ENTOMOLOGY

Antifungal and insecticidal activities of *Raphanus sativus* mediated AgNPs against mango leafhopper, *Amritodus brevistylus* and its associated fungus, *Aspergillus niger*

R. Shanmugapriya, A. Nareshkumar, K. Meenambigai, R. Kokila, A. Shebriya, K. Chandhirasekar, A. Thendral Manikandan, C. Munusamy

Department of Zoology, School of Life Sciences, Periyar University, Salem, India

Abstract

Mangifera indica is an important commercial fruit of India and primary food source for the mango leafhopper, *Amritodus brevistylus* further leading to the development of its associated fungus, *Aspergillus niger*. The present study was done to assess the antifungal and insecticidal activity of biologically synthesized silver nanoparticles using peel extracts of *Raphanus sativus* (Brassicaceae). *Raphanus sativus* peel extract is effective in extracellular reduction of Ag ions and capping the synthesized nanoparticles avoiding further agglomeration. The synthesized AgNPs are characterized by UV-vis spectroscopy, X-ray diffraction analysis, fourier transform infrared spectroscopy, scanning electron microscopy and energy dispersive x-ray analysis. Biologically synthesized AgNPs exhibited significant toxicity (LC_{50} − 7.61 ppm/48h) over mango leafhopper, *Amritodus brevistylus*. Silver nanoparticles synthesized using *Raphanus sativus* is also effective against the fungal pathogen, *Aspergillus niger* (developed on the infestations of mango leafhopper, *Amritodus brevistylus*) showing very strong inhibitory zone (80 mm).

Correspondence: Arjunan Nareshkumar, Department of Zoology, School of Life Sciences, Periyar University, Salem 636011, Tamil Nadu, India.
E-mail: naresh@periyaruniversity.ac.in

Key words: Bio-synthesis; AgNPs; Biology of mango leafhopper; Insecticidal activity; Antifungal activity.

Acknowledgements: the authors are extremely indebted to convey their bouquet of thanks to Department of Science and Technology, Science & Engineering Research Board (SERB), New Delhi-110070, India for providing Young Scientist Award and funds (Award Registration No.SERB/LS-1074/2013) to run the project successfully.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 22 April 2016.
Revision received: 9 January 2017.
Accepted for publication: 18 Janaury 2017.

Journal of Entomological and Acarological Research 2017; 49:5953
doi:10.4081/jear.2017.5953

Introduction

India is the world's largest producer (52%) of mango which is one of the world's most popular fruits with both social and economic importance (Sauco, 2004). The fruit contains essential vitamins and dietary minerals. The unripe mango and its seed constitute a rich source of Vitamin-C, whereas, the ripe mango and its seed constitute a rich source of Vitamin-A with excellent flavor and widespread industrial and commercial use (Benevides *et al*., 2008; Shobana & Rajalakshmi, 2010). The reduction in fruit yield a major issue for mass producers which is due to damage caused by large number of insect pests. Insect pests belonging to a number of species have been reported attacking mango trees. Among them, mango leaf hoppers viz., *Amritodus brevistylus, Amritodus atkinsoni* Leth., *Idioscopus clypealis* Leth., and *Idioscopus niveosparsus* are the most serious and widespread pest throughout the country (Sharma & Sharma, 2011; Gundappa *et al*., 2014).

Insects are the most abundant and diverse organisms of arthropods that attract a variety of pathogens including viruses, bacteria and fungi Mango leaf hoppers secrete honeydew which encourages the development of fungi on leaves, branches and even fruits (Verghese & Jayanthi, 2001). The development of fungal colonies over the leaves interferes with normal photosynthetic activity of the plant, ultimately resulting in non-setting of flowers and dropping of immature fruits (Fletcher *et al*., 2006). *Aspergillus* sp. is a widely distributed fungal pathogen which causes allergic bronchopulmonary aspergillosis, aspergilloma, and invasive aspergillosis. Moreover, *A.niger* is widely distributed in nature with a wide range of hosts. Few strains of *A. niger* are capable of producing Ochratoxin A (OTA), which is a toxic secondary metabolite (Schuster *et al*., 2002) widely spread all over the world. Ochratoxins A is a mycotoxin of Aspergillus and Pencillium molds exerts hazardous effects targeting kidney

and liver in animals and in humans, as well. The mode of action also includes Inhibition of protein synthesis and energy production, induction of oxidative stress, DNA adduct formation, as well as apoptosis/necrosis and cell cycle arrest (Koszegi & Poor, 2016).

