leaves is mixed with milk and sugar candy and given as a remedy for biliousness and intestinal troubles of children. Oil derived from the crushed leaves is applied on itch and the leaf decoction is given to children as an aid to digestion. Leaves, bark, roots and fruit pulp are all used against snakebite

Fig. 7.2 *Heliotropium indicum* L. (Boraginaceae). *Heliotropium indicum*, one of the largest heliotropes found in Texas, is introduced, and is one of the few annuals within this genus (in Texas). India heliotrope grows upright (2–3 ft in height) and is very leafy, when compared to other heliotropes. The leaves are dark green, alternate, entire, and hispid (hairy). The stems are also hispid. Flowers are blue or violet (rarely white), and like all heliotropes, the younger flowers are located towards the tip of the inflorescence (flower cluster), while mature seed are lower on the flower stalk. There are approximately 14 species of *Heliotropium* in Texas. Most are upland species found in the western portions of the state. Six are commonly found in wetlands. Most have white flowers, although blue or violet is not uncommon. Vegetatively, most heliotropes have smallish and narrow leaves and the growth habit is prostrate, or generally so. The seed head, and the way that the flowers are restricted to the tips, is very characteristic of the entire genus. *Heliotropium* from helios (sun) and trope (turn) flowers turn toward the sun. Some species are considered poisonous, while others are considered fair browse for sheep and goats. Although apparently not preferred by waterfowl, some incidental use has been documented

7.2.2 Mosquitoes

The laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with parafilm as membrane for 4 h. *A. albopictus* feeding was done from 12 noon to 4:00 p.m. and *A. subpictus* and *C. tritaeniorhynchus* were fed during 6:00–10:00 p.m. A membrane feeder with the

Fig. 7.3 *Sida acuta* Burm.f. (Family: Malvaceae). Common names: Common *Sida*, Prickly *Sida*, Broomweeds, Wireweed, Cheeseweed; Plant: 1 m; Shrub with slender branches and fibrous stems; Leaves: Slightly concave oval or elongated leaves with a shiny surface and toothed margins; Flower: 10 mm across. The flowers are yellow, conspicuous and borne on short stalks in the leaf axils along the branches; Fruit: The fruit is a dark brown capsule; it splits into 6–10 single-seeded segments when ripe. Native to pantropical regions, it can be found throughout the warm regions of the world. *Sida* is a weed of tropical pastures. Leaves and roots are used in traditional medicine

bottom end fitted with parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C was maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28 ± 2 °C, 70–85 % relative humidity, with a photoperiod of 12-h light and 12-h dark.

7.2.3 Preparation of Plant Extracts

The leaves (*F. elephantum*, *H. Indicum* and *S. acuta*) were dried in shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 50 g of dried leaf powder with 500 mL of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer (Veerakumar et al. 2013). The suspension of dried leaf powder in water was left for 3 h, filtered through Whatman no. 1 filter paper, and the filtrate was stored in amber-colored air-tight bottle at 10 °C temperature till use.

7.2.4 Synthesis of Silver Nanoparticles

The broth solution of fresh plant leaves was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300-mL Erlenmeyer flask along with 100 mL of sterilized double distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1 and stored at −15 °C and could be used within 1 week. The filtrate was treated with aqueous 1 mM $AgNO_3$ (21.2 mg of $AgNO_3$ powder in 125 mL Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight-milliliter aqueous solution of 1 mM of silver nitrate was reduced using 12 mL of leaves extract at room temperature for 10 min, resulting in a brown-yellow solution indicating the formation of Ag NPs (Veerakumar and Govindarajan 2014).

7.2.5 Characterization of the Synthesized Nanoparticles

Synthesis of AgNP solution with leaf extract may be easily observed by UV–Vis spectroscopy. The bioreduction of the Ag ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after 20 times dilution and measuring the UV–Vis spectra of the solution. UV–Vis spectra of these aliquots were monitored as a function of time of reaction on a Shimadzu 1601 spectrophotometer in the 300–800-nm range operated at a resolution of 1 nm. Further, the reaction mixture was subjected to centrifugation at 60,000× g for 40 min; the resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing silver nanoparticles was used for Fourier transform infrared (FTIR). For electron microscopic studies, 25 μL of sample was sputter-coated on a copper stub, and the images of the nanoparticles were studied using scanning electron microscopy (SEM; JEOL, model JFC-1600), and TEM (JEOL, model 1200EX) measurements were operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder. FTIR spectra of the samples were measured using PerkinElmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. An aliquot of this filtrate containing silver nanoparticles was used for X-ray diffraction (XRD) analysis.

7.2.6 Larvicidal Activity

Larvicidal activity of the aqueous crude extract and Ag NPs from of *S. acuta*, *H. Indicum*, *F. elephantum* was evaluated according to WHO protocol (2005). Based on the wide range and narrow range tests, aqueous crude extract was tested at the range of 30–300 μg/mL concentrations and Ag NPs was tested at range of 8–60 μg/mL concentrations. Twenty numbers of late third instar larvae were introduced into a 500-mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired concentrations of leaf extract and silver nanoparticles was added. For each

concentration, five replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. Each test included a set control groups (silver nitrate and distilled water) with five replicates for each individual concentration. The lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis (Finney 1971).

7.2.7 Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95 % confidence limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated using the Statistical Package of Social Sciences 12.0 software. Results with $p<0.05$ were considered to be statistically significant.

7.3 Results

7.3.1 UV–Vis Analysis of Ag NPs

Leaves extracts from all three plants under study (*F. elephantum*, *H. Indicum* and *S. acuta*) showed rapid conversion of silver nitrate into silver nanoparticles indicated by color changes from colorless to red brown within few minutes of extract addition in 100 ppm $AgNO_3$ solution (Figs. 7.4a, b, 7.5a, b, and 7.6a, b). A representative scheme of biosynthesis and UV-vis spectrum is given in Figs. 7.4c, 7.5c and 7.6c. Synthesized silver nanoparticles primarily characterized by UV-visible spectroscopy. Ag NPs give typical spectrum having maximum absorption in range of 420–450 nm. This absorption is unique property of metal nanoparticles called SPR (Surface Plasmon Resonance) arises due to conduction of electrons on surface of AgNPs. After adding leaves extract in $AgNO_3$ solution, the biomolecules are stabilized in medium, interact with each other, and with silver salt, after initial interaction silver salt are consumed and the process of nucleation, reduction and capping starts leading nanoparticles synthesis.

7.3.2 FT-IR Analysis of Ag NPs

Typical IR spectrum of lyophilized powder of *F. elephantum* leaves extract showed presence of C–H bending (671.81, 762.21, and 822.47 cm^{-1}), C–O stretch (1016.97 and 1,120.07), –C–H bending (1,384.10), C=C bending (1,617.90), C–H stretch (2,849.59), and N–H stretch (3,422.14) (Fig. 7.7). FTIR analysis of the purified nanoparticles of *H. Indicum* showed the presence of bands due to O–H group C=H bending (824.98), C=O stretch (1,094.71), N=H bending (1,603.81), –C=O stretch

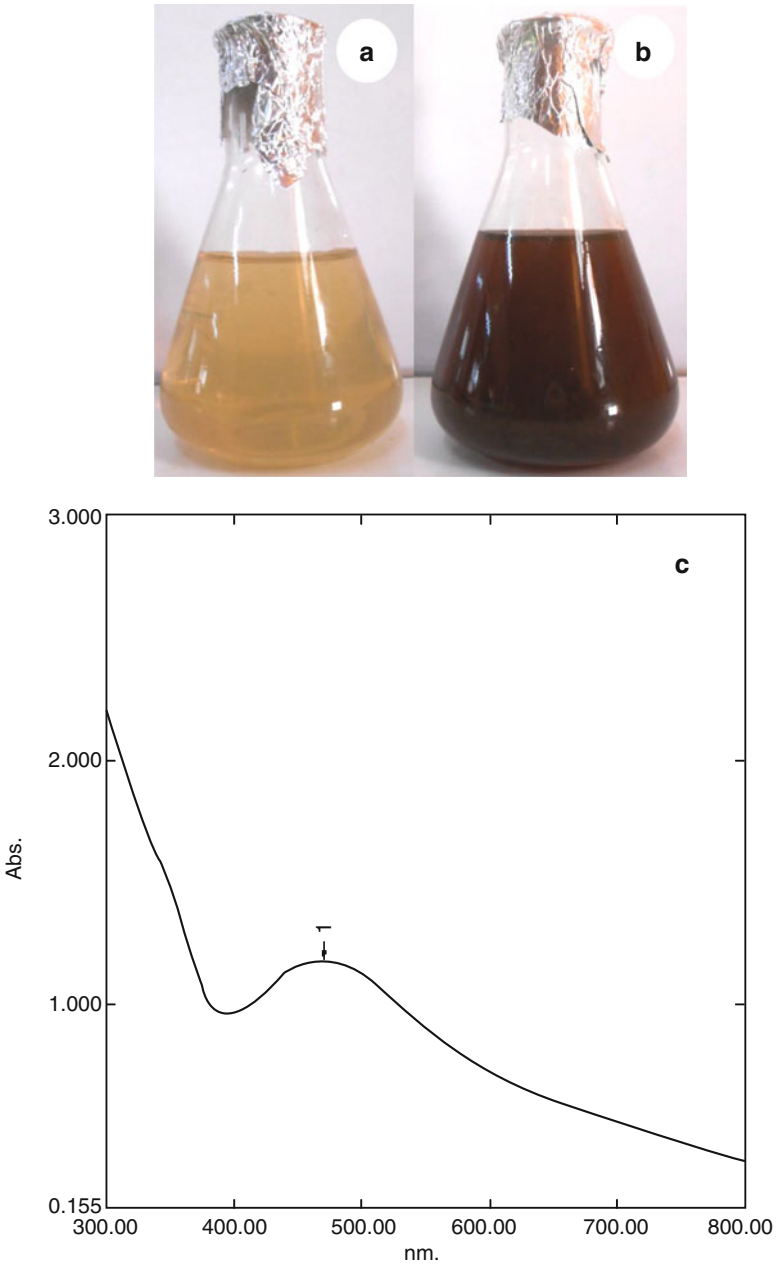

Fig. 7.4 (**a**) Photograph showing change in color after adding $AgNO_3$ before reaction. (**b**) After reaction time of 6 h. (**c**) UV–Vis spectra of aqueous silver nitrate with *F. elephantum* leaf extract

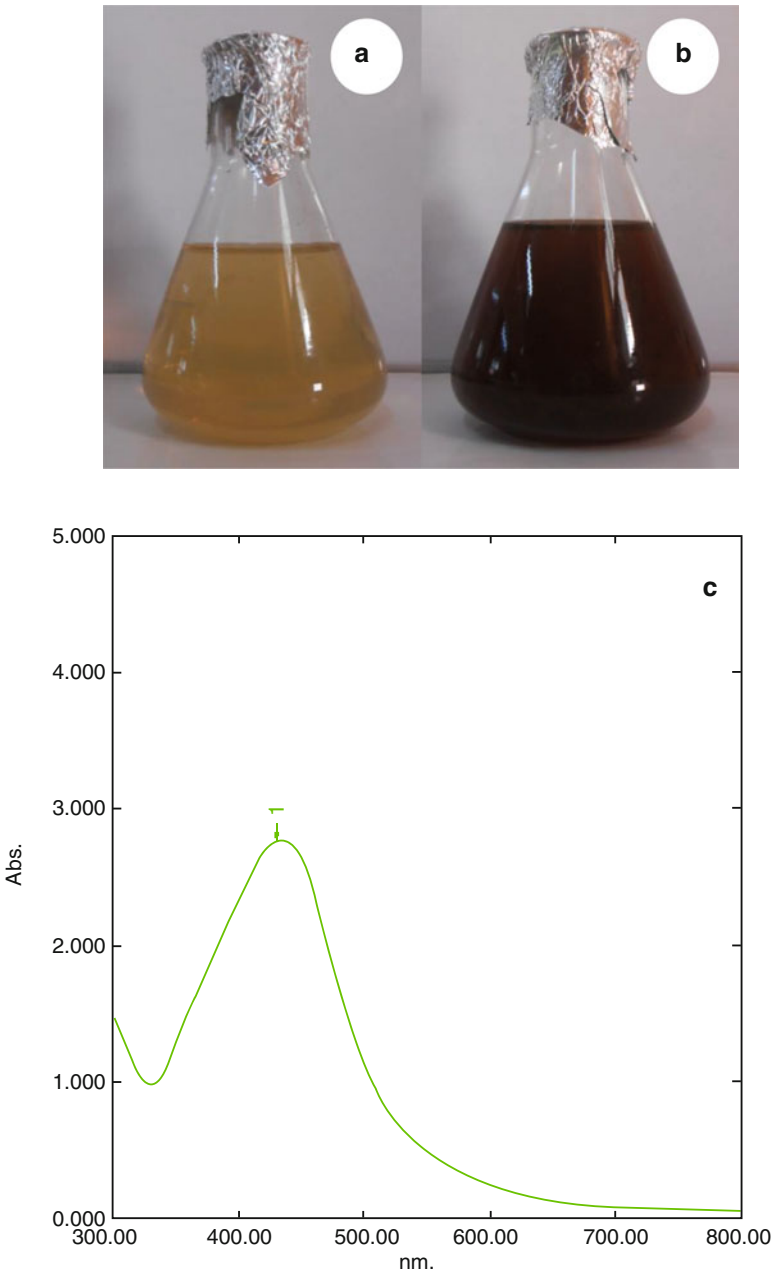

Fig. 7.5 (**a**) Photographs showing change in color after adding $AgNO_3$ before reaction and (**b**) After reaction time of 6 h. (**c**) UV–Vis spectra of aqueous silver nitrate with *H.indicum* leaf extract

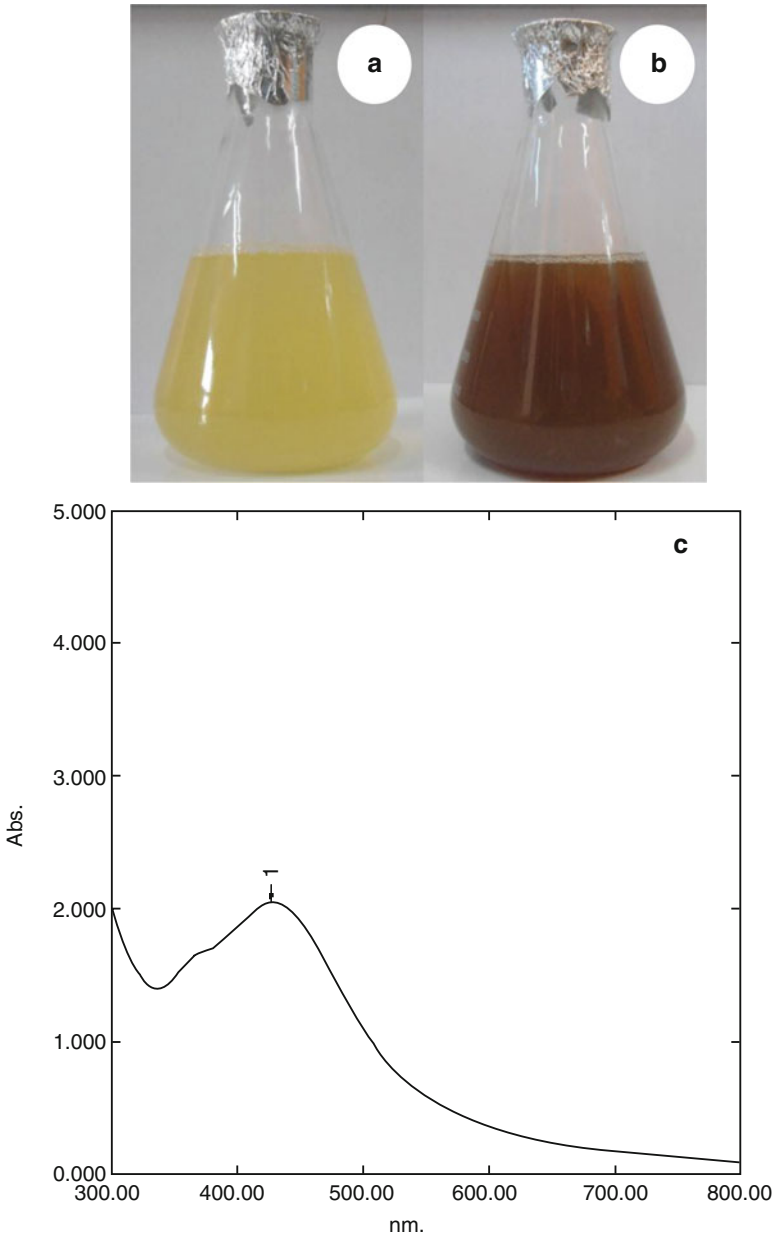

Fig. 7.6 (**a**) Photographs showing change in color after adding $AgNO_3$ before reaction. (**b**) After reaction time of (6 h). (**c**) UV–Vis spectra of aqueous silver nitrate with *S.acuta* leaf extract

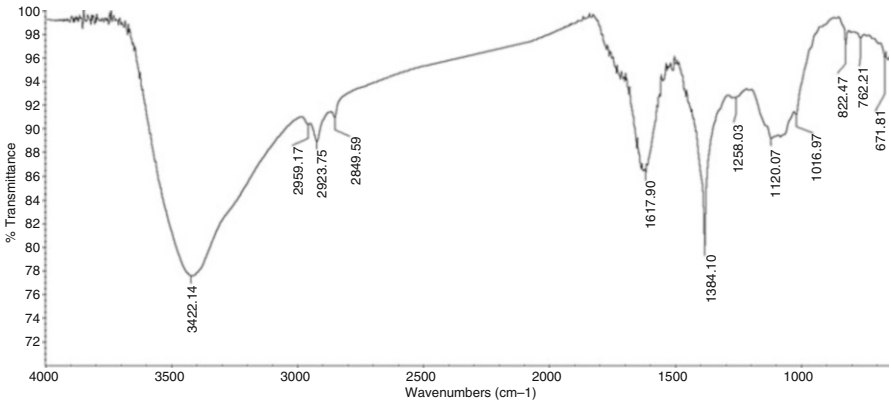

Fig. 7.7 FT-IR spectrum of synthesized AgNPs using *F. elephantum* leaf extract

(1,765.45), C–H stretch (2,851.32), C–H stretch (2,932.36), and O–H stretch (3,396.59) (Fig. 7.8). FT-IR analysis of *S.acuta* Ag NPs showed the presence of bands due to O–H group (1,269.92 cm^{-1}), C═N stretch (1,486.57), – NH2(1636.98), ═NH (2,332.25), −H stretch (2,358.27), and O–H stretch (3,345.57) (Fig. 7.9).

7.3.3 SEM, EDX and TEM Analysis of Ag NPs

SEM micrographs of the synthesized Ag NPs of *F. elephantum*, *H. indicum* and *S. acuta* magnified at ×500, ×3,000 and ×5,000 and measured at 20–60 nm, respectively are shown in Figs. 7.10a, 7.11a, and 7.12a. The triangular, pentagonal, and hexagonal structures are clear. Energy-dispersive X-ray spectroscopy (EDX) proves the chemical purity of the synthesized Ag NPs (Figs. 7.10b, 7.11b, and 7.12b). Transmission electron microscopy has been employed to characterize the size, shape and morphology of synthesized silver nanoparticles. The TEM image of silver nanoparticles is shown in Figs. 7.13a, 7.14a, and 7.15a. The electron microscopic study of the nanoparticles using TEM revealed that the nano-Ag predominates with spherical, triangle, truncated triangles, and decahedral morphologies ranging from 18 to 45 nm. The average particles size measured from the TEM image is 22 nm. Figures 7.13b, 7.14b, and 7.15b shows the histogram of size distribution of silver nanoparticles.

7.3.4 XRD Analysis of Ag NPs

After reaction, the diffraction peaks formed facets of the face- centered cubic crystal structure. A few unassigned peaks were also noticed in the vicinity of the characteristic peaks. These sharp Bragg peaks might have resulted due to the capping agent

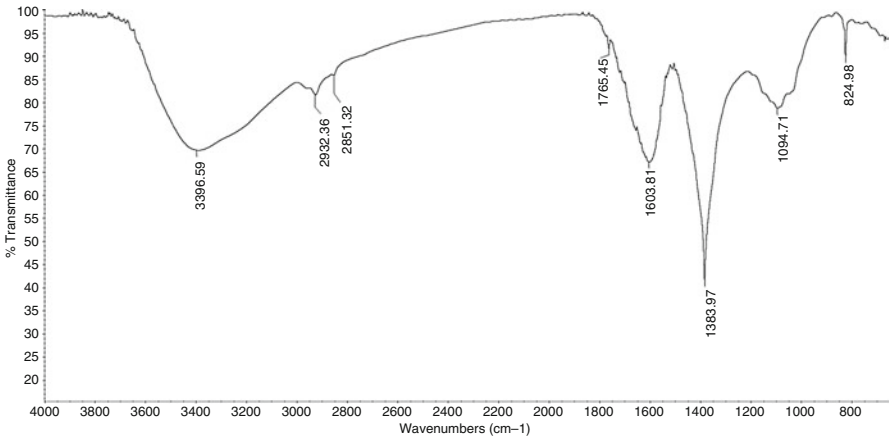

Fig. 7.8 FT-IR spectrum of synthesized AgNPs using *H.indicum* leaf extract

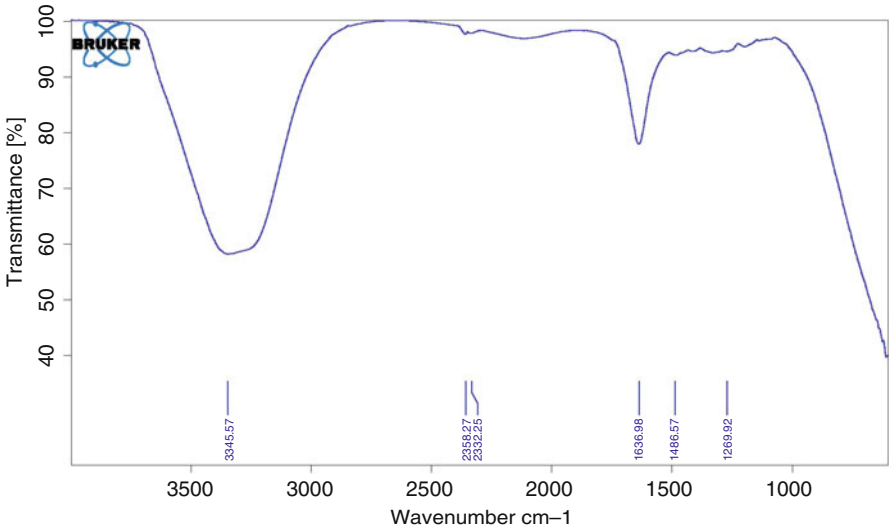

Fig. 7.9 FT-IR spectrum of synthesized AgNPs using *S.acuta* leaf extract

stabilizing the nanoparticles. Figures 7.16, 7.17, and 7.18 depicts the X-ray diffraction (XRD) pattern of *F. elephantum*, *H. Indicum* and *S. acuta* -powdered silver nanoparticles in the 2θ range. It exhibits a broad peak at 38.4°, 44.5°, and 64.2° and 78.4°. The broadening of the peaks clearly indicates that the particles are in the nanoregime. Apart from these, many unidentified peaks at 28°, 29°, 30°, 32°, 35°, 43°, 45° and 52° arise, possibly due to other chemical reactions or organic impurities present in the sample.