Synthetic chemicals used for the control of pests and pathogens are found to be environmentally toxic, reduce the soil fertility and further the insects and pathogens developresistance to these chemicals. Hence, there is a growing emphasis on eco-friendly technologies in pest control, and use of various natural resources to avoid toxic chemicals (Sahab *et al*., 2015). Nanoparticles are defined as: *[...] natural, randomly created or manufactured materials containing particles in a free state or as an aggregate or agglomerate, wherein at least 50% of the particles has one or more dimensions in the range 1-100 nm* (Pulit-Prociak & Banach, 2016). Noble metal nanoparticles have been studied because of their unique optical properties. Gold and silver have a broad absorption band in the visible region of the electromagnetic spectrum. The properties of these metals change depending on their shape, size and the surrounding medium and they have been used in advanced technologies in medicine, optoelectronics, and chemical catalysis, in sensors, for drug delivery, and for etching and cutting (Nareshkumar *et al*., 2012). Nanoparticles have high surface area to its volume ratio and Surface Plasmon Resonance, thus considered as excellent candidate for research in various fields. Silver is an effective antimicrobial agent, exhibits very low toxicity to environment, and has diverse *in vitro* and *in vivo* applications. The AgNPs are reported to possess insecticidal against *Aedes aegypti, Anopheles stephensi,* and *Culex quinquefasciatus*, antibacterial against *Bacillus subtilis, Klebsiella pneumoniae,* and *Salmonella typhi* (Madhiyazhagan *et al*., 2015), antiviral against monkey poxvirus (Rogers *et al*., 2008) and antifungal properties against *Candida* sp. (Panacek *et al*., 2009). The nanoparticles offer an excellent alternative to chemical insecticide as they can be more selective than chemicals (Barik *et al*., 2012; Roni *et al*., 2013; Suresh *et al*., 2015).

Biosynthesis of nanoparticles using plant parts is advantageous over chemical and physical synthesis because it is aneco-friendly and cost-effective method, where there is no need for the high pressure, energy, temperature, and toxic chemicals. Several species of plants viz., *Anthocepholus cadamba* (Nareshkumar *et al*., 2013), *Catharanthus roseus* (Velayutham *et al*., 2012), *Pedilanthus tithymaloides* (Sundaravadivelan *et al*., 2013), *Vincarosea* (Subarani *et al*., 2013), *Sidaacuta* (Veerakumar *et al*., 2013), *Annona squamosa* (Nareshkumar *et al*., 2012), *Leucasaspera* (Suganya *et al*., 2014) and many more have been used for the synthesis of different metal nanoparticles.

In the present study the peels of *Raphanus sativus* are used as a biological reducing agent in synthesis of nanoparticles. Root, stem and leaf of *Raphanus sativus* have showed broad spectrum of antibacterial activity against food-borne pathogens and drug resistant strains (Beevi *et al*., 2009). Raphanin, the main active compound of *R. sativus* is reported to be effective in inhibiting the development of *Escherichia coli, Pseudomonus pyocyaneus, Salmonella typhi, Bacillus subtilis* (Abdou, 1972), *Staphylococcus aureus, Streptococci spp. and Pneumococci spp.* (Yeung, 1985). It is also active against many food borne pathogenic and food spoiling bacteria such as *Listeria, Micrococcus, Enterococcus, Lactobacillus, Pedicoccus*sp. and *Aspergillus oryzae* (Yildium & Johnson, 1998). In the present study *Raphanus sativus* peels were used to synthesis eco-friendly silver nanoparticles for testing their toxicity on the mango pest, *Amritodus brevistylus* and its associated fungus, *Aspergillus niger*.

Materials and methods

Collection and preparation of peel broth

Raphanus sativus peels were collected from households in Salem, Tamil Nadu, India. The *R. sativus* peels were washed well with distilled water and dried for 2 days at room temperature and the broth was prepared by taking 5 g of thoroughly washed and finely cut peels in a 300 mL Erlenmeyer flask with 100 mL of distilled water and then boiling the mixture for 5 min before finally decanting it. They were stored at 4°C and used within a week.

Synthesis of silver nanoparticles

10 mL of *Raphanus sativus* peel broth was added to 190 mL of 1mM aqueous $AgNO_3$ solution for reduction of Ag^+ ions. The synthesis process was carried out in water bath at 90°C with reflux. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min followed by redispersion of the pellet in deionized water (Nareshkumar *et al*., 2012).

Characterization of silver nanoparticles

UV-vis spectra were recorded as a function of reaction time on a UV-3600 Shimadzu spectrophotometer operated at resolution of 1 nm. After freeze drying of the purified silver particles, the structure and composition were analyzed by 10 kV Ultra High Resolution Scanning Electron Microscope (FEI QUANTA- 200 SEM) and energy dispersive X-ray spectroscopy. The surface groups of the nanoparticles were qualitatively confirmed using FTIR spectroscopy. FTIR spectra were recorded on a perkin-Elmer spectrum 2000 FTIR spectrophotometer. X-ray diffraction using Cuk α radiation (PANanlyticalX'pert Pro MPD diffractometer) was used to determine the crystalline structure of silver nanoparticles. Powder X-ray analysis was carried out using a Philips Model PW 1050/37 diffractometer, operating at 40 kV and 30 mA, with a step size of 0.02° (2θ) (Nareshkumar *et al*., 2012).

Biology of mango leafhopper

Twenty pairs of adult leafhoppers were collected random from the mango tree near Periyar University, Salem, Tamil Nadu, India. The pests were released into a cage which enclosed fresh tender leaves; the cage of 45×45×45 diameter was made from fine nylon mesh. The development of the leafhoppers was monitored daily for oviposition. The adults were removed from cage when oviposition was seen. Biology was studied in the laboratory on mango graft enclosed in the rearing cage and observation on incubation, hatching, molting and nymphal period were recorded (Gnaneswaran *et al.,* 2007).

Bioassay

The pests were cultured under laboratory condition. Adults with less than 2 weeks old (Negahban & Moharramipour, 2007) were used for the experiments. Effects of the silver nanoparticles on adult mango leafhoppers were determined by contact toxicity assay at five different doses of 5 ppm, 10 ppm, 20 ppm, 50 ppm and 100 ppm. The experiments were carried out in Completely Randomized Design with five replications, each consisted of 10 adults of mango leafhopper in small plastic screw capped jars containing leaves individually immersed in different concentrations of silver nanoparticles for 5-10 seconds and dried in shade. Then all small plastic screw capped jars were kept at same condition. The effects of silver nanoparticles on leafhoppers were recorded at 12h, 24h, 36h and 48h. In one additional set leaves immersed in distilled water served as control.

The control mortalities were corrected by using Abbott's formula (1925):

$$\text{Corrected mortality} = \frac{\text{observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{number of dead larvae / pupae}}{\text{number of larvae /pupae introduced}} \times 100$$

Fungal isolation and preparation of spore suspension

Fungal species was isolated from the diseased leaves of *Mangifera indica* Linn., The collected leaf samples of *Mangifera indica* Linn., were thoroughly washed in running tap water and only the diseased regions were cut into small square pieces (approximately 0.5 cm) with the aid of a flame-sterilized blade. The leaf pieces were surface-sterilized by immersion in 70% ethanol for 5s, followed by 4% sodium hypochlorite for 90s, then rinsed three times in sterile distilled water for 10s each. Excess moisture was removed by blotting on sterile filter paper. The surface sterilized leaf segments were evenly spaced in petri dishes (9 cm diameter) containing Potato Dextrose Agar (PDA) medium. The petri dishes were sealed using Parafilm and incubated at 28±2°C. The petri dishes were monitored ever day to check for fungal colony growth from leaf segments. 7days old cultures were used for preparation of the spore suspension. The fungal strains were inoculated into potato dextrose agar medium plates and incubated 7 days (28±2°C). After 7 days the young culture developed were diluted at 10-15x10^{5}spores/mL which were identified at Department of Botany, Periyar University, Salem, TN, India and further used for antifungal assay (Jia, 2009).