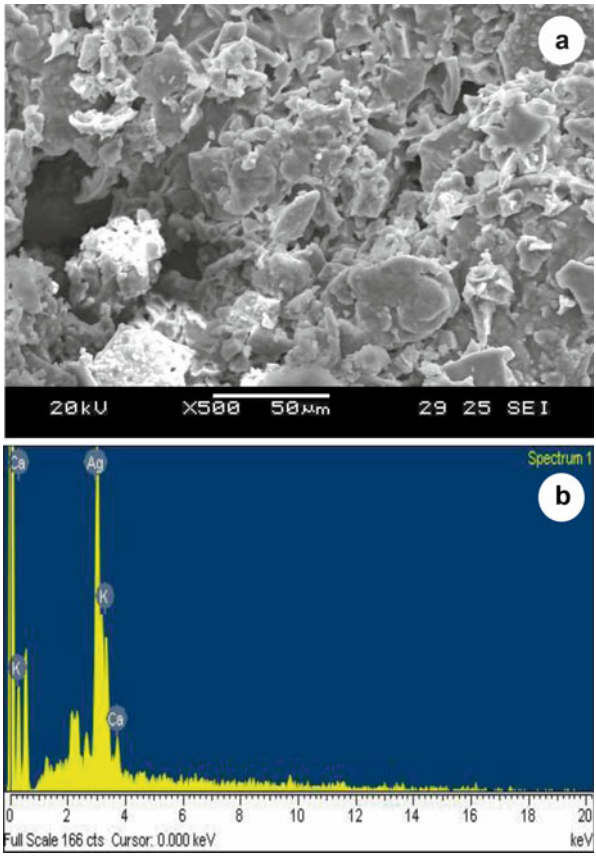

Fig. 7.10 Scanning electron micrographs of AgNPs synthesized with *F. elephantum* leaf extract and 1.0 mM $AgNO_3$ solution and incubated at 60 °C for 6 h at pH 7.0. (**a**) Magnified ×500; *inset bar* represents 50 µm. (**b**) EDX image showing chemical composition

7.3.5 *Larvicidal Efficacy of Aqueous Extract and Synthesized Ag NPs*

The results of larvicidal activity of *S. acuta*, *H. Indicum* and *F. elephantum* aqueous leaf extract and Ag NPs against late third instar *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus* was noted and presented in Tables 7.2, 7.3, 7.4, 7.5, 7.6 and 7.7 (Figs. 7.19, 7.20, and 7.21). From the three plant aqueous leaf extract and Ag NPs tested against late third instar *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*, the highest larvicidal activity was observed in *F. elephantum*, moderate larvicidal activity was observed in *H. Indicum* and lowest larvicidal activity was observed in *S. acuta*. All three plant aqueous leaf extract and synthesized Ag NPs showed the larvicidal efficacy within 24 h of exposure. Mortality rate (Y) is positively related to the concentration of dose (X) indicating that mortality increases with the increasing dose. Among the Ag NPs tested, the Ag NPs of *F. elephantum* were highly effective against third instar larvae of *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*

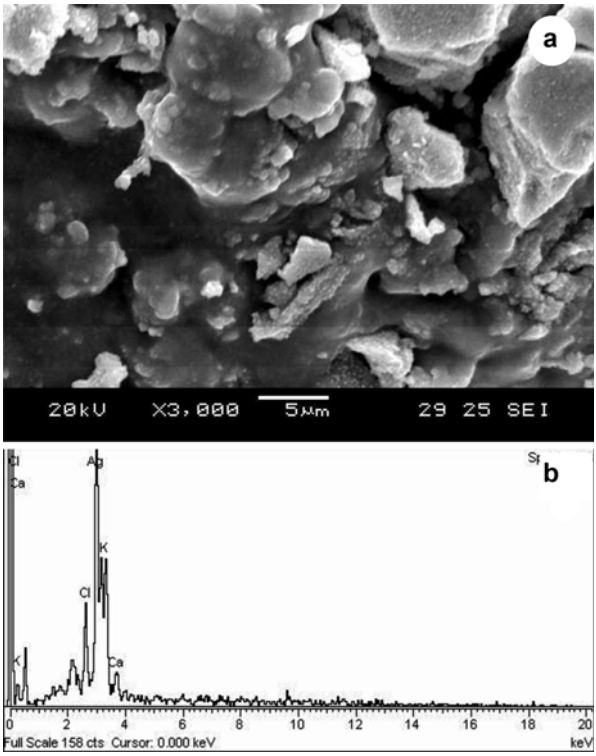

Fig. 7.11 Scanning electron micrographs of AgNPs synthesized with *H.indicum* leaf extract and 1.0 mM $AgNO_3$ solution and incubated at 60 °C for 6 h at pH 7.0. (**a**) Magnified ×3000; *inset bar* represents 5 µm. (**b**) EDX image showing chemical composition

with the LC_{50} and LC_{90} values were 20.01, 21.59, 24.04 µg/mL and 34.76, 37.06, 40.86 µg/mL, respectively. The control showed nil mortality in the concurrent assay. χ^2 value was significant at $p \leq 0.05$ level. High larvicidal activity of *F. elephantum* mediated Ag NPs can be correlated with its lower particle size than other Ag NPs from different plants. Smaller particle size increase surface area to volume ratio and thus increases its action against larvae. The order of effectiveness decreased from *F. elephantum* >*H. Indicum* >*S. acuta* against third instars of *A. subpictus* followed by *A. albopictus* and *C. tritaeniorhynchus*. The larvae of *A. subpictus* were found highly susceptible to the synthesized Ag NPs than the larvae of *A. albopictus* and *C. tritaeniorhynchus*.

7.4 Discussion

Mosquito-borne diseases are one of the most public health problems in the developing countries. Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito-borne disease is by killing mosquito at larval stage. Management of this disease vector using synthetic chemicals has failed because of insecticide resistance, vector resurgence, and environmental pollution

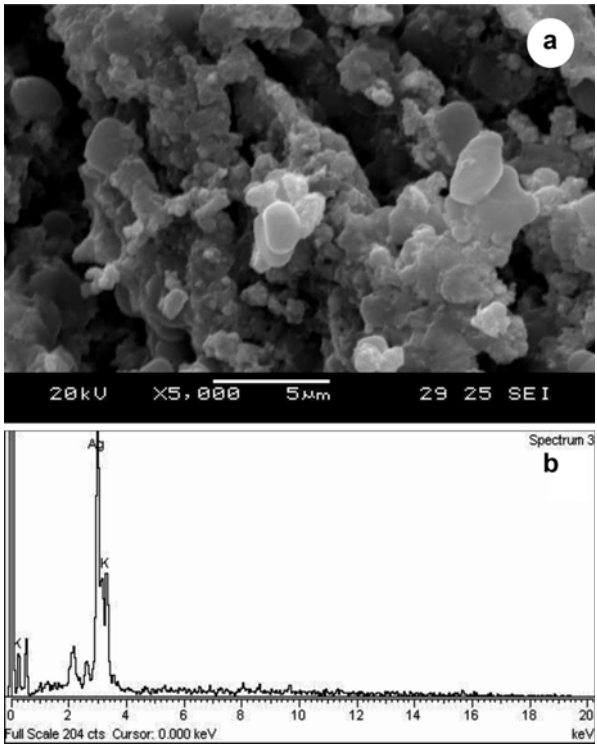

Fig. 7.12 Scanning electron micrographs of AgNPs synthesized with *S.acuta* leaf extract and 1.0 mM $AgNO_3$ solution and incubated at 60 °C for 6 h at pH 7.0. (**a**) Magnified ×5000; *inset bar* represents 5 µm. (**b**) EDX image showing chemical composition

(Wondji et al. 2009). In recent years, silver nanoparticles play a major role in antibacterial, antifungal, and insect control programs. The development of novel technology was in the field of insect control particularly mosquito control due to the resistance behavior of the mosquitoes. Plant materials which synthesized silver nanoparticles also used for the mosquito control (Borase et al. 2013) are more popular. Petroleum ether, acetone, ethyl acetate, aqueous extract, methanol and ethanol fractionate of *Eichhornia crassipes* Solms was tested for their larvicidal efficacy against the different instars (I, II, III and IV) and pupae of *C. quinquefasciatus* . The larval mortality was observed after 24 h of the treatment. Ethanol fractionate of *E. crassipes* showed the highest larvicidal and pupicidal activity against *C. quinquefasciatus* compared to other solvent extracts and fractionates with LC_{50} 71.43, 94.68,120.42, 152.15 and 173.35 ppm for I, II, III, IV and pupae, respectively (Jayanthi et al. 2012). Vinayachandra et al. (2011)) reported that the effect of aril and kernel extracts of *Knema attenuata* on larvae of *A. albopictus* and *A. stephensi* under laboratory conditions. The aril was extracted with chloroform and ethanol; the kernel was extracted with ethanol and hexane. All the graded concentrations (100, 200, 300, 400 and 500 ppm) showed significant larval mortality after 24 h of observation. Chloroform extracts of aril showed 100 % mortality against both larval forms of *A. albopictus* and *A. stephensi* at the concentration of 500 ppm. Among the extracts tested, chloroform extracts of aril and ethanol extracts of kernel exhibited

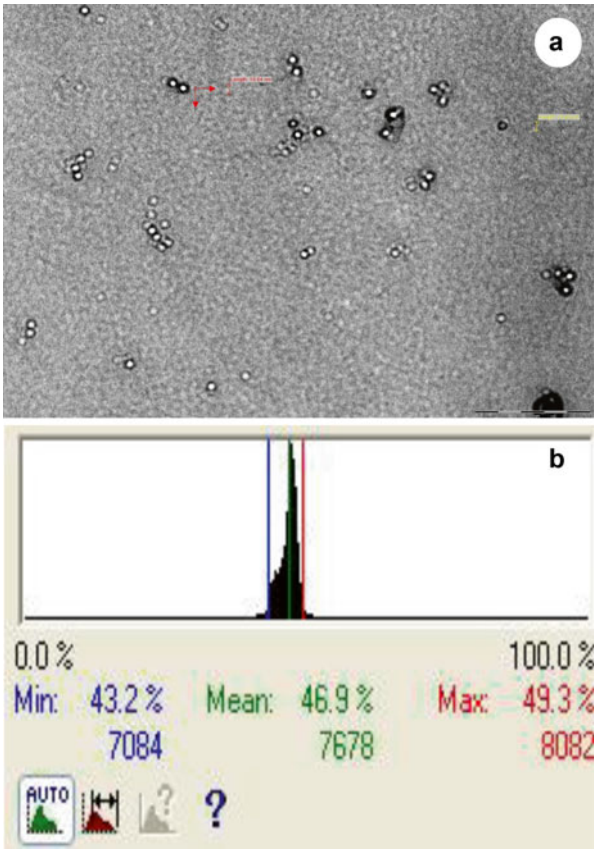

Fig. 7.13 Transmission electron microscopic image (**a**) and histogram (**b**) showing synthesized AgNPs from *F. elephantum*

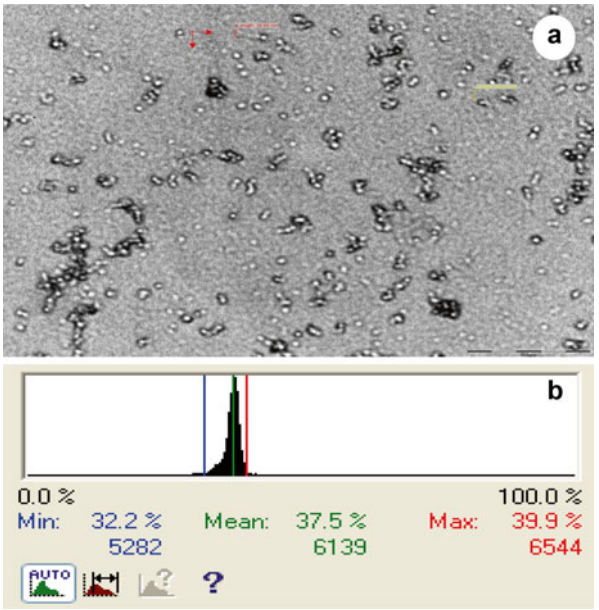

Fig. 7.14 Transmission electron microscopic image (**a**) and histogram (**b**) showing synthesized AgNPs from *H.indicum*

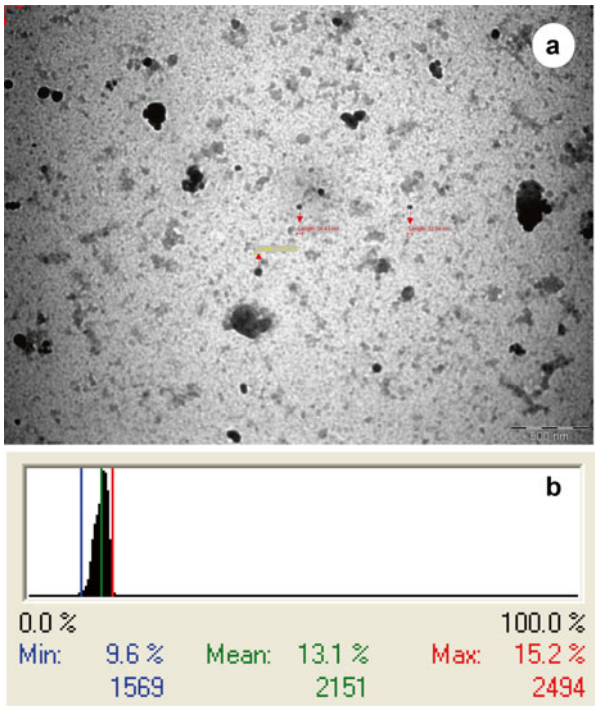

Fig. 7.15 Transmission electron microscopic image (**a**) and histogram (**b**) showing synthesized AgNPs from *S.acuta*

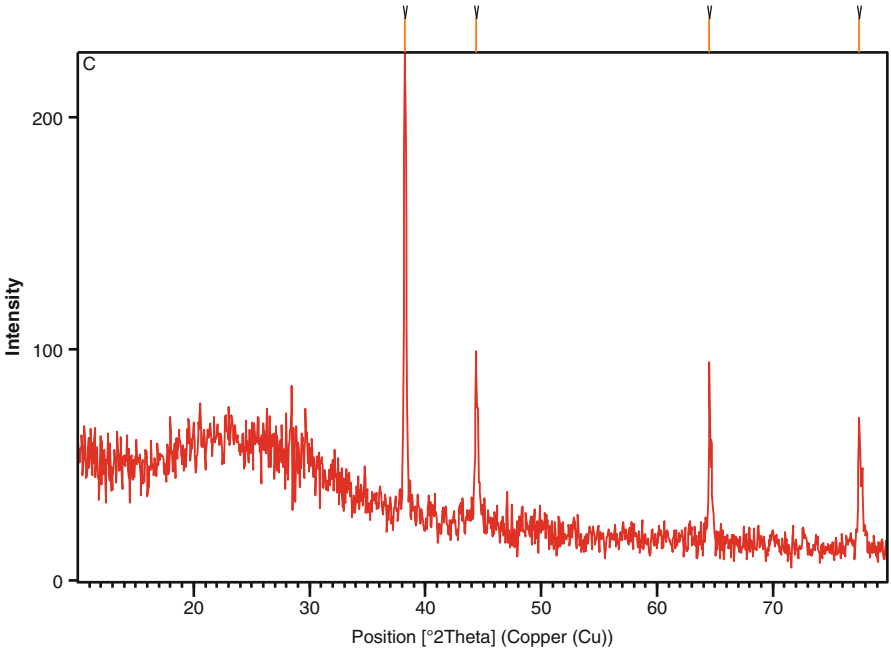

Fig. 7.16 X-Ray diffraction showing synthesized AgNPs from *F. elephantum*

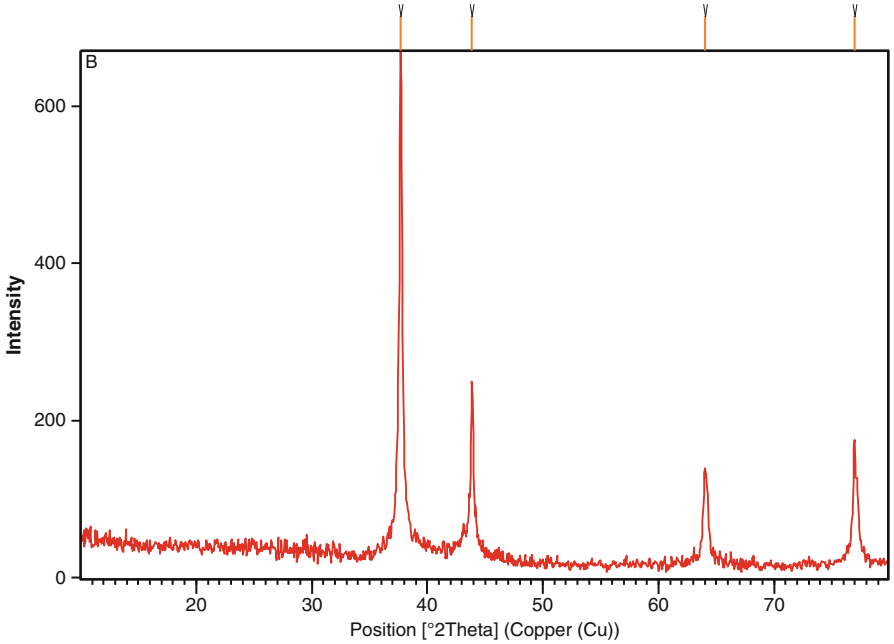

Fig. 7.17 X-Ray diffraction showing synthesized AgNPs from *H.indicum*

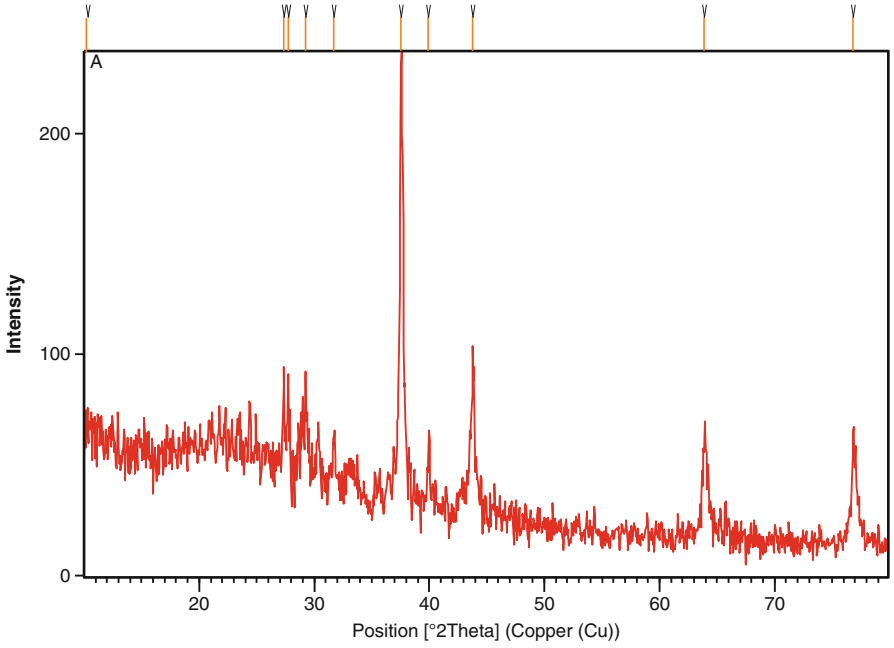

Fig. 7.18 X-Ray diffraction showing synthesized AgNPs from *S. acuta*

Table 7.2 Larvicidal activity of *Sida acuta* aqueous leaf extract against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2
A. subpictus	Control 60 120 180 240 300	0.0 ± 0.0 29.3 ± 0.8 48.5 ± 1.6 70.4 ± 1.2 82.6 ± 0.4 100.0 ± 0.0	131.73 (92.13–168.41)	241.94 (198.89–331.55)	20.320*
A. albopictus	Control 60 120 180 240 300	0.0 ± 0.0 23.8 ± 1.2 41.6 ± 2.0 64.9 ± 0.6 83.2 ± 0.4 96.4 ± 1.3	144.61 (119.08–169.59)	257.66 (224.59–311.47)	9.737*
C.tritaeniorhynchus	Control 60 120 180 240 300	0.0 ± 0.0 20.1 ± 2.0 36.4 ± 1.5 52.5 ± 0.8 78.9 ± 1.6 94.3 ± 0.2	161.44 (135.52–188.06)	280.33 (244.35–340.34)	9.807*

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 Chi-square test
*p < 0.05, level of significance
[a]Values are mean ± SD of five replicates

Table 7.3 Larvicidal activity of silver nanoparticles synthesized using *Sida acuta* against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2
A.subpictus	Control 12 24 36 48 60	0.0 ± 0.0 30.8 ± 1.0 41.6 ± 0.8 62.5 ± 1.6 80.4 ± 1.2 100.0 ± 0.0	25.68 (15.69–36.11)	48.29 (37.42–79.62)	30.914*
A. albopictus	Control 12 24 36 48 60	0.0 ± 0.0 25.2 ± 0.3 38.8 ± 1.2 57.1 ± 2.0 76.4 ± 0.5 98.3 ± 0.2	28.10 (19.61–37.36)	51.71 (41.29–77.14)	24.070*
C.tritaeniorhynchus	Control 12 24 36 48 60	0.0 ± 0.0 21.9 ± 1.6 34.2 ± 1.2 52.1 ± 0.3 70.8 ± 0.8 95.4 ± 1.4	30.19 (22.79–40.24)	56.26 (45.43–81.46)	21.219*

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 Chi-square test
*p < 0.05, level of significance
[a]Values are mean ± SD of five replicates

Table 7.4 Larvicidal activity of *Heliotropium indicum* aqueous leaf extract against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2
A.subpictus	Control 40 80 120 160 200	0.0 ± 0.0 28.6 ± 0.8 45.6 ± 1.6 62.4 ± 1.2 80.3 ± 0.4 100.0 ± 0.0	93.16 (65.00–120.07)	169.67 (138.44–238.76)	22.281*
A. albopictus	Control 40 80 120 160 200	0.0 ± 0.0 24.5 ± 1.2 41.2 ± 2.0 59.6 ± 0.6 78.3 ± 0.4 95.4 ± 1.3	100.83 (80.42–121.13)	182.13 (155.54–229.81)	12.491*
C.tritaeniorhynchus	Control 40 80 120 160 200	0.0 ± 0.0 19.2 ± 2.0 39.5 ± 1.5 54.3 ± 0.8 74.6 ± 1.6 92.1 ± 0.2	109.14 (91.26–127.58)	193.40 (167.97–236.39)	9.662*

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 Chi-square test
*p < 0.05, level of significance
[a]Values are mean ± SD of five replicates

Table 7.5 Larvicidal activity of silver nanoparticles synthesized using *Heliotropium indicum* against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2
A.subpictus	Control 10 20 30 40 50	0.0 ± 0.0 29.5 ± 1.0 45.2 ± 0.8 62.3 ± 1.6 78.4 ± 1.2 100.0 ± 0.0	23.44 (15.80–30.72)	43.04 (34.74–62.53)	24.601*
A. albopictus	Control 10 20 30 40 50	0.0 ± 0.0 23.9 ± 0.3 40.1 ± 1.2 58.5 ± 2.0 74.2 ± 0.5 96.3 ± 0.2	25.84 (20.13–31.62)	46.37 (39.04–60.58)	15.220*
C.tritaeniorhynchus	Control 10 20 30 40 50	0.0 ± 0.0 20.7 ± 1.6 35.3 ± 1.2 52.4 ± 0.3 68.2 ± 0.8 91.5 ± 1.4	28.54 (23.31–34.15)	50.76 (43.20–64.89)	12.184*

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 Chi-square test
*p < 0.05, level of significance
[a]Values are mean ± SD of five replicates

Table 7.6 Larvicidal activity of *Feronia elephantum* aqueous leaf extract against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (μg/mL) (LCL-UCL)	LC_{90} (μg/mL) (LCL-UCL)	χ^2
A.subpictus	Control 30 60 90 120 150	0.0 ± 0.0 22.3 ± 1.0 36.2 ± 1.5 61.4 ± 2.0 82.5 ± 1.8 100.0 ± 0.0	74.14 (58.90–89.38)	126.48 (107.78–160.92)	14.933*
A. albopictus	Control 30 60 90 120 150	0.0 ± 0.0 20.5 ± 1.5 31.6 ± 1.3 56.2 ± 2.0 77.4 ± 1.6 97.3 ± 1.2	80.19 (66.09–94.70)	136.53 (117.81–169.43)	12.224*
C.tritaeniorhynchus	Control 30 60 90 120 150	0.0 ± 0.0 17.2 ± 1.8 26.5 ± 1.2 50.3 ± 0.8 72.4 ± 1.6 94.6 ± 2.0	87.11 (74.29–100.78)	145.38 (127.03–176.38)	10.035*

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 Chi-square test
*$p < 0.05$, level of significance
[a]Values are mean ± SD of five replicates

Table 7.7 Larvicidal activity of silver nanoparticles *Feronia elephantum* against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (μg/mL) (LCL-UCL)	LC_{90} (μg/mL) (LCL-UCL)	χ^2
A.subpictus	Control 8 16 24 32 40	0.0 ± 0.0 23.2 ± 2.0 38.4 ± 1.8 57.5 ± 0.4 79.6 ± 1.6 100.0 ± 0.0	20.01 (15.33–24.85)	34.76 (29.06–46.29)	18.464*
A. albopictus	Control 8 16 24 32 40	0.0 ± 0.0 20.4 ± 1.6 32.5 ± 2.0 54.3 ± 1.9 78.6 ± 1.4 95.2 ± 1.6	21.59 (18.19–25.09)	37.06 (32.36–44.86)	9.846*
C.tritaeniorhynchus	Control 8 16 24 32 40	0.0 ± 0.0 18.4 ± 1.3 26.3 ± 1.2 48.2 ± 0.2 67.5 ± 1.8 92.1 ± 1.9	24.04 (20.17–28.33)	40.86 (35.15–51.25)	11.549*

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 Chi-square test
*$p < 0.05$, level of significance
[a]Values are mean ± SD of five replicates

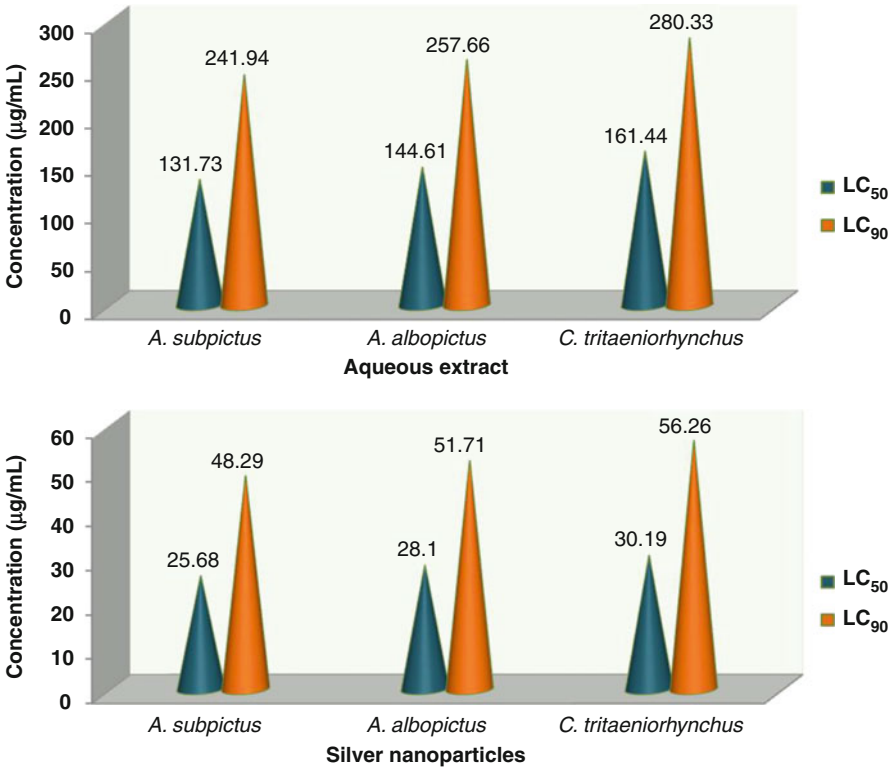

Fig. 7.19 Graph showing the LC_{50} and LC_{90} values of larvicidal activity of *S.acuta* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

higher toxicity against both *A. albopictus* (LC_{50}, 141 and 159 ppm; LC_{90}, 290 and 342 ppm) and *A. stephensi* (LC_{50}, 160 and 162 ppm; LC_{90}, 445 and 458 ppm). Hexane extracts of kernel exhibited least toxicity against *A. albopictus* (LC_{50}, 239 ppm; LC_{90}, 484 ppm), whereas ethanol extracts of aril showed the least toxicity against *A. stephensi* (LC_{50}, 290; LC_{90}, 498).