Antifungal assay (disc diffusion method)

Antifungal activity was conducted using disc diffusion method (Ronald,1990). An inoculum containing fungal spores was applied onto potato dextrose agar plates. The discs were made by cutting discs (10 mm) from a filter paper. Each disc was impregnated with the AgNPs at different concentration (50 µL, 100 µL, 250 µL) using a micro pipette. Discs were also made for the positive controls (0.1% Hydrogen peroxide). These plates are kept at room temperature (28±2°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Jagessar *et al*., 2008). The plates are then inverted and incubated at 28±2°C for 7 days for optimum growth of the organisms. The test materials having antifungal property inhibit fungal growth in the media surrounding the disc and thereby yield a clear, distinct area defined as zone of inhibition. The antifungal activity of the test agent is then determined by measuring the diameters. The experiment was in triplicate and the mean of the reading was taken.

Statistical analysis

The median Lethal Concentration (LC_{50}) observed for the mango leaf hopper were estimated by probit analysis. The level of significance used in all tests was 5%. Statistical significance of mean differences was assessed by Duncan's multiple range test (DMRT). Analyses were made by using SPSS Software package (version 14.0).

Results

UV-visible absorption spectroscopy studies

The UV absorption of silver nanoparticles recorded from the reaction medium at 90°C using 5% *Raphanus sativus* peel extract with 1 Mm $AgNO_3$ exposure to different reaction time (30 min, 60 min, 120 min and 240 min) is shown in Figure 1. The samples showed maximum absorption peak ranging between 400-450 nm with an average of 420 nm. The effect of $AgNO_3$ (1 Mm) on conversion and particle size of silver nanoparticles with 5% *Raphanus sativus* peel extract treated for 120 min at 90°C was observed to be the optimum time when compared with the other reaction times.

X-ray diffraction studies

The biosynthesized silver nanoparticles using 5% *Raphanus sativus* peel extract was supported by X-ray diffraction technique (Figure 2). The diffractometer was operated at 40 kV and 30 Ma, with a step size of 0.02° (2θ). The scanning was done in the region of 10° to 80° for 2θ. The high crystalline nature of Ag nanoparticles was confirmed from the X-ray diffraction analysis with the formation of high intense peak. The intense diffraction peaks due to AgNP at 27.62°, 32.03°, 37.93° and 46.03°were indexed with the planes (110), (150), (200) and (430) of the face centered cubic crystal according to the JCPDS: 89-3722. The broadening of the Bragg peaks indicates the formation of nanoparticles. In addition unassigned peaks were also observed suggesting that the crystallization of bioorganic phase occurs on the surface of the silver nanoparticles. The calculated grain size in accordance with Schrres formula ($T=K\lambda/\beta\cos\theta$) was ~34nm which was well matched with as we calculated in the SEM.

Fourier transform infrared spectroscopy studies

The possible biomolecules of the peel extracts of *Raphanus sativus* involved in the reduction of the Ag^+ ions and capping of the reduced Ag NPs synthesized were analyzed using Fourier Transform Infrared (FT-IR) Spectroscopy (Perkin-Elmer spectrum 2000 FTIR spectrophotometer) in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}. Figure 3 shows the FTIR spectra of aqueous silver nanoparticles prepared from the *Raphanus sativus* peel extract peaks at 2335.80, 1546.91, 1116.78, 678.94, 653.87, 522.71, 468.70, and 455.20. A strong peak was observed at 2335.80 and 1546.91cm^{-1} indicates presence of the CO_2 and C=C stretching, respectively. The IR band observed at 1116.78 indicates the -C-O and -C-O-C stretching modes, respectively. The medium bands at678.94, 653.87 and 522.71 cm^{-1}might be due to the presence of C-N, -CC≡H:C-H band and C-Cl stretching. The active secondary metabolites of *Raphanus sativus* such as alkaloids, phenolic and sulfur compounds would have been the responsible candidates of reduction and capping during the synthesis of Ag NPs.