Mahesh Kumar et al. (2012) have reported that the LC_{50} value of first to fourth instar larvae and pupae was 155.29, 198.32, 271.12, 377.44 and 448.41 ppm, respectively. The LC_{90} value of first to fourth instar larvae and pupae was 687.14, 913.10, 1,011.89, 1,058.85 and 1141.65 ppm, respectively. Patil et al. (2011) evaluated larvicidal activity of extracts of medicinal plants *Plumbago zeylanica* and *Cestrum nocturnum* against *A. aegypti*; the LC_{50} values of both the plants were less than 50 ppm. The larvicidal stability of the extracts at five constant temperatures (19, 22, 25, 28 and 31 °C) evaluated against fourth instars larvae revealed that toxicity of both plant extracts increases with increase in temperature. Prophiro et al. (2012) reported that the susceptibility of the larvae was determined under three different temperatures of 15 °C, 20 °C, and 30 °C with lethal concentrations for *Copaifera* sp.

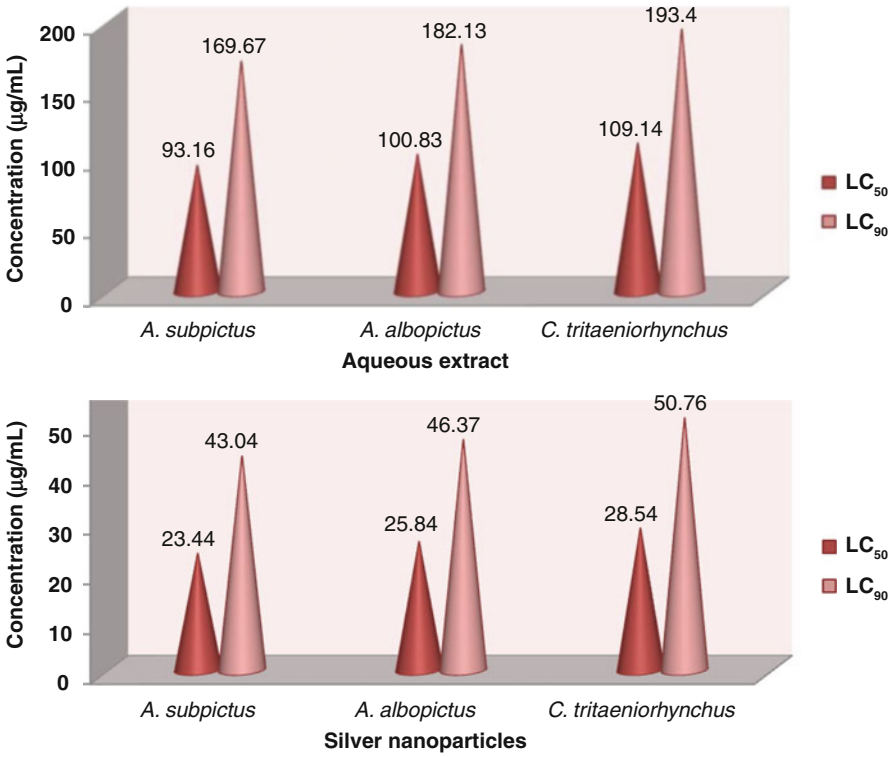

Fig. 7.20 Graph showing the LC_{50} and LC_{90} values of larvicidal activity of *H.indicum* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

ranged from $LC_{50}=47$ to $LC_{90}=91$ (milligrams per liter), and for *Carapa guianensis*, they were $LC_{50}=136$ to $LC_{90}=551$ (milligrams per liter), respectively. The larvicidal activity of crude petroleum ether, ethyl acetate, and methanol extracts of the whole plants of *Phryma leptostachya* was assayed for its toxicity against the early fourth instar larvae of *C. pipiens* pallens. The larval mortality was observed after 24 h of exposure. Among three solvent extracts from *P. leptostachya*, the petroleum ether extract exhibited the best larvicidal activity. The corresponding LC_{50} values of petroleum ether, ethyl acetate, and methanol extracts were 3.23, 5.23, and 61.86 ppm against the early fourth instar larvae of *C. pipiens* (Xiao et al. 2012). The bioefficacy of *Aloe vera* leaf extract and bacterial insecticide, *Bacillus sphaericus* larvicidal activity was assessed against the first to fourth instars larvae of *A. aegypti*, under the laboratory conditions. The LC_{50} of *A. vera* against the first to fourth instars larvae were 162.74, 201.43, 253.30 and 300.05 ppm and the LC_{90} 442.98, 518.86, 563.18 and 612.96 ppm, respectively (Subramaniam et al. 2012).

The hexane extract of *M. koenigii* was found to be the most effective providing 100 % mortality at 750 ppm against the larvae of *A. stephensi* at 48 h followed by

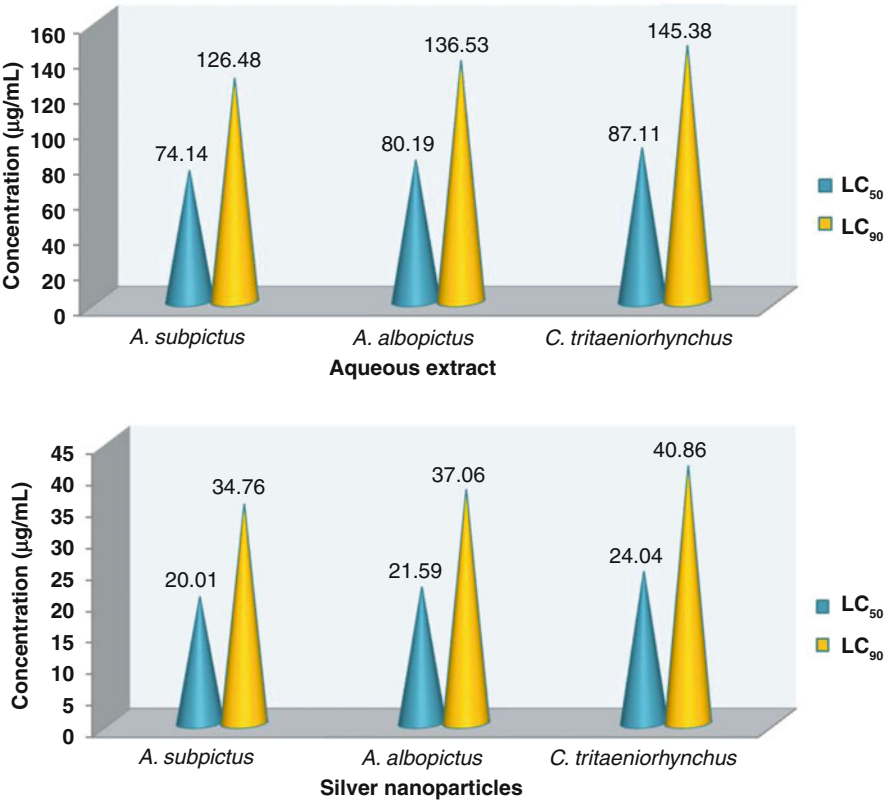

Fig. 7.21 Graph showing the LC_{50} and LC_{90} values of larvicidal activity of *F. elephantum* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

C. quinquefasciatus at 1,000 ppm at 48 h. Hexane extract showed the least LC_{50} value of 418.74 and 466.09 ppm against *A. stephensi* and *C. quinquefasciatus*, but it was diethyl ether in the case of *A. aegypti* with an LC_{50} value of 511.12 ppm (Arivoli and Samuel 2011). Essential oils extracted by steam distillation from rhizome of *Z. officinalis* and leaf and stem of *Achyranthes aspera* were evaluated for larvicidal, attractant/repellent, and oviposition attractant/ deterrent activity against two mosquito species *viz*., *A.aegypti* and *C. quinquefasciatus*. The highest larvicidal activity, i.e., LC_{50} = 154 ppm and LC_{50} = 197 ppm for *A. aegypti* and *C. quinquefasciatus*, respectively was shown by *Z. officinalis*. This oil also offers 5-h protection at the concentration of 0.5 mg/cm^2 from both mosquito species (Khandagle et al. 2011). Khanna et al. (2011) have reported that the larvicidal crude leaf extract of *Gymnema sylvestre* showed the highest mortality in the concentration of 1,000 ppm against the larvae of *A. subpictus* (LC_{50} = 166.28 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50} = 186.55 ppm), and the maximum efficacy was observed in gymnemagenol compound isolated from petroleum ether leaf extract of *G. sylvestre* with LC_{50}

values against the larvae of *A. subpictus* at 22.99 ppm and against *C. quinquefasciatus* at 15.92 ppm.

The larvicidal, ovicidal, and repellent activities of crude benzene and ethyl acetate extracts of leaf of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were assayed for their toxicity against three important vector mosquitoes, *viz*., *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* with the LC_{50} and LC_{90} values were 79.08, 89.59, and 96.15 ppm and 150.47, 166.04, and 174.10 ppm, respectively (Govindarajan et al. 2011b). Mathew et al. (2009) reported that leaf chloroform extracts of *Nyctanthes arbortristis* showed lethal values (LC_{50} = 526.3 and 780.6 ppm (24 h) and LC_{50} = 303.2 and 518.2 ppm (48 h)) against *A. aegypti* and *A.stephensi*, respectively. Elimam et al. (2009) to investigate the larvicidal, adult emergence inhibition and oviposition deterrent activity of aqueous leaves extract of *Calotropis procera* against *A. arabiensis* and *C. quinquefasciatus* as natural mosquito larvicide. LC_{50} and LC_{90} values calculated were 273.53–783.43, 366.44–1018.59 and 454.99–1224.62 ppm for 2nd, 3rd and 4th larval instars, respectively, of *A. arabiensis* and 187.93–433.51, 218.27–538.27 and 264.85–769.13 ppm for 2nd, 3rd and 4th larval instars, respectively, of *C. quinquefasciatus*.

Mathivanan et al. (2010) determine that the LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia coronaria* on *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* larvae in 24 h were 72.41, 65.67, and 62.08 and 136.55, 127.24, and 120.86 mg/L, respectively. The ethanolic leaf extract of *Cassia obtusifolia* was investigated for their larvicidal and oviposition deterrence effects against *A. stephensi*. Concentrations ranging from 25 to 125 mg/l were assessed at 24 h post-treatment against late third instar larvae. The leaf extract had significant larvicidal effect with LC_{50} and LC_{90} values were 52.2 and 108.7 mg/l, respectively. In oviposition behaviour study, four different concentrations ranging from 100 to 400 mg/l were studied against gravid female mosquitoes. Essential oil from *Tagetes filifolia* showed the strongest larvicidal activity against the third instar larvae of *A. aegypti* with the LC_{50} value of 47.7 ppm (Ruiz et al. 2011). Conti et al. (2010) studied *Foeniculum vulgare* essential oil for larvicidal activity against fourth instar larvae of *A. albopictus* and the oil showed larvicidal activity with an IC_{50} value of 142.9 ppm. In *Calotropis procera* against *A. stephensi*, showed 99 % mortality at 64 ppm for *A. stephensi*, only 44 % mortality against *C. quinquefasciatus*, and a maximum of 67 % in 256 ppm, respectively (Shahi et al. 2010). *Clitoria ternatea* leaf methanol extract showed dose-dependent larvicidal activity against *A. stephensi* with LC_{50} values of 555.6 (24 h) and 867.3 (48 h) ppm, also the root extracts with LC_{50} value of 340 ppm (48 h). Seed extract showed larvicidal activity (LC_{50} = 116.8, 195 ppm) after 24 h and (LC_{50} = 65.2, 154.5 ppm) after 48 h treatment against *A. stephensi* and *A. aegypti*, respectively. Larvicidal activity of flower methanol extract showed LC_{50} values 233 and 302.5 ppm against *A. stephensi* and *A. aegypti*, respectively, after 48 h treatment. Methanol extract showed lowest LD values against several instars of larvae and 50 adult (121.59, 142.73, 146.84, 202.98, 290.65, 358.42 and 300.03 µg/

cm^2, respectively) which indicates highest toxicity or insecticidal activity (Ashraful Alam et al. 2009).

Kamalakannan et al. (2011) determined the biological activities of methanol extracts of *Acalypha indica* and *Achyranthes aspera* leaves individually and in combination as botanical insecticides against *A. aegypti*. Based on LC_{50} values for 4th instar *A. aegypti*, the combined extracts showed the strongest larvicidal activity (277 ppm). *Achyranthes aspera* and *Acalypha indica* extracts individually gave similar results (409 and 420 ppm, respectively). Crude extracts of fruits and leaves of *Centratherum anthelminticum* in different solvents were tested for larvicidal activity against *A. stephensi*, the vector of malaria. The petroleum ether crude extract of both fruits and leaves exhibited significant larvicidal activity against III instar larvae with LC_{50} values of 162.60 ppm and 522.94 ppm, respectively after 24 h. The petroleum ether extract of fruit was 11.66, 2.15 and 1.32 times more toxic than that of leaf extract after 24, 48 and 72 h, respectively at LC_{90} level. However, at LC_{50} level the corresponding values were 3.22, 1.83 and 1.19, respectively (Srivastava et al. 2008). Gleiser and Zygadlo (2007) reported that the essential oils of *Lippia turbinata* and *L. polystachya* exhibit LC_{50} values of 74.9 and 121 mg/l, respectively against *C. quinquefasciatus*. A preliminary study was conducted to investigate the effects of the extracts of 112 medicinal plant species, collected from the southern part of Thailand, on *A. aegypti*. Studies on larvicidal properties of plant extracts against the fourth instar larvae revealed that extracts of 14 species showed evidence of larvicidal activity. Eight out of the 14 plant species showed 100 % mosquito larvae mortality. The LC_{50} values were less than 100 μg/mL (4.1–89.4 μg/mL). Six plant species were comparatively more effective against the fourth instar larvae at very low concentrations. Three medicinal plants with promising larvicidal activity, having LC_{50} and LC_{90} values being 4.1 and 16.4 μg/mL for *Mammea siamensis*, 20.2 and 34.7 μg/mL for *Anethum graveolens* and 67.4 and 110.3 μg/mL for *Annona muricata*, respectively (Promisiri et al. 2006).

Cypermethrin and crude extracts of *Solanum xanthocarpum* were both observed for their larvicidal activity against *C. quinquefasciatus*. Petroleum ether extract with lethal concentration LC_{50} and LC_{90} of 41.28 and 111.16 ppm after 24 h and LC_{50} 38.48 and LC_{90} 80.83 ppm after 48 h, respectively, was found to be the most effective, followed by carbon tetrachloride and methanol extracts (Mohan et al. 2006). Sakthivadivel and Daniel (2008) showed the crude petroleum ether leaf extract of *Jatropha curcas* to have larvicidal activity with the LC_{50} of <100 ppm on the early fourth instar larvae of vector mosquitoes including *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti*. Investigations were made to test the larval toxicity and smoke repellent potential of *Albizzia amara* and *Ocimum basilicum* at different concentration (2, 4, 6, 8 and 10 %) against the different instar (I, II, III and IV) larvae and pupae of *A. aegypti*. The LC_{50} values of *A. amara* and *O. basilicum* for I instar larvae was 5.412 and 3.734, II instar 6.480 and 4.154, III instar 7.106 and 4.664, IV instar 7.515 and 5.124, respectively. The LC_{50} and LC_{90} values of pupae were 6.792, 5.449 % and 16.925, 15.474 %. The smoke toxicity of *A. amara* was more effective against *A. aegypti* than the *O. basilicum* (Murugan et al. 2007).

The insecticidal activities of extracts and oils of 17 medicinal plants of Brazil have been determined using an *A. aegypti* larvicidal bioassay. Oils from *Anacardium occidentalis*, *Copaifera langsdorffii*, *Carapa guianensis*, *Cymbopogon winterianus* and *Ageratum conyzoides* showed high activities with LC_{50} values of 14.5, 41, 57, 98 and 148 µg/l, respectively. The most active ethanolic extract tested was that from the stem of *Annona glabra* which presented an LC_{50} value of 27 µg/l (Mendonca et al. 2005). Hidayatulfathi et al. (2005) using the hexane fraction showed the highest larvicidal effect on *A. aegypti* 4th instar larvae with LC_{50} value of 1.88 ppm and the LC_{90} value of 10.76 ppm respectively. Mosquito larvicidal activity of crude carbon-tetra-chloride, methanol and petroleum ether extracts of *Solanum xanthocarpum* fruits was examined against *A. stephensi* and *C. quinquefasciatus*. Among the extracts tested, carbon-tetra-chloride extract was the most effective with LC_{50} values of 5.11 ppm after 24 h and 1.27 ppm after 48 h of treatment against *A. stephensi*. In the case of *C. quinquefasciatus* the petroleum ether extract was observed as most toxic with LC_{50} values of 62.62 ppm after 24 h and 59.45 ppm after 48 h of exposure period, respectively (Mohan et al. 2005).

Sivagnaname and Kalyanasundaram (2004) reported that the methanolic extracts of the leaves of *Atlantia monophylla* were evaluated for mosquitocidal activity against immature stages of three mosquito species, *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti* in the laboratory. Larvae of *C. quinquefasciatus* and pupae of *A. stephensi* were found more susceptible, with LC_{50} values of 0.14 mg/l and 0.05 mg/l, respectively. Insect growth regulating activity of this extract was more pronounced against *A. aegypti*, with EI_{50} value 0.002 mg/l. Larvicidal efficacies of extracts of five species of Cucurbitacious plants, *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* were tested against the late third larval age group of *C. quinquefasciatus*. The larval mortality was observed after 24 h exposure. The LC_{50} values of *M. charantia*, *T. anguina*, *L. acutangula*, *B. cerifera* and *C. vulgaris* were 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm, respectively (Prabakar and Jebanesan 2004). Larvicidal activity of methanol extracts of 22 Australian and 12 Mexican plants against early 4th-instar larvae of *A. aegypti* and *C. pipiens* pallens was examined. At 200 ppm, 100 % mortality in larvae of *A. aegypti* and *C. pipiens* pallens was obtained in extracts of *Kigelia pinnata* and *Ruta chalepensis*. The extract of *K. pinnata* gave 76.3 and 80.3 % mortalities in larvae of *A. aegypti* and *C. pipiens* pallens at 100 ppm but, at 50 ppm, 23.7 and 42.1 % mortality against larvae of *A. aegypti* and *C. pipiens* pallens, respectively. The extract of *R. chalepensis* gave 81.2 and 87.9 % mortality in larvae of *A. aegypti* and *C. pipiens* pallens at 100 ppm but 23.4 and 53.3 % mortality against larvae of *A. aegypti* and *C. pipiens* pallens at 50 ppm, respectively. Larvicidal activities of *K. pinnata* and *R. chalepensis* extracts were significantly reduced when used at 25 ppm (Kim et al. 2002).

The larvicidal effect exhibited by essential oils and the major constituents of *Dianthus caryophyllus*, *Lepidium sativum*, *Pimpinella anisum*, and *Illicium verum* against late third to early fourth instar mosquito larvae of *C. pipiens*. The essential oils of *I. verum* and *P. anisum* demonstrated high larvicidal activity with a LC_{50} $<18\ mgL^{-1}$. The other two essential oils of *D. caryophyllus* and *L. sativum* revealed

moderate larvicidal activity, displaying a LC_{50} value above 50 mgL^{-1}. Among the pure components, the most toxic were eugenol, (E)-anethole, and α-terpinyl acetate, with LC_{50} values 18.28, 16.56, and 23.03 mgL^{-1}, respectively. Eucalyptol (1,8 cineole) and β- caryophyllene were inactive at concentrations even as high as 100 mgL^{-1}, showing the least significant activity against mosquito larvae (Kimbaris et al. 2012). Larvicidal activity of compound pectolinaringenin derived from the chloroform extract of *Clerodendrum phlomidis* against *C. quinquefasciatus* and *A. aegypti* was proved with LC_{50} and LC_{90} values of 0.62 and 2.87 ppm, and 0.79 and 5.31 ppm, respectively (Muthu et al. 2012). Sagnou et al. (2012) reported that the larvicidal activity of isolated curcuminoid compound curcumin from commercially available turmeric extract exhibited LC_{50} and LC_{90} values of 19.07 and 61.63 ppm, respectively, against the fourth instar larvae of *C. pipiens*.

Two coumarins compounds, imperatorin and osthole, from the fruit of *Cnidium monnieri* were effective against the third instar larvae of insecticide-susceptible *C. pipiens* pallens and *A. aegypti* and wild *C. pipiens* pallens with LC_{50} values of 3.14, 2.88, 4.60 ppm and 13.11, 13.14, 15.26 ppm, respectively (Wang et al. 2012); Two natural furocoumarins, 5-methoxypsoralen, and 8- methoxypsoralen, isolated from the milky sap of *Ficus carica* exhibited LC_{50} values of 9.4 and 56.3 ppm, respectively, against the early fourth stage larvae of *A. aegypti* (Chung et al. 2011). A new C_{15} acetogenin isolated from the petroleum ether extract of *Laurencia papillosa* exhibited LC_{50} values of 30.7, 36.9, and 41.8 ppm on the second, third, and fourth instar larvae of *C. pipiens*, respectively (Abou-Elnaga et al. 2011). A sesquiterpene compound, 1α, 3α, 4β- trihydroxy-9-cadinen-8-one, isolated from the chloroform extract of the roots of black galingale (*Kaempferia parviflora*) exhibited LC_{50} and LC_{90} values of 0.7 and 3.8 μM, respectively, against the early fourth stage larvae of *A. aegypti* (Moon et al. 2011). Three alkaloids, namely evodiamine, rutaecarpine, wuchuyuamide I, and two limonoids comprising evodol and limonin, derived from the chloroform extract of *Evodia rutaecarpa* unripe fruits showed LC_{50} values of 12.51, 17.02, 26.16, 52.22, and 32.43 ppm against the early fourth instar larvae of *A. albopictus* (Liu et al. 2012).

Isolated compounds such as prenylated flavonoids derived from *Dodonaea viscosa* showed larvicidal activity against *A. albopictus* and *C. pipiens* (Niu et al. 2010). Jang et al. (2005) also found that b-thujaplicin from *Chamaecyparis obtusa* leaves was effective against fourth-instar larvae of *A. aegypti*, *Ochlerotatus togoi*, and *C. pipiens* with LC_{50} values of 2.91, 2.60, and 1.33 ppm, respectively. Cheng et al. (2004) reported that the cinnamaldehyde, cinnamyl acetate, and eugenol all had excellent larvicidal effect against *A. aegypti* larvae in 24 h with LC_{50} values of 29, 33, and 33 lg/ml, respectively. Neotenone and neorautanone isoflavonoids isolated from *Neorautanenia mitis* display activity against adult *A. gambiae* mosquitoes with LD_{50} values of 80 and 90 ppm, respectively (Joseph et al. 2004).

The petroleum ether extract of dried ground whole fruits of *Piper nigrum* afforded 20 compounds (1–20) including two new insecticidal amides named as pipnoohine (1), and pipyahyine (2), seven reported for the first time from this plant (12, 13, 15–17, 19, 20), and eleven known compounds (3–11, 14, 18). The structure of 1 has been elucidated as (2E,4E,12Z)-N-(4-methylpentyl)octadeca-2,4,12-

trienamide and that of 2 as (2E,4E,11E)-12-(benzo[1,3]dioxol-5-yl)-N-(3-methylbutyl)dodeca-2,4,11-trien-amide through extensive ID-, 2D-NMR spectral studies and chemical reactions. 1 and 2 exhibited toxicity at 35.0 and 30.0 ppm respectively against fourth instar larvae of *A. aegypti* (Siddiqui et al. 2004). Ho et al. (2003) reported that the isolated compounds such as meliternatin (3, 5-dimethoxy-3′, 4′, 6, 7- bismethylendioxyflavone) (6) and six other minor polyoxygenated flavones derived from *Melicope subunifoliolata* showed larvicidal activity against *A. aegypti*. They reported that methanol and ethanol flower extracts exhibited 96 and 100 % larval mortality against the third instar; 88 and 100 % against the fourth instar of *A. aegypti*, respectively, at 0.75 and 1.00-mg/ml concentrations. Yang et al. (2003) reported a similar result, that emodin had strong larvicidal effects against the larvae of *A. aegypti*, *O. togoi* and *C. pipiens* pallens, showing LC_{50} values of approximately 1.4, 1.9, and 2.2 mg/l, respectively. Redwane et al. (2002) reported that gallotannins isolated from *Quercus lusitania* infectoria galls had the LC_{50} value of 373 ppm against *C. pipiens*. The active components dymalol, nymania-3 and triterpenes isolated from the extract of *Dysoxylum malabaricum* act as an oviposition repellent and/or deterrent to *A. stephensi* (Govindachari et al. 1999). The mosquitocidal compound ar-turmerone which isolated from rhizomes of *Curcuma longa* seems 100 % mortality of *A. aegypti* larvae at 50 mg/l concentration (Roth et al. 1998). Seven mosquitocidal compounds isolated from *Magnolia salicifolia* show 100 % mortality at the concentration of 15–100 mg/l (Kelm et al. 1997). They further reported that the higher activity was due to the presence of flavonoid (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one). Pereira and Gurudutt (1990) who observed that (−)-3-epicaryoptin isolated from *C. inerme* inhibited the growth of *C. quinquefasciatus*.

Ag NPs also induced chromosomal aberrations and aneuploidy, which showed that silver nanoparticles were cytotoxic and genotoxic to fish cells (Wise et al. 2010). Earthworms (*Eisenia fetida*) were exposed to $AgNO_3$, (94.21 mg kg^{-1}) and Ag NPs (727.6 mg kg^{-1}) with similar size ranges coated with either polyvinylpyrrolidone (hydrophilic) or oleic acid (amphiphilic) 773.3 mg kg^{-1} during a standard sub-chronic reproduction toxicity test (Shoults-Wilson et al. 2010). The use of nanoparticulate silver, copper, and their oxides will be considered in relation to their effects on bacterial populations. Silver nanoparticles formed exhibited good antibiotic activity against both Gram-positive and Gram-negative pathogens and *Candida albicans*, suggesting their broad-spectrum antimicrobial activity (Kumar et al. 2010). The toxicity of Ag NPs to natural aquatic bacterial assemblages appears to be concentration dependent for concentrations between 0 and 5 μM (Dasari and Hwang 2010). Mechanisms of toxicity are still poorly understood although it seems clear that in some cases, nanoscale-specific properties may cause bio-uptake and toxicity over and above that caused by the dissolved Ag ion (Fabrega et al. 2011). Larvicidal studies were carried out against *C. quinquefasciatus*, and results were compared with bulk permethrin. The LC_{50} of nanopermethrin and bulk permethrin to *C. quinquefasciatus* were 0.117 and 0.715 mg/l, respectively (Anjali et al. 2010).

Sakulku et al. (2009) have reported the low release rate of nanoemulsion with a large droplet size that resulted in prolonged mosquito repellant activity compared to

the nanoemulsion with small droplet size The synthesized zinc oxide nanoparticles showed the LC_{50} and χ^2 values against *R. microplus* (13.41 mg/l; 0.982), *Pediculus humanus* capitis (11.80; 0.966 mg/l), and the larvae of *A. subpictus* (3.19; 0.945 mg/l) and *C. quinquefasciatus* (4.87; 0.970 mg/l), respectively (Kirthi et al. 2011). The highest mortality was found in methanol, aqueous, and synthesized AgNPs, which used *N. nucifera* plant extract against the larvae of *A. subpictus* (LC_{50} = 8.89, 11.82, and 0.69 ppm; LC_{90} = 28.65, 36.06, and 2.15 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50} = 9.51, 13.65, and 1.10 ppm; LC_{90} = 28.13, 35.83, and 3.59 ppm) (Santhoshkumar et al. 2011). Larvicidal activity of synthesized Ag NPs utilizing an aqueous extract from *Eclipta prostrata*, was observed in crude aqueous, and synthesized Ag NPs against *C. quinquefasciatus* (LC_{50} = 27.49 and 4.56 mg/l; LC_{90} = 70.38 and 13.14 mg/l) and against *A. subpictus* (LC_{50} = 27.85 and 5.14 mg/l; LC_{90} = 71.45 and 25.68 mg/l), respectively (Rajakumar and Abdul Rahuman 2011). The synthesized Ag NPs of *Musa paradisiaca* showed the LC_{50} values against *H. bispinosa* (1.87 mg/l), *H. maculata* (2.02 mg/l), and larvae of *A. stephensi* (1.39 mg/l), against *C. tritaeniorhynchus* (1.63 mg/l). Synthesized Ag NPs using *T. cordifolia* extract tested against the larvae of *A. subpictus* (LC_{50} = 6.43 mg/l) and against the larvae of *C. quinquefasciatus* (LC_{50} = 6.96 mg/l) (Jayaseelan et al. 2011).

Microbes and plants are currently used for nanoparticle synthesis. The use of plants for the fabrication of nanoparticles is a rapid, low-cost, eco-friendly, and a singlestep method for biosynthesis process (Huang et al. 2007). The usage of plants can also be suitably scaled-up for large-scale synthesis of nanoparticles in a controlled manner according to their size, shape, and dispersity. Moreover, the use of plants in the process of nanoparticle synthesis is more beneficial than other processes since the nanoparticles are produced extracellularly. Recently, syntheses of silver nanoparticles by using plant extracts are getting more popular (Li et al. 2007; Song and Kim 2009). Chandran et al. (2006) synthesized silver nanoparticles by using the *Aloe vera* extract at 24 h of incubation. Potential antiplasmodial activity of synthesized silver nanoparticle using *Andrographis paniculata* with the inhibitory concentration (IC_{50}) values were 26 ± 0.2 % at 25 µg/ml, 83 ± 0.5 % at 100 µg/ml (Panneerselvam et al. 2011). Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* and their antiplasmodial activities against *P. falciparum* have been reported by Ponarulselvam et al. (2012). The particle shape of plant-mediated Ag NPs was mostly spherical with the exception of neem (*Azadirachta indica*) which yielded polydisperse particles both with spherical and flat plate-likemorphology 5–35 nm in size (Shankar et al. 2004). SEM images of Ag NPs from Emblica officinalis were also predominantly spherical with an average size of 16.8 nm ranging from 7.5 to 25 nm (Ankamwar et al. 2005). Tian et al. (2007) reported that the numerous flavonoids including quercetin or quercetin 3-Oglycosides were isolated from lotus leaves that were used for silver nanoparticle synthesis.

Earlier studies by various authors state that the uses of plant extract, plant-derived essential oils, and bacterial agents especially different strains of *B. thuringiensis* subsp. *varisraelensis* (B175 and B17) are alternative available potential resources for mosquito control. The efficacy of different plant extracts and *B. thuringiensis* subsp. var israelensis (Bti) varies from species to species (Mohana 2010). Levels of

effect of larvicidal activity varied with plant extract depending on the species. In contrast, larvicidal action of aqueous leaf extract of *Cassia obtusifolia* (Rajkumar and Jebanesan 2009) and *Ocimum canum*, *Ocimum sanctum*, and *R. nasutus* (Kamaraj et al. 2008) exhibited their lethal effect against larvae of *A. stephensi* and *A. aegypti*. An effective larval control of neem seed extract against *A. gambiae* was reported by Gianotti et al. (2008). The ethanolic aerial and root extract of *Phyllantus amarus* showed high insecticidal activity against stored grain pest *Triboliumcastaneus* (Khanna et al. 2003). Anti-ecdysteroid and growth inhibition effect of biomolecule Azadirachtin was documented (Zebit 1984), and the isolated compounds, fatty acids, and ricinine from leaf extracts of *R. communis* showed excellent insecticidal activity against the leaf-cutting ant *Acta sexdens* rubropilosa (Bigi et al. 2010). FT-IR studies and XRD analysis showed the presence of bioorganic components which acted as a probable stabilizer for the synthesized Ag NPs (Prathna et al. 2011). FT-IR peaks that were corresponding to aromatic rings, geminal methyls, and ether linkages indicate the presence of flavones and terpenoids responsible for the stabilization of the Ag NPs synthesized by the *Sesuvium portulacastrum* leaf extract (Nabikhan et al. 2010). Kumar et al. (2010) have synthesized Ag NPs using *Syzygium cumini* leaf and seed extract as reducing and stabilizing agent. *B. thuringiensis* subsp. var israelensis known for its crystal toxin being used as commercial larvicide is known to control filarial vector *C. quinquefasciatus*, *C. tritaeniorhynchus*, *C. sitiens*, malarial vector *A. stephensi*, and dengue vector *A. aegypti*. The LC_{50} values for culicines (57.8–300.96 ng/mL), anophelines (740.47–790.61 ng/mL), and *A. aegypti* (514.34–600.03 ng/mL) have been reported (Manonmani and Balaraman 2001).

The maximum efficacy was observed in crude aqueous and synthesized Ag NPs against *C. quinquefasciatus* (LC_{50} 27.49 and 4.56 mg L^{-1}; LC_{90} 70.38 and 13.14 mg L^{-1}) and against *A. subpictus* (LC_{50} 27.85 and 5.14 mg L^{-1}; LC_{90} 71.45 and 25.68 mg L^{-1}), respectively. A biological method has been used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* (Arjunan et al. 2012). The median LC_{50} of silver nanoparticles that killed fourth instars of *A. aegypti*, *C. quinquefasciatus*, and *A. stephensi* were 0.30, 0.41, and 2.12 ppm, respectively. The higher mortality rates at lower doses are comparable with earlier reports of Ag NPs produced by plant *N. nucifera* leaf extracts (LC_{50} = 0.69 ppm, LC_{90} = 2.15 ppm) against *A. subpictus* and *C. quinquefasciatus* (LC_{50} = 1.10 ppm, LC_{90} = 3.59 ppm) (Thirunavukkarasu et al. 2010). The ethyl acetate extract of *E. prostrata* showed an LC_{50} value of 78.28 and LC_{90} value of 360.75 ppm against *A. subpictus* and LC_{50} 119.89 and LC_{90} 564.85 ppm against *C. tritaeniorhynchus*. *Eclipta paniculata* were the most active with a LC_{90} of 17.2 mg L^{-1} and LC_{50} of 3.3 mg L^{-1} against the larvae of *Aedes* fluviatilis (Macedo et al. 1997).

Ag NPs synthesized using *Euphorbia hirta* plant leaf extract against malarial vector *A. stephensi* was determined; the highest larval mortality was found in synthesized Ag NPs against the first to fourth instar larvae and pupae with the following values: LC_{50} (10.14, 16.82, 21.51, and 27.89 ppm, respectively), LC_{90} (31.98, 50.38, 60.09, and 69.94 ppm, respectively), and LC_{50} and LC_{90} of pupae (34.52 and 79.76 ppm, respectively) (Priyadarshini et al. 2012). The mortality effect of silver

nanoparticles on mosquito larvae may be enabled by the small size of the particles, which allows passage through the insect cuticle and into individual cells where they interfere with molting and other physiological processes. The mosquito larvicidal activity of UV irradiationinduced Ag NPs were found to decrease the survival of fourth instar larvae of *A. aegypti* by 88 % after 24 h of exposure at 1-ppm concentration (Sap-Iam et al. 2010). Silver NPs were synthesized using leaf extract of *Acalypha indica*; from the SEM image, the size of the control silver nitrate obtained was greater than 1,000 nm, whereas synthesized silver NPs measured 20–30 nm in size (Krishnaraj et al. 2010). The silver nanoparticles formed were predominantly cubical with uniform shape. It is known that the shape of metal nanoparticles considerably changes their optical and electronic properties (Xu and Käll 2002). The synthesized Ag NPs using *Aloe vera* only after 24 h of reaction and in the presence of ammonia, which in the study mentioned enhances the formation of a soluble silver complex. The sharpening of the peaks clearly indicates that the particles were in the nanoregime (Chandran et al. 2006). The filter paper contact bioassay study showed pronounced pediculicidal activity in the flower bud hexane extract of *Syzygium aromaticum* and the percent mortality were 40, 82, and 100 at 5, 10, and 20 min, and the median lethal time (LT_{50}) value was 5.83 (0.5 mg cm^{-2}); 28, 82, and 100 at 5, 10, and 30 min ($LT_{50} = 6.54$; 0.25 mg cm^{-2}); and 13, 22, 42, 80, and 100 at 5, 10, 20, 40, and 80 min ($LT_{50} = 18.68$; 0.125 mg cm^{-2}), respectively against *P. humanus capitis* (Bagavan et al. 2011). In conclusion, green synthesis shows that the environmentally benign and renewable source of *S. acuta*, *H. Indicum*, *F. elephantum* is used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of silver nanoparticles would be a boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce Ag NPs involving organisms even ranging to higher plants. The formed Ag NPs are highly stable and have significant mosquito larvicidal activity of *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*.

7.5 Conclusion

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friedly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. The screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. The ethno-pharmacological approaches used in the search of new bioactive toxins from plants appear to be predictive compared to the random screening approach. The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and chromatography together with

new pharmacological testing have led to an interest in plants as the source of new larvicidal compounds. Synergestic approaches such as application of mosquito predators with botanical blends and microbial pesticides will provide a better effect in reducing the vector population and the magnitude of epidemiology. Nanotechnology has the potential to revolutionize the existing technologies used in various sectors including agriculture. Nanotechnology may have concrete solutions against many agriculture-related problems like insect pest management using traditional methods, adverse effects of chemical pesticides, development of improved crop varieties, etc. nanomaterials in different forms can be used for efficient management of insect pests and formulations of potential insecticides and pesticides. Nanoparticle-mediated gene transfer would be useful for the development of new insect resistant varieties. Therefore, it can also conclude that nanotechnology can provide green and eco-friendly alternatives for insect pest management without harming the nature.

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Chapter 8
Plant-Synthesized Nanoparticles: An Eco-Friendly Tool Against Mosquito Vectors?

Giovanni Benelli

8.1 Current Control Strategies Against Mosquito Vectors

Arthropods are dangerous vectors of important pathogens and parasites, which may hit as epidemics or pandemics in the increasing world population of humans and animals. Among them, mosquitoes (Diptera: Culicidae) represent a key threat for millions of organisms worldwide, since they act as vectors for important parasites and pathogens, including malaria, dengue and filariasis (Mehlhorn et al. 2012; Benelli 2015a).

Malaria is caused by *Plasmodium* parasites. They are vectored to people and animals through the bites of infected *Anopheles* mosquitoes, which bite mainly between dusk and dawn (Breman 2001; Jensen and Mehlhorn 2009). According to the latest estimates, there were about 198 million cases of malaria in 2013 and an estimated 584,000 deaths. Malaria mortality rates have fallen by 47 % globally since 2000, and by 54 % in the African region. Most deaths occur among children living in Africa, where a child dies every minute from malaria (WHO 2014a).

Dengue is a mosquito-borne viral disease mainly transmitted by *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*. Recently, dengue transmission has strongly increased in urban and semi-urban tropical areas worldwide, becoming a major international public health concern. Over 2.5 billion people are now at risk from dengue. The World Health Organization estimates that there may be 50–100 millions of dengue infections worldwide every year. There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary (WHO 2012). Currently, there is no specific treatment for dengue, even if the development of a vaccine is in progress (Murrell et al. 2011;

G. Benelli
Department of Agriculture, Food and Environment, University of Pisa,
via del Borghetto 80, Pisa 56124, Italy
e-mail: g.benelli@sssup.it; benelli.giovanni@gmail.com

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WHO 2015). Its prevention and control solely depends on effective vector control measures (Suresh et al. 2015; WHO 2015).

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease; more than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million men suffer with genital disease and over 15 million people are afflicted with lymphoedema (WHO 2014b). Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty. Lymphatic filariasis is caused by Filariodidea nematodes, namely *Wuchereria bancrofti*, which is responsible for 90 % of cases, *Brugia malayi*, and *B. timori*. Microfilariae are transmitted to humans by different mosquitoes. *Culex* species, with special reference to *Culex quinquefasciatus*, are the most common vectors across urban and semi-urban areas of Asia (Chadee et al. 2002). Furthermore, mosquitoes also transmit key pathogens and parasites that dogs and horses are very susceptible to, including dog heartworm, West Nile virus and Eastern equine encephalitis (WHO 2012).

In this scenario, mosquito vector control is crucial. Culicidae larvae and pupae are usually targeted using organophosphates, insect growth regulators, and microbial control agents (Benelli 2015a). Indoors residual spraying and insecticide-treated bed nets are also employed to reduce transmission of malaria in tropical countries. However, synthetic chemicals have strong negative effects on human health and the environment, and induce resistance in a number of mosquito species (e.g. Robert and Olson 1989; Wattanachai and Tintanon 1999; Liu et al. 2005).

Eco-friendly tools have been recently implemented to enhance control of mosquitoes. Renewed interest has been devoted to the potential of Sterile Insect Technique (SIT) for suppression of mosquito vectors (Oliva et al. 2014). SIT has been recently combined with auto-dissemination (i.e. adult females contaminated with dissemination stations of juvenile hormone to treat breeding habitats), a technique proved very efficient to control *Aedes* species but that cannot be used at large scales. This has lead to formulate a new control concept, named "boosted SIT" that might enable the area-wide eradication of mosquitoes and other vectors of medical and veterinary importance (Bouyer and Lefrançois 2014). Biological control of mosquito larval populations using aquatic predators, such as insects, copepods and tadpoles also received attention (Bowatte et al. 2013). Furthermore, huge efforts have been carried out to investigate the efficacy of botanical products against mosquito vectors. Many plant extracts, essential oils and pure compounds have been reported as effective against Culicidae, acting as adulticidal, larvicidal, ovicidal, oviposition deterrent, growth and/or reproduction inhibitors and/or adult repellents (e.g. Amer and Mehlhorn 2006a, b; Benelli 2015b; Benelli et al. 2015a, b, c).

8.2 Plant-Mediated Synthesis of Nanoparticles: A Cheap and Single-Step Tool against Mosquitoes?

Nanobiotechnologies have the potential to revolutionize a wide array of applications, including drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, tissue engineering, and pest management (Aurel et al. 2007;

Kim et al. 2007; Rai et al. 2009). The plant-mediated biosynthesis of nanoparticles is advantageous over chemical and physical methods, since it is cheap, single-step, does not require high pressure, energy, temperature, and the use of highly toxic chemicals (Goodsell 2004). A growing number of plant-borne compounds have been proposed for efficient and rapid extracellular synthesis of metal nanoparticles (e.g. Shankar et al. 2004; Song and Kim 2009; Priyadarshini et al. 2012; Ponarulselvam et al. 2012, see Rajan et al. 2015 for a recent review), which showed excellent mosquitocidal properties, also in field conditions (e.g. Santhoshkumar et al. 2011; Marimuthu et al. 2011; Panneerselvam et al. 2012, 2013; Dinesh et al. 2015; Suresh et al. 2015; Murugan et al. 2015a, b; Muthukumaran et al. 2015a, b).

In this review, I focus on the characterization and effectiveness of plant-synthesized nanoparticles against mosquito vectors of medical and veterinary importance, mainly the malaria vector *Anopheles stephensi*, the dengue vector *A. aegypti* and the filariasis vector *C. quinquefasciatus*. In the final section, particular attention is devoted to non-target effects of lethal and sub-lethal doses of plant-synthesized mosquitocidal nanoparticles against aquatic organisms, with special reference to mosquito natural enemies.

8.3 Green Synthesis and Characterization of Mosquitocidal Nanoparticles

In latest years, a growing number of plant part extracts and metabolites have been proposed for the biosynthesis of nanoparticles. The number of publications on the topic is astonishing. A total of 1414 research products were found on SCOPUS database using "plant synthesis nanoparticles" as keywords (May 2015) (Fig. 8.1a). India and China are the most productive countries, with more than 700 and 100 publications, respectively (Fig. 8.1b). Currently, the majority of plant-fabricated metal nanoparticles are silver ones (AgNP) (see Rajan et al. 2015 for a dedicated review).

The biosynthesis of metal nanoparticles for different biological purposes often exploit the reducing and stabilizing potential of plant extracts and metabolites. Two main factors influence the size, shape and stability of nanoparticles, namely the concentration of the plant extract/metabolite and the substrate (metal ions) concentration (Rajan et al. 2015). In the majority of researches, the green synthesis of nanoparticles was confirmed by UV-visualization spectroscopy, followed by scanning electron microscopy (SEM) and/or transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction studies (XRD). Plant-fabricated nanoparticles can be also examined for mean size ranges using nanosizers (Rajan et al. 2015; Murugan et al. 2015b).

For a large number of mosquitocidal nanoparticles synthesized using plant extracts, it has been showed that the color intensity of the plant extract incubated with the aqueous solution of metal ions usually changed from yellowish/pale brown to reddish/dark brown. In the majority of cases, a maximum absorption peak is

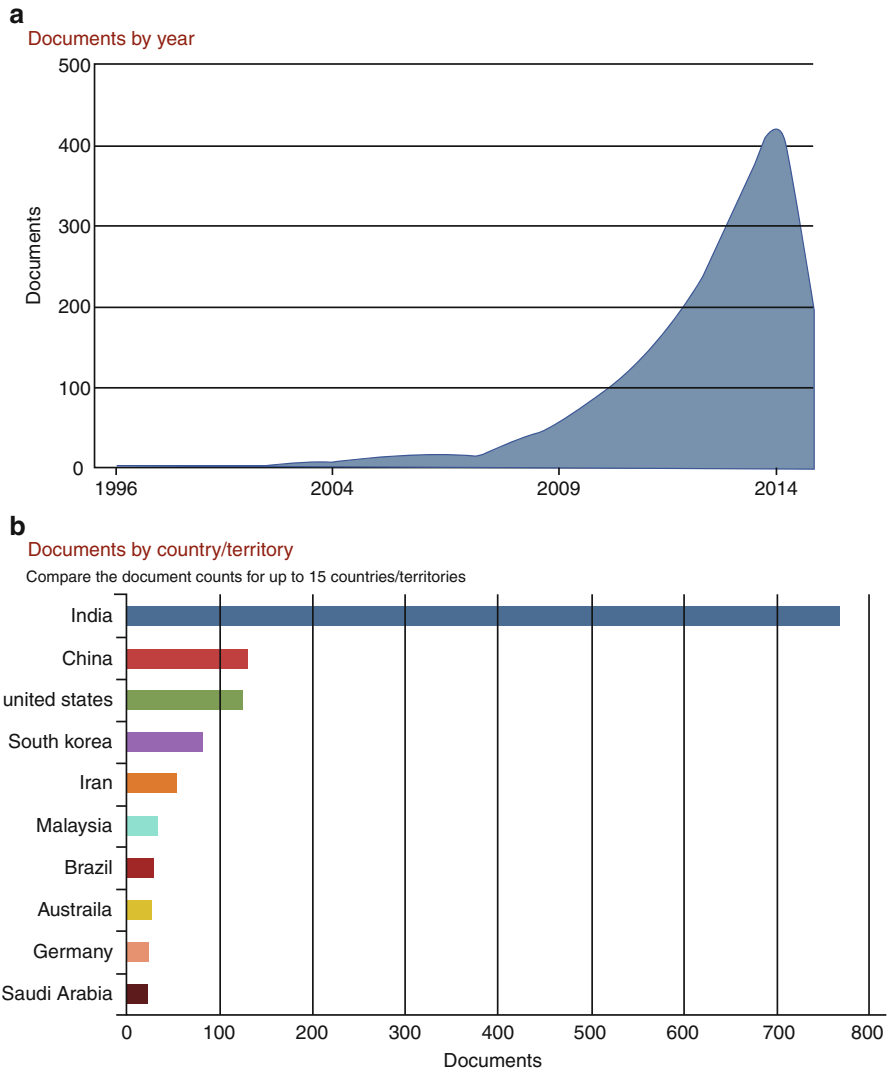

Fig. 8.1 (**a**) Total number and (**b**) country of origin of research products matching the keywords "plant synthesis nanoparticles" on SCOPUS database (May 2015)

observed between 350 and 450 nm, after 60 min or more. The absorption peak varies as the function of reaction time and concentration of metal ions. As the size of ultrafine particles decreases, the energy gap is widened, hence the absorption peaks shifted toward a higher energy (Dinesh et al. 2015; Suresh et al. 2015). The color change may be attributed to the excitation of surface plasmon resonance (SPR) in metal nanoparticles (Natarajan et al. 2010). Bio-synthesized metal nanoparticles have free electrons, which give rise to a SPR absorption band, due to the combined

vibration of electrons of metal nanoparticles in resonance with the light wave (Noginov et al. 2006). Besides UV-vis spectroscopy, the biosynthesis of nanoparticles is confirmed by EDX analysis, which provides information about elemental signals and atomic percent values characterising the reaction mixture (e.g. Murugan et al. 2015a, b). In particular, AgNP display an optical absorption band peaking at 3 keV (due to surface plasmon resonance), which is typical of the absorption of metallic silver nanocrystallites (Shahverdi et al. 2007).

SEM and TEM enable the visualization of the size and shape of plant-fabricated MNP. These two features may vary consistently with the bio-reduction process. As a general trend, the shape of plant-synthesized nanoparticles is spherical, cubic, triangular or rod-like, with the exception of those synthesized using neem leaves, which lead to both flat and spherical nanoparticles (size: 5–35 nm) (Shankar et al. 2004). Recent examples about the morphological features of plant-fabricated mosquitocidal nanoparticles are provided below. *Aloe vera*-synthesized AgNP have spherical and cubic structures with a size range of 35–55 nm. AgNP produced using the leaf extract of *Phyllantus niruri* have a spherical shape, with a mean size of 30–60 nm. *Caulerpa scalpelliformis*-synthesized AgNP are mono-dispersed with spherical and cubic structures, and mean size of 20–35 nm (Murugan et al. 2015a). *Cymbopogon citratus*-synthesized gold nanoparticles showed spherical, triangular, hexagonal, and rod shapes, with size ranging from 20 to 50 nm (Murugan et al. 2015b). Comparable morphological characteristics of metal nanoparticles employed for different purposes have been obtained via plant-mediated synthesis with aqueous extracts from different plant species (Chandran et al. 2006; Rajan et al. 2015)

XRD is carried out to study the crystalline nature of biosynthesized mosquitocidal nanoparticles. Concerning AgNP, a good example is the bio-fabrication of AgNP using the leaf extract of *P. niruri*. The XRD pattern exhibited size-dependent features, with a number of Bragg's reflections corresponding to the (111), (200), (220), (311) and (222) sets of lattice planes. On this basid, it has been pointed out the AgNP formed by the reduction of $AgNO_3$ by *P. niruri* leaf extract were crystalline in nature, and the sharp Bragg's peaks were probably due to the capping agent stabilizing the MNP (Suresh et al. 2015). As regards to gold nanoparticles (AuNP), the XRD pattern of *C. citratus*-synthesized Au nanostructures showed peaks corresponding to (111), (200) and (220) Bragg's reflection based on the face-centered cubic structure of AuNP. Again, XRD highlighted that the nanoparticles formed by the reduction of $HAuCl_4$ with *C. citratus* leaf extract were crystalline in nature (Murugan et al. 2015b).