Scanning electron microscopy studies

Scanning electron microscopy (SEM) technique was employed to visualize the size and shape of Ag nanoparticles. Figure 4 shows the SEM images of Ag nanoparticles obtained using *Raphanus sativus* peel extract. The 10 kV Ultra High Resolution Scanning Electron Microscope (FEI QUANTA-200 SEM) was been used. SEM grids were prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp. The formation of silver nanoparticles as well their morphological dimensions in the SEM study demonstrated that the size was ranging from 20-40 nm with an average size of about 35 nm. The observed silver nanoparticles were irregular in shape.

Energy dispersive X-ray analysis of silver nanoparticles

The energy dispersive X-ray analysis (EDAX) pattern shows the crystalline and elemental composition of silver nanoparticles synthesized from the peel extract of *Raphanus sativus.* EDAX reveals strong signal in the silver region and confirms the formation of silver nanoparticles. Metallic silver nanocrystals generally

show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance. This analysis revealed that the nanostructures were formed solely of silver (Table 1).

Biology of *Amritodus brevistylus*

Amritodus brevistylus was kept under observation for the measurement of total life span. The fresh white eggs of *Amritodus brevistylus* were placed over fresh and young mango leaves for incubation and hatching. The incubation period was 4.2 days. The hatching process underwent for around 30 to 50 min after which the yellowish nymph came completely out from the egg case. The freshly hatched nymph was active and yellowish (Figure 5). As exposure to environment the body turned darker. The nymph were nocturnal, most preferred the darker portion during the day. The 1st instar nymph was active in feeding and developed in 2.1 days. The 2nd, 3rd and 4th instars took around 3.5, 2.2 and 3.3 days respectively for development. The adult was emerged in around 11 days from the date of emergence from egg. The winged adults were brownish with yellow and black spots. The adult male (4.8 mm) and female (5.2 mm) survived for around 6.6 days feeding on fresh tender mango leaves replaced daily.

Table 1. Energy dispersive X-ray analysis spectrum recorded of silver nanoparticles synthesized using peel extract of *Raphanus sativus*.