The bio-reduction of metal ions by plant extracts is a chemically complex phenomenon involving a wide array of plant compounds, such as vitamins, enzymes/proteins, organic acids such as citrates, amino acids, and polysaccharides for the reduction and capping. Notably, recent studies showed that "capped" AgNP are stable in solution for more than 8 weeks (Suganya et al. 2013). The phytochemical screening of secondary metabolites also revealed the presence of terpenoids, flavonoids, phenols, alkaloids, proteins, and carbohydrates in the plant extracts. These plant metabolites had hydroxyl, carbonyl, and amine functional groups (Rajan et al. 2015). Results of FTIR studies showed that the functional groups of the diverse metabolites react with metal ions and reduced their size into nano-range. Moreover, it has been elucidated that the

mentioned functional groups acted as capping agents around the bio-synthesized metal nanoparticles, providing stability as well as biocompatibility (Rajan et al. 2015). For instance, the FTIR spectrum of AgNP fabricated using the *P. niruri* leaf extract showed transmittance peaks at 3327.63, 2125.87, 1637.89, 644.35, 597.41 and 554.63 cm^{-1}, indicating that the carbonyl groups from amino acid residues probably acted as capping agents on nanoparticles, prevent agglomeration, thereby stabilizing the medium (Suresh et al. 2015). Furthermore, the peaks at 1027–1092 cm^{-1} corresponded to the C–N stretching vibration of aliphatic amines or to alcohols/phenols, representing the presence of polyphenols (see also Song et al. 2009). A further example is the synthesis of AuNP using the lemongrass leaf extract; AuNP showed FTIR peaks at 1448.54 cm^{-1} (C=C stretch nitro groups of aromatics), 1643.35 cm^{-1} (C=O stretch amides), 2360.87 cm^{-1} (P-H stretch phosphines), 2856.58 cm^{-1} and 2918.30 cm^{-1} (bending carboxylic acids) (Murugan et al. 2015b).

8.4 Effectiveness of Plant-Synthesized Nanoparticles against Mosquito Vectors

A growing number of plant-synthesized nanoparticles have been reported as effective larvicidals, pupicidals and adulticidals against a number of mosquito species of medical and veterinary importance. A survey conducted on SCOPUS (May 2015) using "nanoparticles mosquito" as keywords, lead to 101 research results (Fig. 8.2a). The most productive countries are India and United States. Of 101 contributions, 36 have been published on *Parasitology Research* (Springer), followed by *Environmental Science and Pollution Research* (Springer), *Acta Tropica* (Elsevier), *Asia-Pacific Journal of Tropical Disease* (Elsevier), and *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (Fig. 8.2b). Restricting the analysis with keywords "plant nanoparticles mosquito" 48 research products have been found. India was confirmed the leading country and *Parasitology Research* the journal at the forefront in this field. Of these studies, 7 have been discarded since they were not focused on plant-mediated-synthesis of mosquitocidal nanoparticles or did not contain proper calculation of LC_{50}. In addition, 5 research articles more have been found searching on Web of Science with the above-mentioned keywords (Raman et al. 2012; Velayutham et al. 2013; Murugan et al. 2015a; Santhosh et al. 2015; Suresh et al. 2015).

8.4.1 Ovicidal, Larvicidal and Pupicidal Toxicity in Laboratory Conditions

To the best of my knowledge, there are no published evidences about the ovicidal toxicity of green-synthesized nanoparticles. Concerning larvicidal and pupicidal nanoparticles, it has been pointed out that few parts per million of different

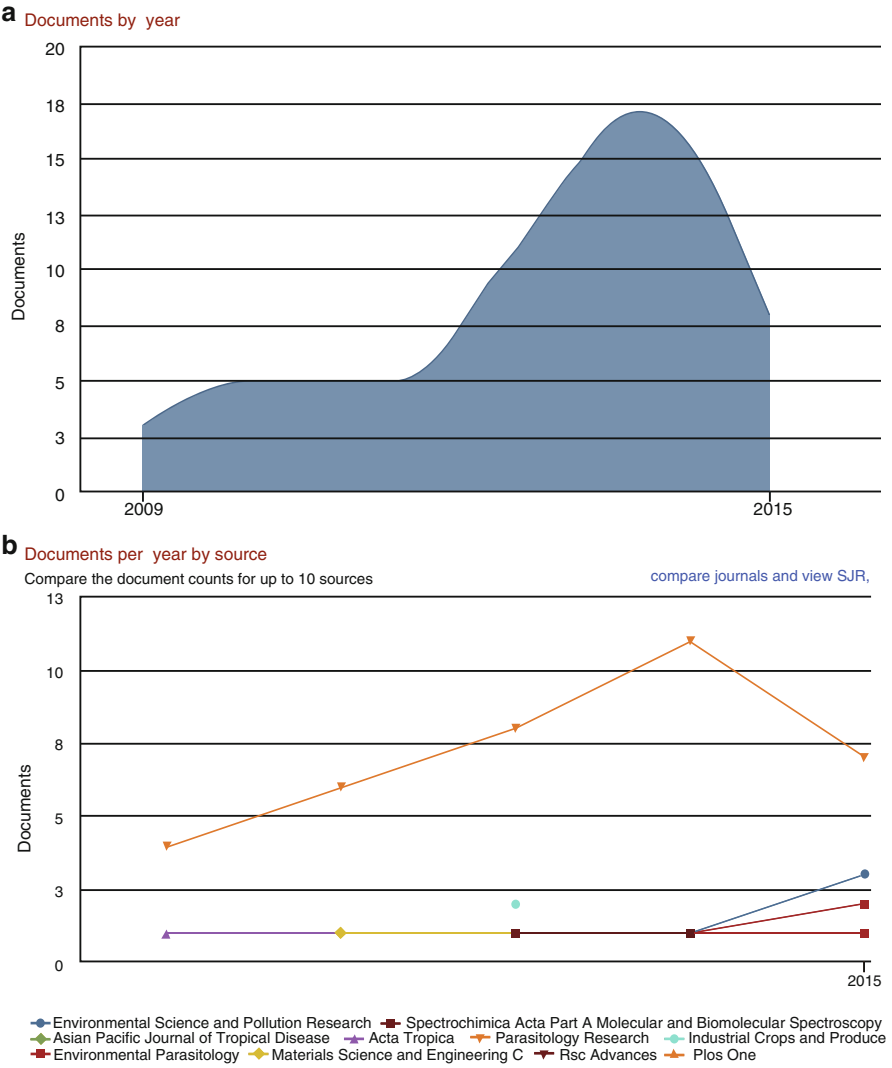

Fig. 8.2 (**a**) Total number and (**b**) publication source of research products matching the keywords "nanoparticles mosquito" on SCOPUS database (May 2015)

plant-synthesized metal nanoparticles possess acute toxicity (i.e. 24 h of exposure, unless specified differently) against different mosquito vectors.

In 2011, five researches investigated the toxicity of plant-synthesized nanoparticles against mosquito larvae. The first article dealing with this issue was from Santhoshkumar et al. (2011), which showed that AgNP synthesized using the aqueous leaf extract of *Nelumbo nucifera* were toxic to IV instar larvae of *Anopheles subpictus* (LC_{50} = 0.69 ppm) and *C. quinquefasciatus* (LC_{50} = 1.10 ppm). Marimuthu

et al. (2011) determined the efficacy of AgNP synthesized with the aqueous leaf extract of *Mimosa pudica* against IV instar larvae of *A. subpictus* and *C. quinquefasciatus,* with LC_{50} of 13.90 and 11.73 mg/L, respectively. Rajakumar and Rahuman (2011) studied the toxicity of AgNP synthesized using the aqueous leaf extract from *Eclipta prostrata* towards IV instar larvae of *C. quinquefasciatus* (LC_{50} 4.56 mg/L) and *Anopheles subpictus* (LC_{50} = 5.14 mg/L). AgNP fabricated with the leaf aqueous extract of *Tinospora cordifolia* are toxic to IV instar larvae of *A. subpictus* and *C. quinquefasciatus*, with LC_{50} of 6.43 and 6.96 mg/L, respectively (Jayaseelan et al. 2011). AgNP synthesized using *Rhizophora mucronata* leaf extract have been tested to IV instar larvae of *A. aegypti* and *C. quinquefasciatus*, with LC_{50} of 0.585 and 0.891 mg/L, respectively (Gnanadesigan et al. 2011).

In 2012, five studies focused on the toxicity of plant-synthesized nanoparticles against mosquito larvae and pupae. *Annona squamosa*-synthesized AgNP were toxic to IV instar larvae of *A. aegypti*, *C. quinquefasciatus* and *A. stephensi*, LC_{50} were 0.30, 0.41, and 2.12 ppm, respectively (Arjunan et al. 2012). AgNP fabricated with the *Euphorbia hirta* leaf extract were toxic to *A. stephensi* larvae and pupae, with LC_{50} values of 10.14 (I), 16.82 (II), 21.51 (III), 27.89 (IV) and 34.52 ppm (pupae) (Priyadarshini et al. 2012). AgNP produced using the *Plumeria rubra* plant latex were toxic to II and IV instar larvae of *A. aegypti* and *A. stephensi*; LC_{50} values were 1.49 (II) and 1.82 ppm (IV) for *A. aegypti* and 1.10 (II) and 1.74 ppm (IV) for *A. stephensi* (Patil et al. 2012a). AgNP synthesized with the *Pergularia daemia* latex were toxic to *A. aegypti* and *A. stephensi* larvae; LC_{50} values were 4.39 (I), 5.12 (II), 5.66 (III) and 6.18 ppm (IV) for *A. aegypti*, and 4.41 (I), 5.35 (II), 5.91 (III) and 6.47 ppm (IV) for *A. stephensi* (Patil et al. 2012b). Lastly, AgNP fabricated using the aqueous leaf extract of *Pithecellobium dulce* showed toxicity against IV instar larvae of *C. quinquefasciatus* (LC_{50} = 21.56 mg/L) (Raman et al. 2012).

In 2013, the productivity in this field duplicated, 11 studies were published. AgNP produced using *Pedilanthus tithymaloides* aqueous leaf extract showed anti-developmental activity and acute toxicity towards *A. aegypti*, with LC_{50} values of 0.029 (I), 0.027 (II), 0.047 (III), 0.086 (IV), and 0.018 % (pupae) (Sundaravadivelan et al. 2013). After 48 h of exposure, AgNP synthesized with the aqueous leaf extract of *Vinca rosea* were toxic to IV instar larvae of *A. stephensi* and *C. quinquefasciatus,* with LC_{50} values of 12.47 and 43.80 mg/mL, respectively (Subarani et al. 2013). AgNP fabricated using *Nerium oleander* leaf extract was toxic to *A. stephensi* larvae and pupae, with LC_{50} values of 20.60 (I), 24.90 (II), 28.22 (III), 33.99 (IV) and 39.55 ppm (pupae) (Roni et al. 2013). The larvicidal activity of *Anthocephalus cadamba-synthesized* AuNP has been ascertained against III instar larvae of *C. quinquefasciatus,* with LC_{50} of 1.08 ppm (Naresh Kumar et al. 2013). AgNP produced with the *Murraya koenigii* leaf extract were toxic to *A. stephensi* and *A. aegypti. A. stephensi* LC_{50} values were 10.82 (I), 14.67 (II), 19.13 (III), 24.35 (IV), and 32.09 ppm (pupae), while *A. aegypti* LC_{50} were 13.34 (I), 17.19 (II), 22.03 (III), 27.57 (IV) and 34.84 ppm (pupae) (Suganya et al. 2013). AgNP synthesized using dried green fruits of *Drypetes roxburghii* have been found toxic against *A. stephensi* and *C. quinquefasciatus*; LC_{50} for II, III and IV larval instars were 0.863, 1.162 and 1.281 ppm against *C. quinquefasciatus* and 0.7329, 0.8397 and 0.9848 ppm against

A. stephensi, respectively (Haldar et al. 2013). AgNP fabricated using the aqueous aerial extract of *Ammannia baccifera* as reducing agent showed toxic effects against IV instar larvae of *A. subpictus* (LC_{50} = 29.54 ppm) and *C. quinquefasciatus* (LC_{50} = 22.32 ppm) (Suman et al. 2013). The mesocarp layer extract of *Cocos nucifera* has been employed to produce AgNP toxic to IV instar larvae of *A. stephensi* and *C. quinquefasciatus;* after 72 h of exposure, LC_{50} was 87.24 mg/L for *A. stephensi* and 84.85 mg/L for *C. quinquefasciatus* (Roopan et al. 2013). The larvicidal activity of AgNP synthesized using the aqueous bark extract of *Ficus racemosa* was successfully tested against IV larvae of the filariasis vector *C. quinquefasciatus* and the Japanese encephalitis vectors *Culex gelidus* (LC_{50} = 12.00 and 11.21 mg/L, respectively) (Velayutham et al. 2013). AgNP fabricated with the *Sida acuta* leaf extract were tested against III instar larvae of *A. stephensi*, *A. aegypti,* and *C. quinquefasciatus*, with LC_{50} values of 21.92, 23.96 and 26.13 µg/mL, respectively (Veerakumar et al. 2013). AgNP were produced using the leaf and berry extracts of *Solanum nigrum* and tested against II and III instar larvae of *A. stephensi* and *C. quinquefasciatus*. Concerning II instar larvae, *A. stephensi* LC_{50} values were 2.12, 2.04 and 1.67 ppm for dry leaves, fresh leaves and berries, respectively. *C. quinquefasciatus* LC_{50} values were 2.62, 2.20 and 2.88 ppm for dry leaves, fresh leaves and berries, respectively. Concerning III instar larvae, *A. stephensi* LC_{50} values were 1.33, 1.59 and 1.54 ppm for dry leaves, fresh leaves and berries, respectively. *C. quinquefasciatus* LC_{50} values were 1.26, 1.33 and 2.44 ppm for dry leaves, fresh leaves and berries, respectively (Rawani et al. 2013).

In 2014, productivity was constant; ten studies have been conducted to evaluate the toxicity of plant-synthesized nanoparticles against mosquito larvae and pupae. Silver nanoparticles fabricated with the aqueous extract of *Citrullus colocynthis* were toxic to III instar larvae of *C. pipiens*, with LD_{50} of 0.5 mg/mL (Shawky et al. 2014). *Feronia elephantum*-synthesized silver nanoparticles are toxic against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*; *A. stephensi* has LC_{50} of 11.56 µg mL^{-1}; *A. aegypti* has LC_{50} of 13.13 µg mL^{-1}; and *C. quinquefasciatus* has LC_{50} of 14.19 µg mL^{-1} (Veerakumar et al. 2014a). *Morinda tinctoria* acetone leaf extract has been used to produce AgNP that achieved an LC_{50} of 1.442 ppm towards III instar larvae of *C. quinqufasciatus* (Kumar et al. 2014). AgNP fabricated with the leaves of *Melia dubia* were toxic to IV instar larvae of *C. quinquefasciatus* (LC_{50} = 11.27 ppm), and it has been showed that the larvicidal effect of these AgNP was probably due to the different phytoconstituents coating AgNP (Karthikeyan et al. 2014). The aqueous leaf extracts of neem has been employed to produce AgNP active as larvicides and pupicides against *A. stephensi* and *C. quinquefasciatus*. After exposure times shorter than 24 h, LC_{50} values against *C. quinquefasciatus* were 6 (II), 10 (III) and 1 ppm (pupae). No values have been calculated for I and IV instar larvae. LC_{50} values against *A. stephensi* were 2 (I), 2 (II), 2 (III) and 1 (IV). No values have been calculated for pupae (Soni and Prakash 2014). AgNP produced using the aqueous leaf extract of Leucas aspera were toxic against IV instar larvae of *A. aegypti*, with LC_{50} of 8.563 mg/L (Suganya et al. 2014). The aqueous leaf extracts of *Aegle marmelos* has been used to synthesize nickel nanoparticles toxic to *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*; LC_{50} were 534.83, 595.23 and 520.83 ppm, respectively

(Angajala et al. 2014). AgNP fabricated with the leaf extract of *Helitropium indicum* have been tested against III instar larvae of *A. stephensi* (LC_{50} = 18.40 µg/mL), *A. aegypti* (LC_{50} = 20.10 µg/mL) and *C. quinquefasciatus* (LC_{50} = 21.84 µg/mL) (Veerakumar et al. 2014b). AgNP produced using the seed extract of *Sterculia foetida* showed mosquitocidal activity against IV instar larvae of *A. aegypti* (LC_{50} = 67.75 mg/mL), *A. stephensi* (LC_{50} = 57.36 mg/mL) and *C. quinquefasciatus* (LC_{50} = 71.54 mg/mL) (Rajasekharreddy and Rani 2015). AgNP synthesized using the aqueous root extract of *Delphinium denudatum* exhibited toxic activity towards II instar larvae of *A. aegypti*, with a LC_{50} value of 9.6 ppm (Suresh et al. 2014).

From January 2015 to May 2015, 13 researches were published. *A. vera-fabricated* AgNP were toxic against *A. stephensi*; LC_{50} vales were 3.825 ppm (I instar lavae), 4.119 ppm (II), 4.982 ppm (III), 5.711 ppm (IV), and 6.113 ppm (pupae) (Dinesh et al. 2015). *P. niruri*-fabricated AgNP have been reported as toxic to larvae and pupae of *A. aegypti*, with LC_{50} of 3.90 ppm (I), 5.01 ppm (II), 6.2 ppm (III), 8.9 ppm (IV) and 13.04 ppm (pupae) (Suresh et al. 2015). *Caulerpa scalpelliformis-synthesized* AgNP were effective against *C. quinquefasciatus* larvae and pupae, with LC_{50} of 3.08 ppm (I instar larvae), 3.49 (II), 4.64 (III), 5.86 ppm (IV) and 7.33 (pupae) (Murugan et al. 2015a). *C. citratus*-produced AuNP were toxic against *A. stephensi* and *A. aegypti*; LC_{50} against *A. stephensi* were 18.80 ppm (I), 21.32 ppm (II), 25.92 ppm (III), 31.46 ppm (IV) and 38.32 ppm (pupae); LC_{50} against *A. aegypti* were 20.27 ppm (I), 23.24 ppm (II), 8.63 ppm (III), 35.09 ppm (IV) and 41.52 ppm (pupae) (Murugan et al. 2015b). AgNP synthesized using *Chomelia asiatica* leaf extract were toxic to *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus,* with LC_{50} and LC_{90} values; *A. stephensi* LC_{50} was 17.95 µg/mL, *A. aegypti* LC_{50} was 19.32, *C. quinquefasciatus* LC_{50} was 20.92 µg/mL (Muthukumaran et al. 2015a). AgNP obtained with the *Gmelina asiatica* leaf extract appeared to be effective against *A. stephensi* (LC_{50} = 22.44 µg/mL), *A. aegypti* (LC_{50} = 25.77 µg/mL) and *C. quinquefasciatus* (LC_{50} = 27.83 µg/mL) (Muthukumaran et al. 2015b). AgNP fabricated using leaf and fruit extracts from *Couroupita guianensis* were toxic to IV instar larvae of *A. aegypti*, with LC_{50} values of 2.1 ppm (leaf extract) and 2.09 ppm (fruit extract) (Vimala et al. 2015). The aqueous leaf extract of neem, *Azadirachta indica*, has been tested against III instar larvae of *A. aegypti* and *C. quinquefasciatus*, LC_{50} values were 0.006 and 0.047 mg/l, respectively (Poopathi et al. 2015). AgNP biosynthesized using 2,7.bis[2-[diethylamino]-ethoxy]fluorence isolate from *Melia azedarach* leaves have been tested against III instar larvae of *A. aegypti* and *C. quinquefasciatus*, with LC_{50} of 4.27 µg/mL and 3.43 µg/mL, respectively (Ramanibai and Velayutham 2015). Extremely stable AgNP have been synthesized using the leaf aqueous extract of *Mukia maderaspatana*; LC_{50} values against *A. aegypti* and *C. quinquefasciatus* IV instar larvae were 0.211 and 0.094 ppm, respectively (Chitra et al. 2015). AgNP synthesized with *Avicennia marina* leaf extract have been tested against I-IV larvae of *A. stephensi* and *A. aegypti*, with LC_{50} values of 4.374 and 7.406 mg/L, respectively (Balakrishnan et al. 2015). Green synthesized AgNP produced using the *Annona muricata* leaf extract were toxic to III instar larvae of *A. aegypti* (LC_{50} = 12.58 µg mL^{-1}), *A. stephensi* (LC_{50} = 15.28 µg mL^{-1}) and *C. quinquefasciatus* (LC_{50} = 18.77 µg mL^{-1}) (Santhosh

et al. 2015). Recently, AgNP synthesized from the seed extract of *Moringa oleifera* have been reported as toxic towards *A. aegypti* young instars, with LC_{50} of 10.24 ppm (I), 11.81 ppm (II), 13.84 ppm (III), 16.73 ppm (IV) and 21.17 ppm (pupae). In addition, these AgNP were able to inhibit the growth of dengue virus, serotype DEN-2 (Sujitha et al. 2015).

Overall, as a general trend, the majority of biosynthesized mosquitocidal nanoparticles have been produced exploiting the reducing potential of terrestrial plant extracts, while algae and seaweeds have been scarcely employed (Murugan et al. 2015a). Most of metal nanoparticles tested against mosquito larval populations were AgNP. *C. quinquefasciatus* larvae and pupae seemed more resistant to the toxic activity of plant-synthesized AgNP.

8.4.2 Larvicidal Toxicity in the Field

Interestingly, field experiments confirmed the effectiveness of plant-synthesized mosquitocidal nanoparticles. Indeed, *A. aegypti* and *A. stephensi* III instar larvae have been recently eliminated from water storage reservoirs 72 h after a single treatment with *P. niruri*- and *A. vera*-synthesized AgNP ($10\times LC_{50}$), respectively (Dinesh et al. 2015; Suresh et al. 2015). It has been hypothesized that the death of mosquito larvae and pupae may be related to the ability of metal nanoparticles to penetrate through the exoskeleton. In the intracellular space, nanoparticles can bind to sulphur from proteins or to phosphorus from DNA, leading to the rapid denaturation of organelles and enzymes. Subsequently, the decrease in membrane permeability and disturbance in proton motive force may cause loss of cellular function and cell death (Rai et al. 2009).

8.4.3 Adulticidal Toxicity and Ovideterrent Properties

Moderate knowledge is available about the adulticidal properties of plant-synthesized metal nanoparticles. In laboratory conditions, AgNP synthesized using *F. elephantum* leaf extract were toxic against adults of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. *A. stephensi* LD_{50} and LD_{90} were 18.041 and 32.575 μg mL^{-1}. *A. aegypti* LD_{50} and LD_{90} were 20.399 and 37.534 μg mL^{-1}. *C. quinquefasciatus* LD_{50} and LD_{90} were 21.798 and 39.596 μg mL^{-1} (Veerakumar and Govindarajan 2014). The adulticidal activity of AgNP synthesized using *H. indicum* leaf extract has been evaluated against adults of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*; the maximum efficacy has been observed against the adults of *A. stephensi* ($LD_{50}=26.712$ μg/mL), followed by *A. aegypti* ($LD_{50}=29.626$ μg/mL) and by *C. quinquefasciatus* ($LD_{50}=32.077$ μg/mL) (Veerakumar et al. 2014c). AgNP prepared using the neem leaf extract were toxic for *C. quinquefasciatus* adults, with LC_{50} of 0.53 ppm calculated after 4 h of exposure (Soni and Prakash 2014). *P. niruri-synthesized* AgNP

tested against *A. aegypti* adults achieved LC_{50} and LC_{90} values of 6.68 ppm and 23.58 ppm, respectively (Suresh et al. 2015).

Little information is available on the impact of metal nanoparticles on oviposition behavior of mosquito vectors. To the best of my knowledge, only Barik et al. (2012) investigated the oviposition behavior of three mosquito species in presence of different types of nanosilica. Complete ovideterrence activity of hydrophobic nanosilica was observed at 112.5 ppm in *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*, while there was no effect of lipophilic nanosilica on oviposition behavior of the three vectors (Barik et al. 2012).

8.5 Non-target Effects against Mosquito Natural Enemies and Other Aquatic Organisms

In latest years, extensive efforts have been done to investigate non-target effects of nanoparticles against aquatic organisms (e.g. Oberdorster et al. 2006; Park et al. 2014; see Baun et al. 2008 and Fabrega et al. 2011 for reviews). However, few attempts have been conducted to shed light on the toxicity of plant-synthesized mosquitocidal nanoparticles against aquatic organisms sharing the same ecological niche of mosquito vectors, with special reference to Culicidae natural enemies (Patil et al. 2012a, b; Haldar et al. 2013; Rawani et al. 2013; Subarani et al. 2013; Murugan et al. 2015a, b).

Little knowledge is available about the acute toxicity towards aquatic non-target species. *P. rubra*- and *P. daemia*-synthesized AgNP did not exhibit any evident toxicity effect against *Poecilia reticulata* fishes, after 48 h of exposure to LC_{50} and LC_{90} values calculated on IV instar larvae of *A. aegypti* and *A. stephensi* (Patil et al. 2012a, b). Subarani et al. (2013) did not reported toxicity effects of *V. rosea-synthesized* AgNP against *P. reticulata*, after 72 h of exposure to dosages toxic against *A. stephensi* and *C. quinquefasciatus*. Similarly, Haldar et al. (2013) did not detected toxicity of AgNP produced using dried green fruits of *D. roxburghii* against *P. reticulata*, after 48 h-exposure to LC_{50} of IV instar larvae of *A. stephensi* and *C. quinquefasciatus*. Rawani et al. (2013) showed that mosquitocidal AgNP synthesized using *Solanum nigrum* berry extracts were not toxic against two mosquito predators, *Toxorhynchites* larvae and *Diplonychus annulatum*, and *Chironomus circumdatus* larvae, exposed to lethal concentrations of dry nanoparticles calculated on *A. stephensi* and *C. quinquefasciatus* larvae. AgNP biosynthesized using the 2,7.bis[2-[diethylamino]-ethoxy] fluorence isolate from the *Melia azedarach* leaves did not show acute toxicity against *Mesocyclops pehpeiensis* copepods (Ramanibai and Velayutham 2015).

Scarce information is available about how low dosages of these mosquitocidals may impact behavioral traits of aquatic organisms sharing the same ecological niche of mosquitoes, such as their predators (Murugan et al. 2015a, b). Notably, these investigations unveiled fascinating scenarios. For instance, Murugan et al. (2015b) showed that very low doses (i.e. 1 ppm) of lemongrass-synthesized AuNP may help to control malaria and dengue vectors boosting early instar mosquito larvae predation by copepods (*Mesocyclops aspericornis*) in an aquatic environment contaminated with ultra-low doses of plant-synthesized AuNP.

8.6 Conclusions

Overall, despite the extensive number of published papers on plant-mediated synthesis of nanoparticles for mosquito control there is a strong gap between theory and practical applications. Much remains to know about this fast-growing research area, with special reference to the following issues: (i) chemical characterization and standardization of plant-borne botanicals used from nano-synthesis (Heng et al. 2013); (ii) the potential of industrial by-products of plant origin for bio-fabrication of nano-mosquitocidals (e.g. neem-cake, see Benelli et al. 2015c for a dedicated review); (iii) field evaluation of larvicidal and pupicidal properties of green nanoparticles against Culicidae (e.g. Dinesh et al. 2015; Suresh et al. 2015); (iv) the potential of plant-synthesized nanoparticles as mosquito ovicidals; (v) the non-target effects and environmental fate of plant-synthesized nanoparticles used against mosquito vectors. This latter point is of peculiar importance. To deal with these key challenges, cooperation among parasitologists, entomologists, behavioral and chemical ecologists is encouraged.

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Chapter 9
Do Nanomosquitocides Impact Predation of *Mesocyclops edax* Copepods Against *Anopheles stephensi* Larvae?

Balamurugan Chandramohan, Kadarkarai Murugan, Kalimuthu Kovendan, Chellasamy Panneerselvam, Palanisamy Mahesh Kumar, Pari Madhiyazhagan, Devakumar Dinesh, Udaiyan Suresh, Jayapal Subramaniam, Duraisamy Amaresan, Thiyagarajan Nataraj, Devaraj Nataraj, Jiang-Shiou Hwang, Abdullah A. Alarfaj, Marcello Nicoletti, Angelo Canale, Heinz Mehlhorn, and Giovanni Benelli

B. Chandramohan • K. Murugan • K. Kovendan • C. Panneerselvam • P.M. Kumar
P. Madhiyazhagan • D. Dinesh • U. Suresh • J. Subramaniam • D. Amaresan • T. Nataraj
Division of Entomology, Department of Zoology, School of Life sciences, Bharathiar University, Coimbatore, Tamil Nadu 641 046, India

D. Nataraj
Department of Physics, Bharathiar University, Coimbatore, Tamil Nadu 641 046, India

J.-S. Hwang
Institute of Marine Biology, National Taiwan Ocean University, Keelung 202-24, Taiwan

A.A. Alarfaj
Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

M. Nicoletti
Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, Rome 00185, Italy

A. Canale • G. Benelli (✉)
Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, Pisa 56124, Italy
e-mail: g.benelli@sssup.it; benelli.giovanni@gmail.com

H. Mehlhorn
Department of Parasitology, Heinrich Heine University, Building 26.13.01 Room 42, Universitätsstr. 1, Düsseldorf D-40225, Germany

H. Mehlhorn (ed.), *Nanoparticles in the Fight Against Parasites*,
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9.1 Introduction

Mosquitoes (Diptera: Culicidae) represent a huge threat for millions of humans and animals worldwide, since they act as vectors for malaria, dengue, yellow fever, West Nile virus, Japanese encephalitis, and filariasis (Mehlhorn et al. 2012; Benelli 2015). According to the latest estimates, there were about 198 million cases of malaria in 2013 and an estimated 584,000 deaths. Malaria mortality rates have fallen by 47 % globally since 2000, and by 54 % in the African region. Most deaths occur among children living in Africa, where a child dies every minute from malaria. Malaria is caused by *Plasmodium* parasites; they are vectored to people and animals through the bites of infected *Anopheles* mosquitoes, which bite mainly between dusk and dawn (WHO 2014).

People entering into regions where malaria, dengue or yellow fever risks exist may protect themselves by use of chemical or plant derived repellents (Mehlhorn et al. 2005, 2012; Mehlhorn 2011; Amer and Mehlhorn 2006b). However, people living in endemic regions have to protect themselves by several strategies at the same time, since infection rates of mosquitoes may be extremely high (Amer and Mehlhorn 2006a, c; Rahuman 2011). *Anopheles* populations are usually targeted using synthetic insecticides. However, these chemicals have important negative effects on human health and the environment, and induce resistance in a number of targeted species (Benelli 2015). In this scenario, eco-friendly tools have been recently implemented to enhance control of mosquito vectors, with special reference of botanical mosquitocidals (Azizullah et al. 2014). Recently, a growing number of plant-borne compounds have been reported as excellent toxics against mosquitoes, acting as adulticidal, larvicidal, ovicidal, oviposition deterrent, growth and/or reproduction inhibitors and/or adult repellents (e.g. Amer and Mehlhorn 2006a, b, c, d; Semmler et al. 2009; Rahuman 2011; Benelli et al. 2015a, b).

Nanobiotechnologies have the potential to revolutionize a wide array of applications, including drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, tissue engineering, and pest management (Elechiguerra et al. 2005). The plant-mediated biosynthesis (i.e. "green synthesis") of nanoparticles is advantageous over chemical and physical methods, since it is cheap, single-step, does not require high pressure, energy, temperature, and the use of highly toxic chemicals (Song and Kim 2009). In particular, a growing number of plants and fungi have proposed for efficient and rapid extracellular synthesis of silver and gold nanoparticles, which showed excellent mosquitocidal properties, also in field conditions (e.g. Amerasan et al. 2015; Dinesh et al. 2015; Suresh et al. 2015).

However, while extensive efforts have been conducted to investigate non-target effects of nanoparticles against aquatic organisms, little has been done to shed light about the toxicity of green-synthesized mosquitocidal nanoparticles against predators of mosquito larval and pupal populations (see Benelli 2016 for a recent review). Most importantly, scarce information is available about the impact of mosquitocidal nanostructures on al traits of mosquito natural enemies, such as copepods (Murugan et al. 2015a, b, c).

Acorus calamus (Acorales: Acoraceae) has been used in the Indian and Chinese system of medicine for hundreds of years to cure a wide array of diseases (Mukherjee and Kumar 2007). *A. calamus* rhizome is the source of an essential oil, which is a unique source of oxygenated sesquiterpenes of great structural variety, responsible for antibacterial, antifungal, and insecticidal properties (Meena et al. 2010; Dahiya and Purkayastha 2011). The alcoholic extract of *A. calamus* rhizome exhibited antiviral activity against herpes viruses that is HSV-1 and HSV-2 (Mamgain and Signh 1994). The ethanolic extract of the rhizome is also used as antiulcer agent that inhibits gastric secretion and protects gastroduodenal mucosa against the injuries caused by pyloric ligation (Keller et al. 1985). Recently, Nakkala et al. (2014) reported that silver nanoparticles (AgNP) synthesized using the aqueous extract of *A. calamus* exhibited free radical quenching ability in antioxidant assays and antibacterial activity against different pathogenic bacteria. Furthermore AgNP showed anticancer effects in HeLa cells and in A549 cells.

In this study, we reported a cheap method to synthesize silver nanoparticles (Ag NP) using the rhyzome extract of *Acorus calamus*, a nontoxic and eco-friendly material, that worked as reducing and stabilizing agent during the biosynthesis. AgNP were characterized by UV–vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). We investigated the ovicidal, larvicidal, pupicidal and adulticidal properties of *A. calamus* aqueous rhyzome extract and green-synthesized AgNP against the malaria vector *Anopheles stephensi*. Furthermore, we evaluated the predation efficiency of the cyclopoid copepod *Mesocyclops edax* against larvae of *A. stephensi*, both in standard laboratory conditions and in an aquatic environment treated with AgNP.

9.2 Materials and Methods

9.2.1 Plant Materials

Fresh rhizomes of *A. calamus* were collected from campus of Bharathiar University (Coimbatore, India). Plants were identified by an expert taxonomist at the Department of Botany (Bharathiar University, Coimbatore). Voucher specimens (ID: ACO1-3) were stored in our laboratories and are available under request.

9.2.2 Mosquito Rearing

Following the method by Dinesh et al. (2015), the eggs of *A. stephensi* were collected from National Centre for Disease Control (NCDC) field station of Mettupalayam, Tamil Nadu, India. Eggs were transferred to laboratory conditions [27 ± 2 °C, 75–85 % R.H., 14:10 (L:D) photoperiod] and placed in 18 × 13 × 4 cm

plastic containers containing 500 mL of tap water, waiting for hatching. Larvae were fed daily with a mixture of dog biscuits (Pedigree, USA) and hydrolyzed yeast (Sigma Aldrich, Germany) (3:1, *w*:*w* ratio). Pupae were collected and transferred to plastic containers with 500 mL of water. Each container was placed inside a cubic chiffon cage (90×90×90 cm) to wait for adult emergence. Adults were fed *ad libitum* on 10 % (v:v) sucrose solution. Five days after emergence, the adults were deprived of sugar feeding for 12 h and then supplied with artificial blood feeding. The blood meal was furnished, by means of a professional heating blood (lamb blood), at fixed temperature of 38 °C and provided with a membrane of cow gut. After 30 min, the blood meal was removed, due to blood drying phenomena, and gut membrane was substituted with a new fresh one for the following utilization (Nicoletti et al. 2012). Petri dishes (diameter 60 mm) lined with filter paper and containing 50 mL of water were placed inside each cage, allowing oviposition by females.

9.2.3 Acorus calamus-*Mediated Synthesis of Silver Nanoparticles*

The *A. calamus* aqueous rhizome extract was prepared adding 10 g of washed and finely cut rhizomes in a 300-mL Erlenmeyer flask filled with 100 mL of sterilized double distilled water and then boiling the mixture for 5 min, before finally decanting it. The extract was filtered using Whatman filter paper n. 1, stored at −4 °C and tested within 5 days. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature. A brown-yellowish solution indicated the formation of AgNP, since aqueous silver ions were reduced by the *A. calamus* extract generating stable AgNP in water. Silver nitrate was purchased from the Precision Scientific Co. (Coimbatore, India).

9.2.4 Characterization of Green-Synthesized Silver Nanoparticles

Synthesis of AgNP was confirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV–vis, at the wavelength of 200–600 nm in UV-3600 Shimadzu spectrophotometer at 1 nm resolution. Then, the reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min, resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 μm). An aliquot (2 mL) of this filtrate containing silver nanoparticles was used for SEM, EDX, FTIR and XRD analyses. The structure

and composition of freeze-dried purified AgNP was analyzed using a 10 kV ultra high-resolution scanning electron microscope. 25 μL of sample were sputter coated on copper stub and the images of nanoparticles were studied using FEI QUANTA-200 SEM. The surface groups of the AgNP were qualitatively confirmed by FTIR spectroscopy, using a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. EDX assays confirmed the presence of metals in the analyzed AgNP samples.

9.2.5 Ovicidal Activity

Following Su and Mulla (1998), mosquito eggs were collected placing ovitraps (i.e., Petri dishes, diameter 60 mm, lined with filter paper and containing 50 ml of water) inside each cage. All ovitraps were stored in the cages for 2 days from the blood meal of mosquito females. The eggs laid on filter paper lining were examined using a photomicroscope (Leica ES2, Germany). For each mosquito species, the eggs were placed in a cage with six glass cups (diameter: 6 cm). Five of them were filled with water plus AgNP treatments as follows: 10, 20, 30, 40 and 50 ppm. The control cup was filled with distilled water. 100 eggs were placed in each cup. Five replicates were done for each dosage. After treatment, the eggs from each concentration were transferred to distilled water cups for hatching assessment after counting the eggs under microscope. The percent egg mortality was calculated on the basis of non-hatchability of eggs with unopened opercula (Chenniappan and Kadarkarai 2008). The hatch rates were assessed 48 h post-treatment using the following formula (Govindarajan et al. 2011):

$$\%\,\text{of egg mortality} = \left(\text{number of hatched larvae / total number of eggs}\right) \times 100$$

9.2.6 Larvicidal and Pupicidal Activity

Following the method reported by Suresh et al. (2015), 25 *A. stephensi* larvae (I, II, III or IV instar) or pupae were placed in a glass beaker filled with 250 mL of dechlorinated water plus the desired concentration of *A. calamus* extract or green-synthesized AgNP. Larval food (0.5 mg) was provided for each tested concentration. Each concentration was replicated five times against all instars. In control treatments, 25 larvae or pupae were transferred in 250 mL of dechlorinated water. Percentage mortality was calculated as follows:

$$\%\,\text{mortality} = \left(\text{number of dead individuals / number of treated individuals}\right) \times 100$$

9.2.7 *Adulticidal Toxicity*

Adulticidal bioassay was performed following the method by Suresh et al. (2015). The *A. calamus* aqueous crude extract was tested at 100, 200, 300, 400 and 500 ppm, and AgNP nanoparticles were tested at 3.125, 6.25, 12.5, 25 and 50 ppm. *A. calamus* aqueous crude extract or AgNP were applied on Whatman n. 1 filter paper (size 12×15 cm) lining a glass holding tube (diameter 30 mm; length 60 mm). Control filter paper was treated with distilled water and silver nitrate (1 mM), respectively. In each test, 20 *A. stephensi* females were gently transferred into another glass holding tube. The mosquitoes were allowed to acclimatize in the tube for 1 h and then exposed to test tube lined with treated or control paper for 1 h. At the end of exposure period, the mosquitoes were transferred back to the original holding tube and kept for a 24 h recovery period. A pad of cotton soaked with 10 % (w:w) glucose solution was placed on the mesh screen at the top of the holding tube (Suresh et al. 2015).

9.2.8 *Predation of* Mesocyclops edax *against* Anopheles stephensi *Larvae*

The predation efficiency of *M. edax* was assessed against *A. stephensi* larvae. For each instar, 100 mosquitoes were introduced, with 10 copepods, in a glass beaker containing 250 mL of dechlorinated water. Mosquito larvae were replaced daily with new ones. For each mosquito instar, four replicates were conducted. Control was 250 mL of dechlorinated water without copepods. All beakers were checked after 1, 2, 3, 4, and 5 days and the number of prey consumed by copepods was recorded. Predatory efficiency was calculated using the following formula:

$$\text{Predatory efficiency} = \left(\text{number of consumed mosquitoes / number of predators}\right) \\ \text{total number of mosquitoes} \times 100$$

9.2.9 *Predation of* Mesocyclops edax *against* Anopheles stephensi *Larvae Post-treatment with Silver Nanoparticles*

Here the predation efficiency of *M. edax* adults was assessed against *A. stephensi* larvae, after a mosquitocidal treatment with AgNP. For each instar, 100 mosquitoes were introduced with 10 copepods in a glass beaker filled with 250 mL of dechlorinated water plus the desired concentration of *A. calamus*-synthesized AgNP (i.e., 1/3 of the LC_{50} calculated against first instar larvae of *A. stephensi*). Mosquito larvae were replaced daily with new ones. For each mosquito instar, four replicates were

conducted. Control was dechlorinated water plus AgNP, without copepods. All beakers were checked after 1, 2, 3, 4, and 5 days and the number of prey consumed by copepods was recorded. Predatory efficiency was calculated using the above-mentioned formula.

9.2.10 Data Analysis

Ovicidal, larvicidal, pupicidal and adulticidal percentage data were transformed into arcsine $\sqrt{}$ proportion values and analyzed using ANOVA. Means were separated using Tukey's HSD ($P<0.05$). The average mosquito mortality data were subjected to probit analysis. In larvicidal, pupicidal and adulticidal experiments, LC_{50} and LC_{90} were calculated using the method by Finney (1971).

Copepod predation data were analyzed by JMP 7 using a weighted generalized linear model with one fixed factor: $y = X\beta + \varepsilon$ where y is the vector of the observations (i.e. the number of consumed preys), X is the incidence matrix, ß is the vector of fixed effects (i.e. the targeted instar), and ε is the vector of the random residual effect. A probability level of $P<0.05$ was used for the significance of differences between values.

9.3 Results and Discussion

9.3.1 Characterization of Green-Synthesized Silver Nanoparticles

When the $AgNO_3$ aqueous solution was added to the *A. calamus* rhizome extract, the color changed from yellowish to brownish, indicating the reduction from Ag^+ to Ag^0, and the formation of AgNP (Fig. 9.1a, b). After 240 min, a maximum absorption peak was observed at 430 nm (Fig. 9.1c), which is characteristic of silver nanomaterials, and probably arises from the excitation of longitudinal plasmon vibrations of AgNP in the solution (Dhas et al. 2014). XRD patterns showed intense peaks corresponding to (111), (200) and (220) Bragg reflection based on the face-centered cubic structure of AgNP. XRD highlighted that AgNP formed by the reduction of $AgNO_3$ with *A. calamus* rhizome extract were crystalline in nature (Fig. 9.2). SEM analysis (Fig. 9.3) highlighted that AgNP synthesized using the rhizome of *A. calamus* were spherical in shape, with a mean size ranging from 30 to 80 nm. In addition, the SEM analysis predicted the distribution of size of AgNP from 50 to 80 nm, which is in good agreement with the absorbance observed in UV–vis spectrophotometer (Zahir and Rahuman 2012).

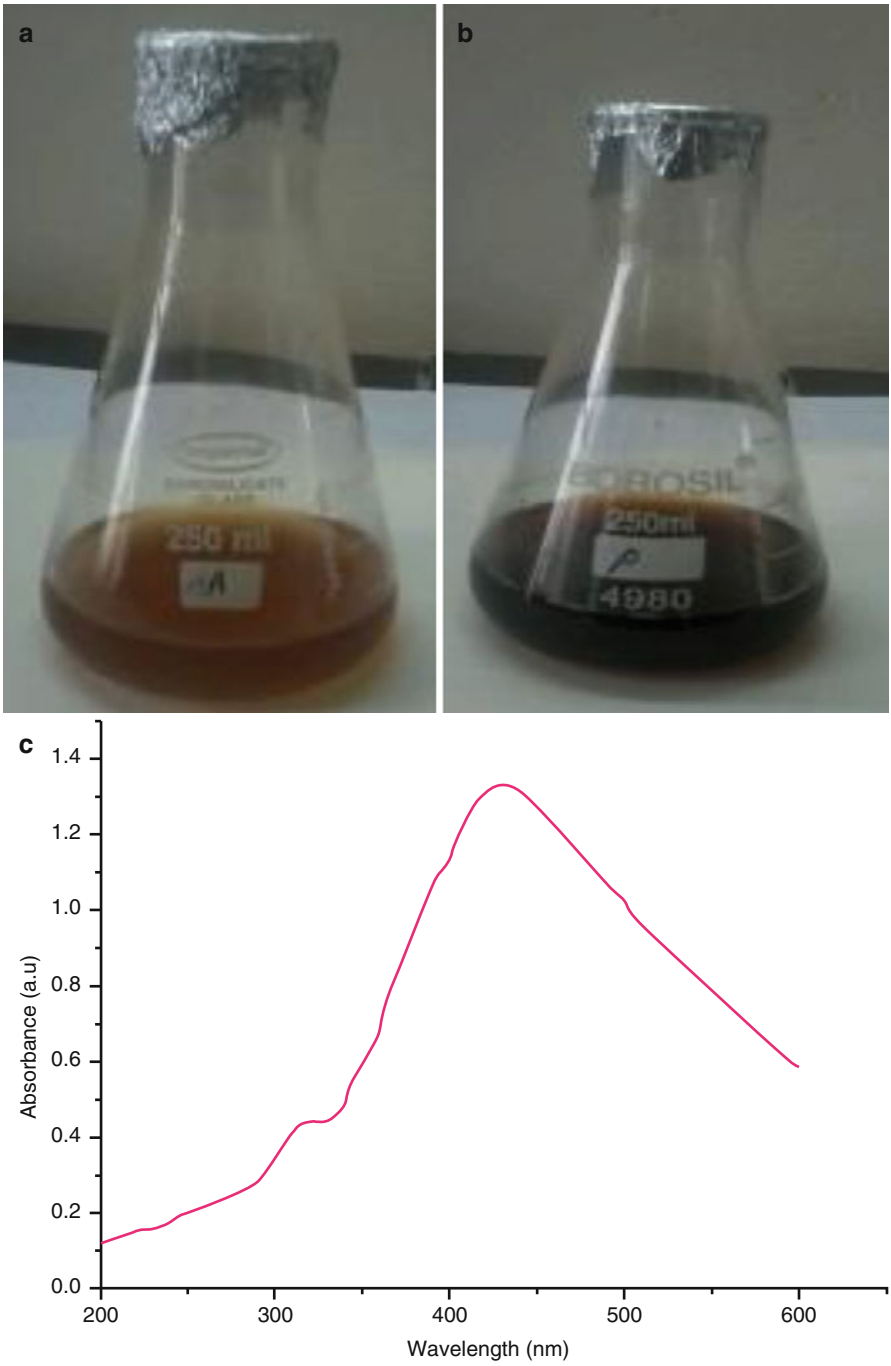

Fig. 9.1 Chromatic variations of the rhizome extract of *Acorus calamus* before (**a**) and after (**b**) the process of reduction of Ag^{+} to Ag nanoparticles. (**c**) UV-visualization of the absorption spectrum of silver nanoparticles synthesized using different dosages of *A. calamus* rhizome extract plus an aqueous solution $AgNO_3$ (1 mM) after 240 min

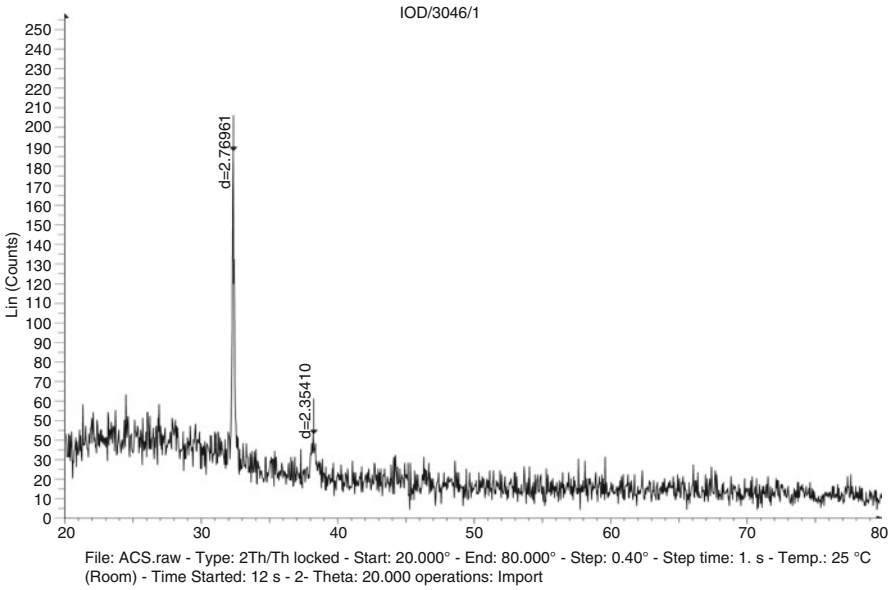

Fig. 9.2 X-ray diffraction pattern of silver nanoparticles green-synthesized using the aqueous rhizome extract of *Acorus calamus*

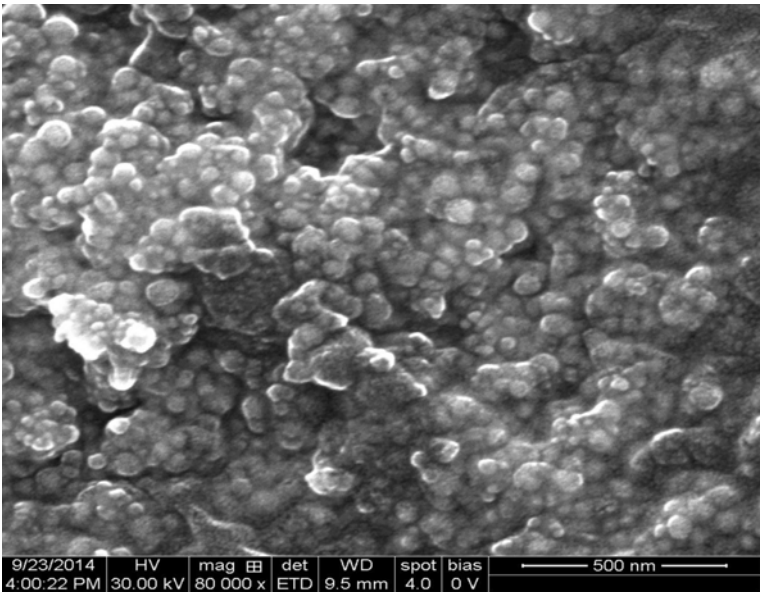

Fig. 9.3 Scanning electron micrograph of *Acorus calamus*-synthesized silver nanoparticles

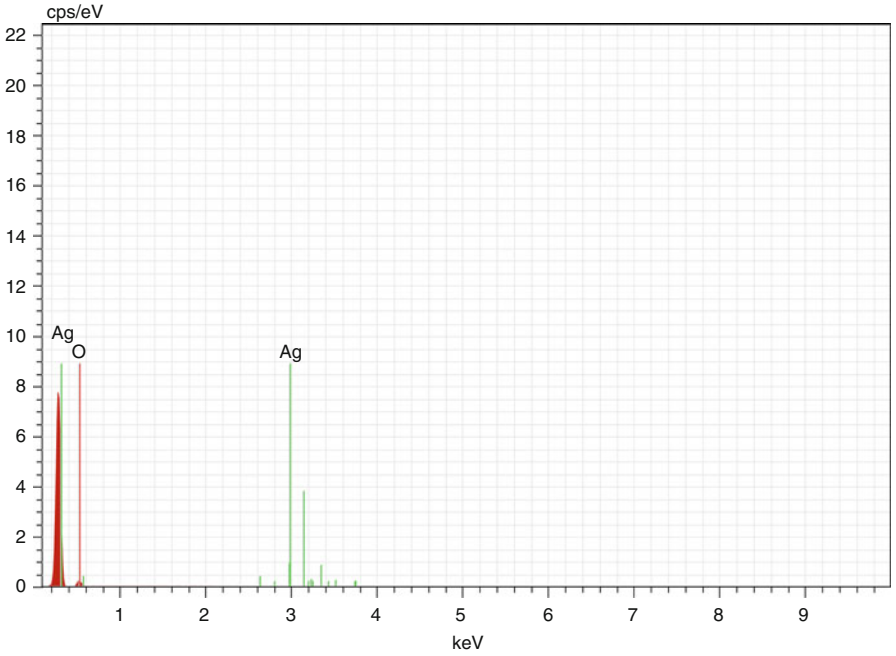

Fig. 9.4 Energy dispersive X-ray spectrum of *Acorus calamus*-synthesized silver nanoparticles

The EDX spectrum recorded from *A. calamus*-synthesized AgNP was reported in Fig. 9.4. An absorption peak approximately at 3 KeV was detected, which is typically originated by surface plasmon resonance of silver nanocrystallites (Fayaz et al. 2010). FTIR spectroscopy analysis was carried out to identify the biomolecules capping the AgNP. FTIR spectrum showed main peaks at 666.36, 1217.11, 1636.87, 2129.78 and 3329.97 cm^{-1} (Fig. 9.5). The absorption peak close to 1640 cm^{-1} may be due to the amide bond from the carbonyl group of proteins (Macdonald and Smith 1996), while peak at 3359 cm^{-1} may be due to OH groups from alcohols and phenols (Theivasanthi and Alagar 2012). The band at 1053 cm^{-1} corresponds to C–N stretching vibrations of aliphatic amines that are commonly found in proteins, indicating the presence of proteins as ligands for AgNP, which increase the stability of biosynthesized nanoparticles (Sanghi and Verma 2009).

9.3.2 Mosquitocidal Activity

Egg hatchability of *A. stephensi* was reduced by 100 % after treatment with 25 and 30 ppm of AgNP; the *A. calamus* extract exerted 100 % mortality post-treatment with 500 ppm, while control eggs showed the 100 % hatchability (Table 9.1). To the best of our knowledge, no efforts have been carried out to shed light on ovicidal

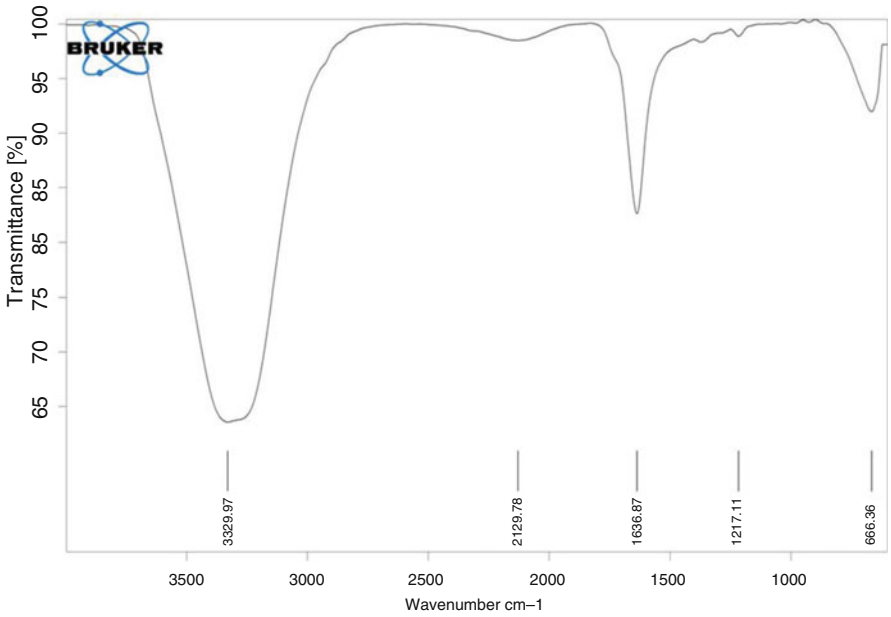

Fig. 9.5 Fourier transform infrared spectrum of vacuum-dried powder of silver nanoparticles fabricated using the rhizome extract of *Acorus calamus*

Table 9.1 Ovicidal activity of *Acorus calamus* rhizome extract and green synthesized silver nanoparticles against the malaria vector *Anopheles stephensi*

Treatment	Egg hatchability (%)					
Acorus calamus extract	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
	84.6 ± 1.1^{a}	61.8 ± 1.3^{b}	57.2 ± 1.9^{b}	45.4 ± 0.89^{c}	32.4 ± 2.0^{d}	NH
Silver nanoparticles	Control	3 ppm	6 ppm	12 ppm	25 ppm	50 ppm
	91.0 ± 2.1^{a}	56.4 ± 1.1^{b}	43.2 ± 2.3^{b}	20.0 ± 2.2^{c}	NH	NH

Values are means ± SE from five replicates
Within each row, different letters indicate significant differences ($P < 0.05$)
NH no egg hatchability (100 % mortality)

properties of plant-synthesized AgNP, and limited information is available about the impact of nanoparticles on oviposition behavior of mosquito vectors. Only Barik et al. (2012) investigated oviposition behavior of three mosquito species in presence of different types of nanosilica. Complete ovideterrence activity of hydrophobic nanosilica was observed at 112.5 ppm in *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*, while there was no effect of lipophilic nanosilica on oviposition behavior of the three vectors. The ovicidal efficiency of the tested compounds seems to be linked to their ability to penetrate inside the eggshell, and this could be influenced by the exposure period (Grosscurt 1977).

In larvicidal and pupicidal assays, the *A. calamus* rhyzome extract was toxic against all larval instars (I-IV) and pupae of *A. stephensi*. LC_{50} values were 219.06 ppm (I instar), 246.01 ppm (II), 285.79 ppm (III), 345.19 ppm (IV) and 470.93 ppm (pupa) (Table 9.2). Comparable mortality rates have been recently reported testing a wide array of botanical extracts and plant-borne compounds (Amer and Mehlhorn 2006a, b; see Azizullah et al. 2014 and Benelli et al. 2015b for recent reviews). The larvicidal and pupicidal effect is probably due to the impact of secondary metabolites present in *A. calamus*, on membrane integrity, damage in the lipid components of the gill membrane (Nivsarkar et al. 2001).

A. calamus-synthesized nanoparticles were highly effective against *A. stephensi* larvae and pupae, with LC_{50} values of 8.94 ppm (I), 11.64 ppm (II), 14.94 ppm (III), 19.15 ppm (IV) and 28.66 ppm (pupa) (Table 9.3). In latest years, a growing number of green-synthesized AgNP showed comparable larvicidal and pupicidal toxicity against a number of mosquito vectors. *Aloe vera*-synthesized AgNP were toxic

Table 9.2 Larvicidal and pupicidal toxicity of *Acorus calamus* rhizome extract against the malaria vector *Anopheles stephensi*

Target	LC_{50} (LC_{90})	Regression equation	95 % Confidence Limit		χ^2 (*d.f.* = 4)
			Lower LC_{50} (LC_{90})	Upper LC_{50} (LC_{90})	
Larva I	219.06 (463.23)	$y = 1.150 + 0.005x$	191.513 (426.168)	243.099 (513.724)	2.495 *n.s.*
Larva II	246.01 (512.31)	$y = 1.184 + 0.005x$	218.234 (469.106)	271.083 (572.580)	1.616 *n.s.*
Larva III	285.79 (590.52)	$y = 1.202 + 0.004x$	256.795 (534.204)	313.838 (672.919)	0.117 *n.s.*
Larva IV	345.19 (714.60)	$y = 1.198 + 0.003x$	311.998 (629.879)	383.007 (850.038)	0.791 *n.s.*
Pupa	470.93 (917.94)	$y = 1.350 + 0.003x$	420.190 (775.479)	551.387 (1178.556)	1.957 *n.s.*

No mortality was detected in the control
χ^2 chi-square, *d.f.* degrees of freedom, *n.s.* not significant ($\alpha = 0.05$)

Table 9.3 Larvicidal and pupicidal toxicity of *Acorus calamus*-synthesized silver nanoparticles against the malaria vector *Anopheles stephensi*

Target	LC_{50} (LC_{90})	Regression equation	95 % confidence limit		χ^2 (*d.f.* = 4)
			Lower LC_{50} (LC_{90})	Upper LC_{50} (LC_{90})	
Larva I	8.94 (30.11)	$y = 0.542 + 0.061x$	6.561 (26.26)	11.056 (35.885)	5.170 *n.s.*
Larva II	11.64 (39.47)	$y = 0.537 + 0.046\ x$	8.768 (14.29)	34.60 (46.49)	4.808 *n.s.*
Larva III	14.94 (46.84)	$y = 0.600 + 0.040\ x$	11.86 (17.92)	40.98 (55.37)	4.940 *n.s.*
Larva IV	19.15 (59.76)	$y = 0.605 + 0.032\ x$	15.44 (22.98)	51.29 (72.89)	5.182 *n.s.*
Pupa	28.66 (75.84)	$y = 0.779 + 0.027\ x$	18.37 (49.32)	53.13 (164.48)	6.611 *n.s.*

No mortality was detected in the control
χ^2 chi-square, *d.f.* degrees of freedom, *n.s.* not significant ($\alpha = 0.05$)

against *A. stephensi*, with LC_{50} ranging from 3.825 ppm (larva I) to 6.113 ppm (pupae) (Dinesh et al. 2015). Low doses of AgNP synthesized using the *Euphorbia hirta* leaf extract have been reported as toxic to *A. stephensi*, with LC_{50} ranging from 10.14 (I) to 34.52 ppm (pupae) (Priyadarshini et al. 2012). Suresh et al. (2015) highlighted that AgNP synthesized using the aqueous extract of *Phyllanthus niruri* leaves were effective against larvae and pupae of *Aedes aegypti*, with LC_{50} ranging from 3.90 ppm (I) to 13.04 ppm (pupae). Low doses of *Caulerpa scalpelliformis*-synthesized AgNP were toxic also to the filariasis vector *Culex quinquefasciatus*, with LC_{50} ranging from 3.08 ppm (I) to 7.33 ppm (pupae) (Murugan et al. 2015a). We hypothesize that the toxicity of AgNP against dengue vectors may be enabled by the small size of nanoparticles, which allows passage through the insect cuticle and into individual cells where they interfere with molting and other physiological processes (Murugan et al. 2015b).

Lastly, in adulticidal experiments, the *A. calamus* rhizome extract and AgNP showed LC_{50} of 251.71 ppm and 12.74 ppm, respectively (Table 9.4). In both cases, at the highest concentrations tested, the adults showed restless movements for some times with abnormal wagging, and then died. While AgNP have been extensively studied as larvicides and pupicides, moderate efforts have been carried out to shed light on adulticidal properties. In laboratory conditions, AgNP synthesized using *Feronia elephantum* leaf extract were toxic against adults of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus. A. stephensi* LD_{50} and LD_{90} were 18.041 and 32.575 μg mL^{-1}. *A. aegypti* LD_{50} and LD_{90} were 20.399 and 37.534 μg mL^{-1}. *C. quinquefasciatus* LD_{50} and LD_{90} were 21.798 and 39.596 μg mL^{-1} (Veerakumar and Govindarajan 2014). The adulticidal activity of AgNP synthesized using *Heliotropium indicum* leaf extract has been evaluated against adults of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*; the maximum efficacy has been observed against the adults of *A. stephensi* (LD_{50} = 26.712 μg/mL), followed by *A. aegypti* (LD_{50} = 29.626 μg/mL) and by *C. quinquefasciatus* (LD_{50} = 32.077 μg/mL) (Veerakumar et al. 2014). AgNP prepared using the neem leaf extract were toxic for *C. quinquefasciatus* adults, with LC_{50} of 0.53 ppm calculated after 4 h of exposure (Soni and Prakash 2014). *Phyllanthus niruri*-synthesized AgNP tested against *A. aegypti* adults achieved LC_{50} and LC_{90} values of 6.68 ppm and 23.58 ppm, respectively (Suresh et al. 2015).

Table 9.4 Adulticidal toxicity of *Acorus calamus* rhizome extract and green synthesized silver nanoparticles against the malaria vector *Anopheles stephensi*

Treatment	LC_{50} (LC_{90}) ppm	Regression equation	95 % confidence limit		χ^2 (*d.f.* = *4*)
			Lower LC_{50} (LC_{90})	Upper LC_{50} (LC_{90})	
Acorus calamus extract	251.71 (525.83)	$y = 1.177 + 0.005x$	223.51 (277.30)	480.46 (589.67)	0.091 *n.s.*
Silver nanoparticles	12.74 (40.09)	$y = 0.597 + 0.047x$	9.998 (15.344)	35.188 (47.155)	2.263 *n.s.*

χ^2 chi-square, *d.f.* degrees of freedom, *n.s.* not significant ($P < 0.05$)

9.3.3 *Predation of* Mesocyclops edax *against* Anophels stephensi *Larvae before and After Treatment with Silver Nanoparticles*

In our experiments, *M. edax* adults actively predate *A. stephensi* young larval instars. The predatory efficiency per copepod per day was 7.1, 5.8, 2.4 and 1.2 larvae (I, II, III, and IV, respectively) (Table 9.5). Post-treatment with *A. calamus-synthesized* AgNP, the predatory efficiency of a single *M. edax* per day was 8.4, 6.9, 4.6, and 2.4 larvae (I, II, III, and IV, respectively) (Table 9.5). In agreement with Murugan et al. (2015a), copepods were effective predators of first and second instars of mosquitoes, while they are not active control agents against late larval instars. Our results highlighted that a combined approach using green synthesized AgNP and predaceous aquatic organisms is effective against the malarial vector against *A. stephensi*.

Scarce information is available about how low dosages of green nanosynthesized mosquitocidals may impact behavioral traits of aquatic organisms sharing the same ecological niche of mosquitoes, such as their predators (Murugan et al. 2015a, b, c). Notably, these investigations unveiled fascinating scenarios. For instance, Murugan et al. (2015b) showed that very low doses (i.e. 1 ppm) of lemongrass-synthesized AuNP may help to control malaria and dengue vectors boosting early instar mosquito larvae predation by copepods (*Mesocyclops aspericornis*) in an aquatic environment contaminated with ultra-low doses of plant-synthesized AuNP.

9.4 Conclusions

Our research showed that the AgNP synthesized using the rhizome extract of *A. calamus* are highly effective as ovicides, larvicides, pupicides and adulticides against malaria mosquitoes. No detrimental effects were found on the predatory efficacy of cyclopoid crustaceans predating *A. stephensi* larvae. Overall, the chance to use *A. calamus*-synthesized AgNP for control of mosquito vectors seems promising, since they are effective at low doses, and may constitute an advantageous alternative to build newer and safer mosquito control tools.

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Compliance with Ethical Standards All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Table 9.5 Predation of the cyclopoid crustacean *Mesocyclops edax* against *Anopheles stephensi* larvae in standard laboratory conditions and after a treatment with *Acorus calamus*-synthesized silver nanoparticles (3 ppm)

Treatment	Target	Consumed larvae (n)					Total predation (n)	Consumed larvae per copepod per day (n)
		Day 1	Day 2	Day 3	Day 4	Day 5		
Control	I instar	80 ± 2.1^{b}	76 ± 2.5^{b}	69 ± 2.3^{b}	$67 \pm 2.9b^{b}$	64 ± 1.5^{b}	356	7.1^{b}
	II instar	71 ± 2.2^{c}	67 ± 2.7^{c}	58 ± 1.5^{c}	51 ± 2.5^{c}	45 ± 2.1^{d}	292	$5.8^{b,c}$
	III instar	49 ± 2.3^{d}	39 ± 2.7^{e}	28 ± 2.1^{e}	21 ± 2.5^{e}	15 ± 1.5^{f}	122	2.4^{d}
	IV instar	22 ± 1.5^{f}	16 ± 2.5^{g}	11 ± 2.3^{g}	8 ± 2.1^{g}	5 ± 2.7^{h}	62	1.2^{a}
Silver nanoparticles	I instar	94 ± 2.3^{a}	88 ± 2.5^{a}	81 ± 1.5^{a}	80 ± 2.1^{a}	77 ± 1.5^{a}	420	8.4^{a}
	II instar	85 ± 2.5^{b}	79 ± 2.3^{b}	69 ± 2.9^{b}	63 ± 3.5^{b}	51 ± 1.5^{c}	347	6.9^{b}
	III instar	72 ± 2.1^{c}	55 ± 2.5^{d}	42 ± 1.5^{d}	36 ± 2.8^{d}	26 ± 3.3^{e}	231	4.6^{c}
	IV instar	45 ± 2.7^{e}	28 ± 3.6^{f}	20 ± 2.3^{f}	15 ± 2.7^{f}	12 ± 3.1^{g}	120	2.4^{d}

Predation rates were means ± SD of four replicates (10 copepods vs. 100 mosquitoes per replicate)
Within each column means followed by the same letter(s) were not significantly different ($P < 0.05$)

Informed Consent Informed consent was obtained from all individual participants included in the study.

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Chapter 10
Nanoparticles Against Schistosomiasis

Mohamed A. Dkhil, Dina A. Nafady, Marwa S.M. Diab, Amira A. Bauomy, and Saleh Al-Quraishy

10.1 Introduction

Schistosomiasis is a neglected parasitic disease and a major public health concern in developing countries (WHO 2015, Fig. 10.1). Globally, 232 million people in 78 countries require annual treatment for schistosomiasis (WHO 2015). The use of praziquantel (PZQ) is effective in clearing the infestation but, in large and widespread population in endemic areas it has been proven to be insufficient to stop disease transmission, prevent reinfection, or reduce parasite-induced illness (King 2009; Matthews 2001). Thus, synergistic approach of using drug and vaccination can serve as an alternative to the current treatment (Fuaad et al. 2015). Akbarieh

M.A. Dkhil (✉)
Department of Zoology, College of Science, King Saud University,
2455, Riyadh 11451, Saudi Arabia

Department of Zoology and Entomology, Faculty of Science, Helwan University,
Cairo, Egypt
e-mail: mohameddkhil@yahoo.com

D.A. Nafady
Department of Graphic Design, Faculty of Architectural Engineering and Digital Design,
Dar Al Uloom University, Riyadh, Saudi Arabia

The Higher Institute of Applied Arts, 6th of October City, Cairo, Egypt

M.S.M. Diab
Molecular Drug Evaluation Department, National Organization for Drug Control & Research
(NODCAR), Giza, Egypt

A.A. Bauomy
Department of Zoology and Entomology, Faculty of Science, Helwan University,
Cairo, Egypt

S. Al-Quraishy
Department of Zoology, College of Science, King Saud University,
2455, Riyadh 11451, Saudi Arabia

H. Mehlhorn (ed.), *Nanoparticles in the Fight Against Parasites*,
Parasitology Research Monographs 8, DOI 10.1007/978-3-319-25292-6_10

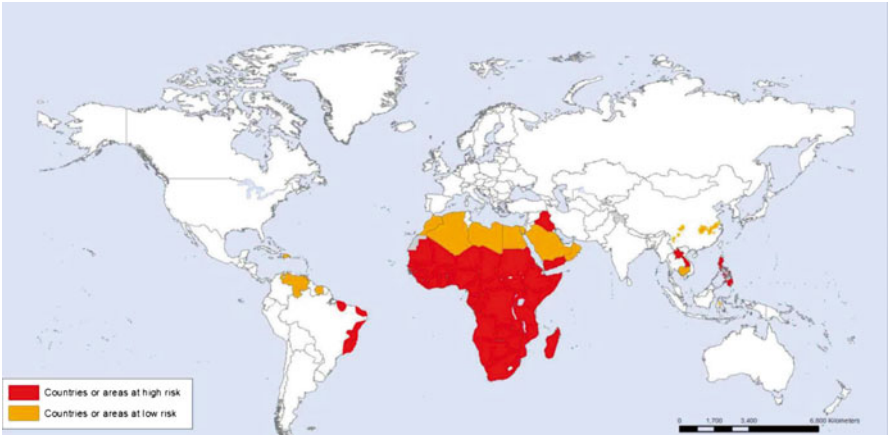

Fig. 10.1 Schistosomiasis, countries or areas at risk, 2014 (WHO 2015)

et al. (1992) reported that, the failure of mass treatment to control schistosomiasis has been attributed to the fact that therapy is not sufficiently long lasting. This effect can occur because of the low bioavailability of praziquantel due to its low hydrosolubility (el-Arini et al. 1998).

Recently, a powerful emerging technology based on the unique properties of nanoscale materials was introduced, which presents a great opportunity to develop fast, accurate, and cost-effective diagnostics and treatment for the detection of infectious agents (Jain 2005; Rosi and Mirkin 2005; Kaittanis et al. 2010). In this chapter we will concentrate on the neuroschistosomiasis induced by *Schistosoma mansoni* and the effect of gold nanoparticles on the brain.

10.2 Nanotechnology and Nanomedicine Applications in Schistosomasis

Nanoparticles are a collective name for nanospheres and nanocapsules. Nanospheres have a matrix type structure, where active compounds can be adsorbed at their surface, entrapped or dissolved in the matrix. Nanocapsules have a polymeric shell and an inner core. In this case, the active substances are usually dissolved in the core, but may also be adsorbed at their surface (Nishioka and Yoshino 2001; Soppimath et al. 2001; Panyam and Labhasetwar 2003). Nanoparticles or colloidal carriers have been extensively investigated in biomedical and biotechnological areas, especially in drug delivery systems for drug targeting because their particle size (ranging from 10 to 1000 nm) is acceptable for intravenous injection (Allemann et al. 1998; Jeon et al. 2000; Soppimath et al. 2001).

Depending on the desired administration way, the size of the carriers should be optimized. Thus, if the carrier size is under 1 μm, an intravenous injection (the diameter of the smallest blood capillaries is 4 μm) is enabled and this carrier size is

also desirable for intramuscular and subcutaneous administration, minimizing any possible irritant reactions (Görner et al. 1999; Hans and Lowman 2002).

10.2.1 Nanotechnology-Based Drug Delivery Systems in Schistosomasis Treatment

The studies concerning the production of new drugs that are more effective and that provoke fewer side effects are undoubtedly important and have been the focus of several researchers and industries. However, problems shown by the actual drugs, such as toxicity and poor solubility in water, may be overcome through their incorporation into drug delivery systems (DDS). The DDS should deliver a biologically active molecule at a desired rate for a desired duration and at a desired target, so as to maintain the drug level in the body at optimum therapeutic concentrations with minimum fluctuation (Kumar et al. 2009; Lakshmi and Cato 2006).

There are several types of medicine applying the DSS technology, and some are already approved by the Food and Drug Administration (FDA). DDS applied for cancer treatment, for example, has been attracting much attention, but fewer studies are available on the literature relating to the incorporation of antiprotozoal drugs into DDS. These studies involve the use of DDS to treat Leishmaniasis, Malaria and trypanosomiasis diseases, but fewer efforts are made for schistosomiasis (Das et al. 2011; Nayaka et al. 2010; Romero and Morilla 2010). De Araújo et al. (2007) reported the *in vitro* schistosomicidal activity of the nanoemulsion containing 2-(butylamino)-1-phenyl-1-ethanethiosulfuric acid (BphEA) and compared its activity with that of free BphEA.

Luz et al. (2012) evaluated the in vitro schistosomicidal activity of curcumin incorporated into poly (lactic-co-glycolic) acid (PLGA) nanoparticles. The schistosomicidal activity of the curcumin-loaded PLGA nanoparticles was evaluated where; the curcumin-loaded PLGA nanoparticles caused the death of 100 % of parasites at 50 and 100 μM at 12 and 24 h. Besides the lethal effect, the curcumin-loaded PLGA nanoparticles caused a decrease of motor activity in the first 24 h of incubation at 40 μM. This effect was also observed at 30 μM in 12 h of incubation. The worms in the negative control groups maintained normal movements with no evident alterations. In addition, the results showed that curcumin-loaded PLGA nanoparticles caused partial alterations in the tegument of the adult worms in concentrations higher than 40 μM, revealed by the presence of vesicles in its structure and this alteration was observed after 48 h of incubation (Luz et al. 2012).

10.2.2 Nanoparticle Gene Delivery System against Schistosomiasis

Until now, there is still urgent need for a vaccine against schistosomiasis, especially in *Schistosoma japonicum* endemic areas where even a vaccine that will interrupt zoonotic transmission will be potentially effective as an intervention tool.

Thus, Mbanefo et al. (2015) developed a novel nanoparticle gene delivery system, which has proven efficacious in gene transfection to target immune cells with complementary adjuvant effect and high protective efficacy in several diseases and had applied this nanoparticle system in combination with S. japonicum glutathione S-transferase (SjGST) DNA vaccine to show the immunogenicity and anti-fecundity effect of the nanoparticle coated vaccine formulation against murine schistosomiasis. The nanoparticle-coated DNA vaccine formulation induced desired immune responses. In comparison with the nanoparticle coated empty vector, it produced significantly increased antigen-specific humoral response, T-helper 1 polarized cytokine environment, higher proportion of IFN-γ producing CD4+ T-cells and the concomitant decrease in IL-4 producing CD4+ T-cells. Although there was no effect on worm burden, a marked reduction in tissue egg burden has been recorded. There was up to 71.3 % decrease in tissue egg burden and 55 % reduction in the fecundity of female adult worms. Their data showed that SjGST DNA vaccine, delivered using the nanoparticle gene delivery system, produced anti-fecundity effect on female adult schistoomes as previously described by using conventional subunit vaccine with adjuvant, proving this DNA vaccine formulation as a promising candidate for anti-pathology and transmission blocking application (Mbanefo et al. 2015).

The combination of the nanoparticle gene delivery system and SjGST was very immunogenic, inducing higher levels of antibodies and a dominant Th1 type of immune response as compared to the nanoparticle coated blank vector. Several workers have identified that the presence of polarizing cytokines environment at the time of initial CD4 T-cell activation is the determining factor influencing Th phenotype (Zhu and Paul 2008; Steinfelder et al. 2009). This vaccine formulation preferentially induced Th1 type of immune response by inducing IL-12 and IFN-γ production. By a characteristic feedback mechanism, persistent exposure to the antigens by an effective gene delivery system activates macrophages by the classical activation pathway to produce more IL-12, which in turn further drives Th1 type of immune response (Mosser 2000; Watford 2003; Cardoso et al. 2008). Such dominant Th1 type of immune response is arguably required for protective immunity against human schistosomiasis (Brito et al. 2000; Acosta et al. 2002); and sustenance of this Th1 skewed response till the onset of egg production when immune response in schistosomiasis becomes Th2 polarized is correlated with reduced liver egg burden (Zhang et al. 2001; Cardoso et al. 2008; Xu et al. 2009), and is thought to be a potential regulator of egg induced pathology (Sher et al. 1996). Indeed, protective immunity elicited by vaccination with radiation-attenuated cercariae and other promising vaccine candidates have consistently proved to be Th1 mediated (Anderson et al. 1998; Hewitson et al. 2005; Wang et al. 2008; Cardoso et al. 2008; He et al. 2010; Farias et al. 2010).

In 2012, researchers found a new strategy based on Smrho protein loaded chitosan nanoparticles as a candidate oral vaccine against Schistosomiasis (Oliveira et al. 2012). Moreover, Luz et al. (2012) reported that curcumin-loaded poly(lacticco-glycolic)acid nanoparticles could decreased the motor activity and caused partial alterations in the tegument of Schistosoma.

10.2.3 Nanodiagnostic Assay for Shistosomasis

The diagnosis of schistosomiasis is traditionally achieved through the use of parasitological methods (urine filtration for *S. haematobium* and Kato-Katz thick smears for *S. mansoni* and *S. japonicum* infections). Nevertheless, the parasitological methods of diagnosis have low sensitivity in patients with the acute phase of the illness or with low- intensity infection (Corachan 2002). In addition, day-to-day and circadian variation in egg excretion may lead to incorrect estimates in prevalence and intensity of infection (Salah et al. 2006). To overcome this problem, several immunological tests have been developed for diagnosis of schistosomiasis (Rabello et al. 2002). Moustafa et al. (1998) reported that antigen detection assays may facilitate earlier diagnosis than antibody tests, as production of detectable levels of specific immunoglobulin needs time. Also, Aly et al. (2013) had demonstrated that the use of nanotechnology can provide a novel diagnostic assay for Schistosomasis. By using the magnetic nanoparticles beads which can utilize larger surface area, a higher sensitivity can be achieved for detection of Schistosoma infections in serum samples as compared with Sandwich ELISA. The use of magnetic nanoparticles in immunoassay (nanomagnetic assay) combines the use of magnetic nanoparticles with a high binding capacity as a solid phase and the rapid reaction kinetics of solutions with the simple separation of bound and unbound materials on the solid phase, which provides the chance of enhancing the sensitivity of *Schistosoma* antigen detection (Aly et al. 2013).

10.3 Gold Nanoparticles against *S. mansoni*-Induced Neuroschistosomiasis

Neuroschistosomiasis is the infection of the central nervous system by *Schistosoma spp*. Both the brain and the spinal cord can be affected (Ferrari and Moreira 2011). There are substantial differences in the pathogenesis, clinical presentation, and outcome of the neurological disorder, depending on the phase and clinical form of schistosomiasis in which it occurs (Ferrari and Moreira 2011).

Central nervous system involvement can occur during acute primary infections (Chitsulo et al. 2000; Amaral et al. 2006). Neurological complications generally occur during chronic hepatointestinal schistosomiasis (Lambertucci 2010). Most pathology in schistosome infected animals is attributed to the host's reaction to the eggs which is maximal by the 8th week of infection.

Gold nanoparticles have received special attention because they have found potential application in many fields of chemistry, physics and biology because they possess an excellent biocompatibility and low toxicity and also because of their unique optical, electrical, and photothermal properties (Sadowski 2010; Isaac et al. 2013).

Recent interest in using gold nanoparticles (GNPs) in medicine has altered the methods of diagnosis and treatment (Chen et al. 2008; Peng et al. 2012). However,

gold has a long history of use in the western world as nervine; a substance that could revitalize people suffering from nervous conditions. In addition, in the sixteenth century it was recommended for the treatment of epilepsy (Richards et al. 2002).

GNPs have attracted great attention due to their unique electronic, optical, thermal, chemical, biological properties and their potential catalytic applications in various fields such as biology, medicine, physics, chemistry, material science and other interdisciplinary fields (Panyala et al. 2009). Although physicochemical properties of nanoparticles are well studied, their biological properties largely remain unexplored (Saritha et al. 2014).

The properties of colloidal gold nanoparticles, and thus their applications, depend strongly upon their size and shape (Zeng et al. 2011). The size could be determined by transmission electron microscopy (Fig. 10.2).

10.3.1 Gold Nanoparticles Induced Changes in Brain Neurotransmitters Content during Neuroschistosomiasis

Dkhil et al. (2015) found a histopathological impairments, neuronal loss, vacuolated cytoplasm, nuclear hyperchromasia, marked dilated congested blood capillaries accompanied with vessel wall edema and presence of extravasated red blood cells in brain tissue of schistosome infected mice as compared to non-infected control group. On the other hand, the treatment of the schistosome infected mice with three different doses of gold nanoparticles recorded histological alleviations.

Also, schistosomiasis resulted in a significant reduction in both brain norepinephrine (NE) and dopamine (DA) contents were recorded in schistosome infected mice as compared to non-infected mice (Abdel Ghafar et al. 1996; Bauomy et al. 2014; Dkhil et al. 2015). The treatment of schistosome infected mice with different doses of gold nanoparticles (Table 10.1) caused a significant alleviation in content of NE and DA (Dkhil et al. 2015). Ancient cultures in Egypt, India and China used gold to treat some diseases (Chen et al. 2008). Moreover, gold complexes with different organic drugs have been tested as ligands against malaria, trypanosomiasis, and leishmaniasis (Navarro et al. 2007). It is worth mentioning that gold nanoparticles have also been used for the diagnosis of Alzheimer's disease and activated microglia (Nunes et al. 2012).

10.3.2 Gold Nanoparticles and Oxidative Damage during Neuroschistosomiasis

The oxidative damage in stress could contribute to the degenerative diseases of aging, including brain dysfunction (Liu et al. 1996). The early-onset decline in learning and memory is associated with a very significant increase in two

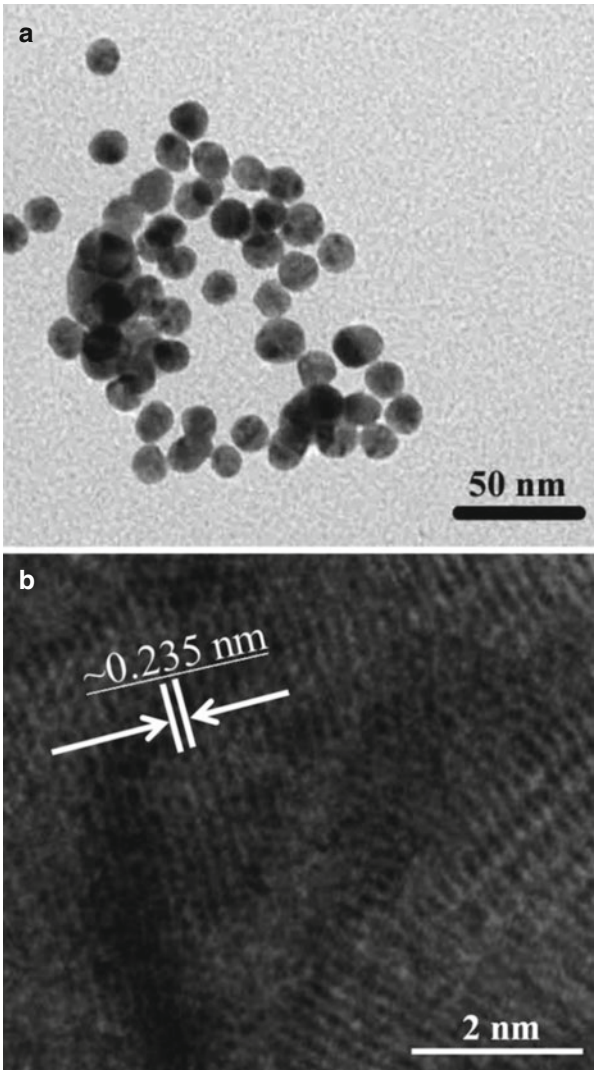

Fig. 10.2 Typical TEM and their corresponding HR-TEM images of synthesized GNPs: (**a**) shows the low magnification image of spherical GNPs (~15–20 nm), whereas (**b**) presents the HR-TEM image of difference between two lattice fringes, which is ~0.235 nm (Dkhil et al. 2015)

parameters of oxidative stress in the brain, levels of lipid peroxidation and of protein oxidation (Liu et al. 2003). However, shistosomiasis induced a reduction in brain glutathione (GSH) level and an elevation in both levels of nitric oxide (NO) and malondialdehyde (MDA) (de Oliveira et al. 2013; Diab et al. 2013; Bauomy 2014; Bauomy et al. 2014; Dkhil 2014). Also, de Oliveira et al. (2013) cleared that *S. mansoni* had altered non-enzymatic antioxidant status in brain. In addition, Bauomy (2014) reported that schistosomiasis induced brain oxidative stress as evidenced by the decrease of GSH level, total antioxidant capacity and the activity of catalase significantly, while a significant elevation in the levels of NO and MDA. Furthermore, in different mice organs *S. mansoni* infection decreased GSH level, the activities of

Table 10.1 Effect of gold nanoparticles (GNPs) on brain neurotransmitters Norepinephrine (NE) and Dopamine (DA) on *S. mansoni* infected mice (Dkhil et al. 2015)

Group	Norepinephrine (μg/g tissue)	Dopamine (μg/g tissue)
Non-infected	290.2 ± 2.93	877.8 ± 2.67
Infected (-GNP)	263.3 ± 2.80[a]	778.6 ± 2.08[a]
Infected (+0.25 mg/kg GNP)	339.0 ± 2.59[a,b]	842.8 ± 2.85[a,b]
Infected (+0.5 mg/kg GNP)	331.5 ± 2.62[a,b]	838.1 ± 2.50[a,b]
Infected (+ 1 mg/kg GNP)	324.0 ± 2.44[a,b]	1011.2 ± 4.58[a,b]
Infected (+ PZQ)	330.6 ± 2.08[a,b]	798.4 ± 3.54[a,b]

Values are means ± SEM
[a]Significant against non-infected (-GNPs) group at $P \leq 0.05$
[b]Significant against infected (-GNPs) group at $P \leq 0.05$

catalase and superoxide dismutase significantly, on contrary, increased NO and MDA levels significantly (Diab et al. 2013; Bauomy et al. 2014; Dkhil 2014). Moreover, treatment with gold nanoparticles at different doses to schistosome infected mice resulted in a significant increase in GSH level and a significant decrease in NO and MDA levels as compared to schistosome infected mice (Fig. 10.3). The mechanisms of action of gold drugs are poorly understood (Best and Sadler 1996). Typically, after systemic administration, the nanoparticles are small enough to penetrate very small capillaries throughout the body, and therefore they could offer the most effective approach to target certain tissues (Braydich-Stolle et al. 2005); such as brain and can affect the physiology of any cell in an animal body (Brooking et al. 2001).

10.3.3 Gold Nanoparticles and Gene Expression during Neuroschistosomiasis

Comparing gene expression profile of mice genes during schistosomiasis and after treatment with gold nanoparticles of infected mice will explain different molecular mechanisms of action and regulated genes by such nanoparticles during infection. Dkhil et al. (2015) studied the gene expression in the brain of mice infected with *S. mansoni* and after the treatment of mice with gold nanoparticles. They found that, schistosomiasis increased brain Adam23 (a disintegrin and metallopeptidase domain 23) gene expression. Sagane et al. (1999) and Mitchell et al. (2001) reported that disruption of the mouse Adam23 induced neurological defects, ataxia and premature death indicating that this protein is important for normal brain development. On the other hand, injection of gold nanoparticles recorded a significant down regulation in brain Adam23 gene.

Glrb gene (glycine receptor, beta subunit) encodes the inhibitory human glycine receptor β subunit (Lee et al. 2013). Inhibitory glycinergic synapses are located predominantly in the spinal cord and brainstem (Chalphin and Saha 2010) and disruptions to their function increase the general level of excitability of motor neurons, thus

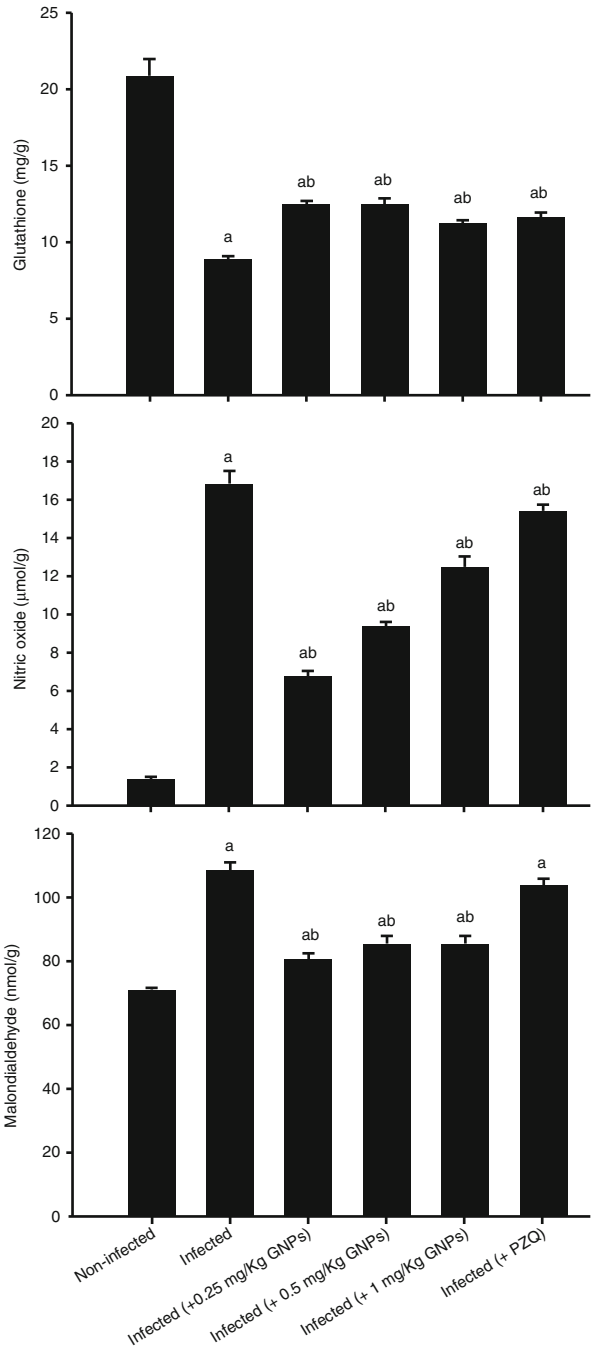

Fig. 10.3 Changes in the level of reduced glutathione (GSH), nitric oxide and malondialdehyde in infected mice brain tissue with *S. mansoni* and treated with different doses of gold nanoparticles. Values are means ± SEM. *a*: Significant against non-infected (-GNPs) group at $P \leq 0.05$, *b*: Significant against infected (-GNPs) group at $P \leq 0.05$ (Dkhil et al. 2015)

accounting for neonatal hypertonia. Glrb is one of the adult walking behaviors which recorded high expression in the malarial brain (Desruisseaux et al. 2010). These results are in agreement with our records which schistosomiaisis induced a significant over expression in brain Glrb gene. On contrary, gene amelioration resulted in GNPs treatment. In the present result, Vdac3 gene (voltage-dependent anion channel 3) recorded a significant overexpression as a result of schistosomiasis. Cízková et al. (2008) reported that Vdac3 is a mitochondrial gene is regulated in the malaria brain whose dysfunction is associated with neurological disorders. Our results, pointed that GNPs and PZQ treatment to schistosome infected mice induced downregulation in Vdac3 brain gene.

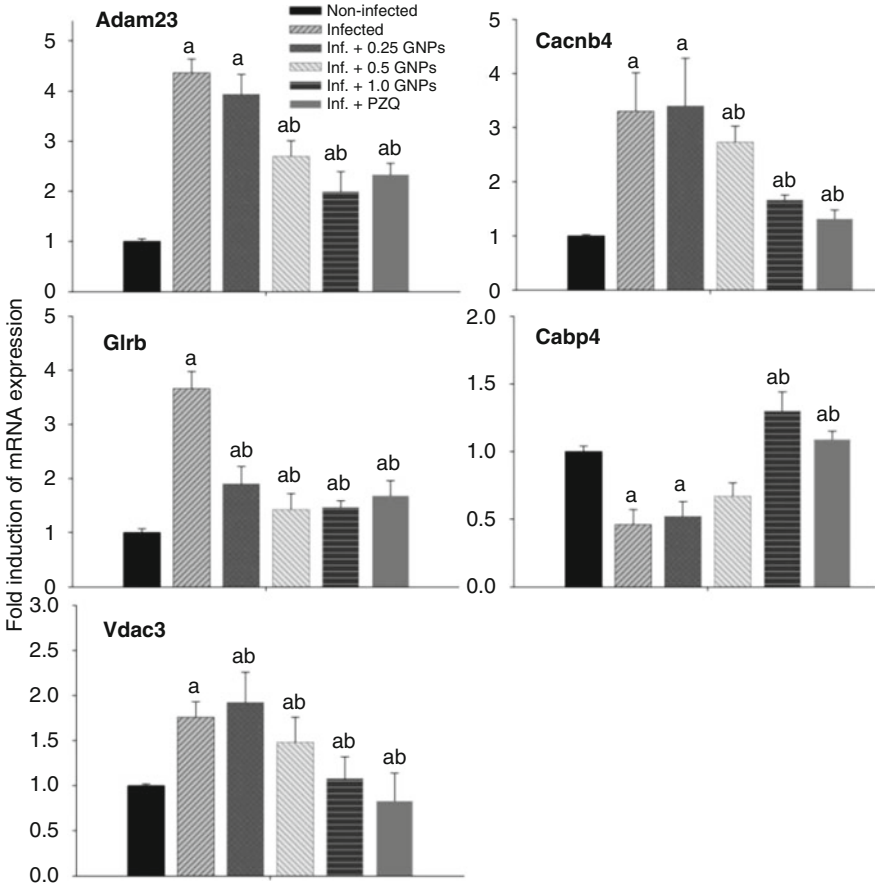

Fig. 10.4 Gold nanoparticles induced changes in gene expression of mice brain infected with *S. mansoni*. Expression of Adam23, Glrb, Vdac3, Cacnb4 and Cabp4 in brain tissues was analyzed by quantitative RT-PCR in noninfected mice and *S. mansoni*-infected mice on day 59 p.i. with and without GNPs treatment. Relative expression is given as fold increase in comparison with noninfected control mice. Values are means ± SD. *a*: Significant against non-infected (-GNPs) group at $P \leq 0.05$, *b*: Significant against infected (-GNPs) group at $P \leq 0.05$ (Dkhil et al. 2015)

Cacnb4 gene (calcium channel, voltage-dependent, beta 4 subunit) mutations encoding voltage-gated calcium channels (VGCCs) induce diverse neuronal pathologies, such as epilepsy, ataxia, autism and migraine (Bidaud et al. 2006). It is known neuroschistosomiasis induced ataxia, headache as common manifestations (Ferrari 2004).

In the present work, Cacnb4 gene recorded a significant upregulation while, Cabp4 gene showed a significant downregulation as a result of neuroschistosomiasis. It was reported that, calcium current through VGCCs controls gene expression (Tadmouri et al. 2012). Barnes and Kelly (2002) deduced that calcium influx through calcium channels triggers neurotransmitter release where increase the release of the neurotransmitters by exocytosis; and this may explain reduced contents of DA and NE in our investigations. VGCCs mediate the influx of calcium ions into the cell upon membrane polarization which control multiple neuronal functions including excitability, synaptic transmission and activity-dependent gene regulation (Catterall and Few 2008). Cabp4 (calcium binding protein 4) may be an important regulator of calcium influx and transmitter release in synaptic terminals (Haeseleer et al. 2004). Cabp4 interacts with calmodulin-binding sequences in VGCC which weakly inhibits calcium-dependent inactivation (Fig. 10.4).

It can be concluded that the developed nanoparticles have great potential to overcome the limitations associated with products currently available in the market for the treatment of schistosomiasis because of their ability to modulate drug release with the highest rate of absorption into the body and to mask the unpleasant taste of the drug, which are considered important steps for increasing the efficiency of pediatric treatments.

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Chapter 11
Nanoparticles Against Eimeriosis

Mohamed A. Dkhil and Saleh Al-Quraishy

11.1 Introduction

11.1.1 Problems of Eimeriosis

Eimeriosis is one of the factors that interfere the development of livestock including cattle, rabbits, sheep, goats, and, particularly, poultry (Dkhil 2013; Wunderlich et al. 2014). This disease is caused by protozoans of the coccidian genus Eimeria, striking the digestive tract of their hosts (Daugschies and Najdrowski 2005; Blake and Tomley 2014; Chapman 2014; Mehlhorn 2014). Eimeriosis is characterized by diarrhea, fluid loss, dehydration, inflammation, malabsorption of nutrients, as well as increased susceptibility to microbial pathogens (Dkhil et al. 2013; Alnassan et al. 2014). The mortality due to eimeriosis may reach up to 80 %, depending on the Eimeria species (Fossum et al. 2009). Reduced body weight gain and death of infected animals cause enormous economic losses. Worldwide, the costs for control measures of coccidiosis only in cattle and poultry are estimated to exceed about two billion dollars annually (Williams 1999; Shirley et al. 2005; Dalloul and Lillehoj 2006; Blake and Tomley 2014).

M.A. Dkhil (✉)
Department of Zoology, College of Science, King Saud University,
P.O. Box 2455, Riyadh 11451, Saudi Arabia

Department of Zoology and Entomology, Faculty of Science, Helwan University,
Cairo, Egypt
e-mail: mohameddkhil@yahoo.com

S. Al-Quraishy
Department of Zoology, College of Science, King Saud University,
P.O. Box 2455, Riyadh 11451, Saudi Arabia

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11.1.2 Anticoccidial Agents

Treatment of infected hosts with known anti-coccidial drugs causes several adverse side effects. There is a critical need for the development and evaluation of new drugs. Although a number of drugs are commercially available for the treatment of eimeriosis, their efficacy is being increasingly impaired due to emerging parasite resistance and also these drugs causes several adverse side effects (Mehlhorn 2014). This led us to the search for novel anti-Eimeria agents with a focus on low-cost medications. During the last two decades, many attempts have been made to develop effective new compounds for treatment of eimeriosis that would be economically applicable and could avoid development of resistance.

11.2 Trace Elements Nanoparticles

Trace elements such as selenium and zinc have been found to possess anti-Eimeria activity in various animal models (Wunderlich et al. 2014). These elements when used in nanosize, became more effective (Dkhil et al. 2015). For e example zinc oxide nanoparticles (ZNPs) are characterized by their high catalytic efficiency and high adsorbing ability (Bouwmeester et al. 2009). Moreover, zinc is considered to be an essential trace element for various cellular activities (Wunderlich et al. 2014; Dkhil et al. 2015). Moreover, silver nanoparticles were used as additive in poultry feeds, but the price of silver nanoparticles could not compete with that of antibiotics this is because silver is considered to be a non-toxic, safe, inorganic antibacterial agent used for many years for its capability of killing about 650 types of pathogens (Jeong et al. 2005). Recently, it was reported that silvernanoparticles used in drinking water could reduce the oocyst out put in chicken to about 50 % after 7 days postinfection with *E. tenella* (Chauke and Siebrits 2012).

Recently, Dkhil et al (2015) showed that, mice infected with *Eimeria papillata* produced $29.7 \times 10^3 \pm 1{,}500$ oocysts/g feces on day 5 postinfection. This output was significantly decreased, to $12.5 \times 10^3 \pm 1{,}000$ oocysts, in mice treated with ZNPs. In the same experiment the infection induced a moderate inflammatory damage to the infected mice jejuna and ZNPs was able to improve the jejuna histopathology (Dkhil et al. 2015). The fact that ZNPs possess anticoccidial activity has not been reported before, but Bafundo et al. (1984) demonstrated clearly that zinc utilization is diminished by *Eimeria acervulina* infection.

It was reported that, ZNPs may be indicative of improved innate responses to *E. papillata* infection (Dkhil et al. 2015) where the number of goblet cells, the major intestinal immunocompetent cells secreting mucous, could be elevated after the treatment of the infected mice with ZNPs. At the same time ZNPs could protect from the eimeria induced intestinal oxidative damage (Dkhil et al. 2015). In 2009, Scrimgeour and Condlin (2009) reported that, zinc could be in the treatment of gastrointestinal inflammatory disorders, and ZNPs are known to be able to prevent

the loss of GSH during oxidative damage induced by infection, as has been described by Dkhil et al. (2011). It is postulated that the active oxygen species generated by these metal oxide particles could be the main mechanism of their antibacterial activity (2014).

Generally, it is known that dietary trace elements/antioxidants can help maintain an appropriate antioxidant balance in the case of many infections (Evans and Halliwell 2001). Wunderlich et al. (2014) for example, reported that zinc is fundamentally important for a balanced redox state, for the immune system, as well as for growth and development.

11.3 Nanoparticle Based Vaccine

Vaccination is considered to be an alternative way to control coccidiosis (Dalloul and Lillehoj 2006; Michels et al. 2011). Dalloul and Lillehoj (2006) reported that compared with virulent or attenuated live vaccine, recombinant protein vaccine can induce good antibody response and has more efficiency to protect birds against challenge of Eimeria oocysts. In 2012, Zahng et al. investigated the adjuvant effect of ginsenoside-based nanoparticles (ginsomes) on the immune responses induced by profiling recombinant vaccine and subsequent protection against *E. tenella*. The results of this study showed that vaccination with profilin plus ginsomes induced an antigen-specific antibody response, induced changes in the local lymphocyte subpopulations, and significantly reduced the total oocysts production following challenge infection. Also, Jang et al. (2011) investigated protection against *Eimeria acervulina* following vaccination of chickens with an Eimeria recombinant profilin in conjunction with nanoparticle adjuvants, or by changing the route of administration of the adjuvants. They indicated that experimental immunization of chickens with the recombinant profilin subunit vaccine in conjunction with adjuvants increases protective mucosal immunity against *E. acervulina* infection.

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Index

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