Element	Weight %	Atomic %
O K	29.71	74.03
Ag L	70.29	25.97
Total		100.00

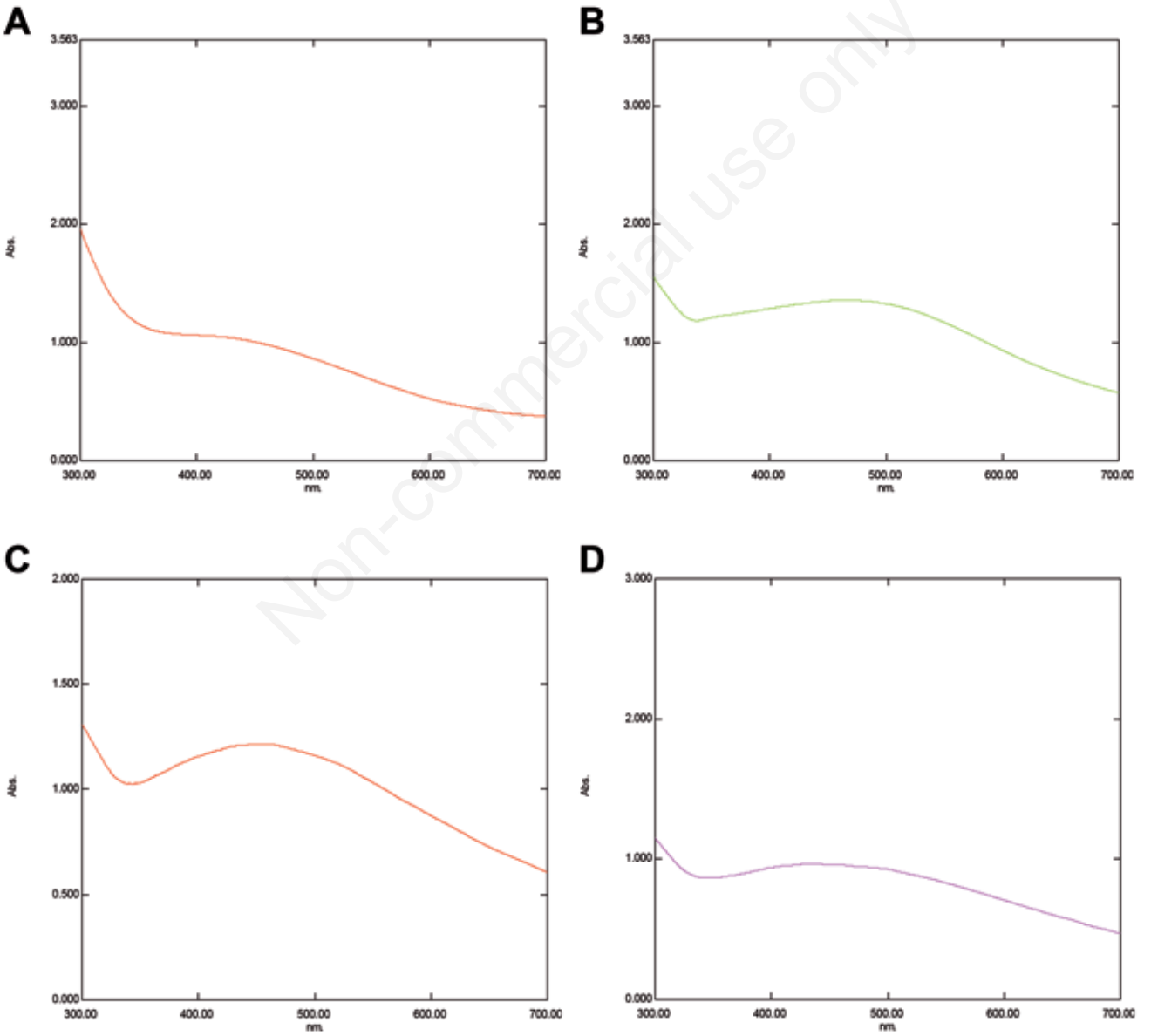

Figure 1. Ultraviolet-visible spectra of silver nanoparticles synthesized using *Raphanus sativus* peel extract at 30, 60, 120 and 240 min reaction time (A, B, C and D, respectively).

Bioassay

Bioassay was conducted to test the efficacy of silver nanoparticles on adult mango leafhopper, *Amritodus brevistylus*. The results presented in Table 2 and Figure 6 show silver nanoparticles are effective against the adult mango leafhopper, *Amritodus brevistylus.* Percentage mortality was calculated for the adult mango leafhopper treated with various concentrations (5 ppm, 10 ppm, 20 ppm, 50 ppm and 100 ppm) of silver nanoparticles. The median Lethal Concentration (LC_{50}) of the silver nanoparticles against the adult mango leafhopper, *Amritodus brevistylus* are 181.83 ppm, 135.75 ppm, 40.89 ppm and 7.61 ppm for 12h, 24h, 36h and 48h respectively.

Silver nitrate has long been considered as a powerful and natural antibiotic and antifungal agent. The antifungal activity of the synthesized silver particles has been investigated against *Aspergillus niger.* Silver nanoparticles synthesized using *Raphanus sativus* showed very strong inhibitory zone (Table 3 and Figure 7) against *Aspergillus niger* (80 mm/120 µL).

Discussion

Environment friendly, cost effective and target specific nanoparticles can been obtained/synthesized using environmentally acceptable, wide spread and eco-friendly reducing as well as capping agents (Marimuthu *et al*., 2011). The plant mediated silver nanoparticles are nontoxic and environmentally acceptable pesticides as well as antimicrobial agents which disperse uniformly in water and highly stable without any side effects (Salunkhe *et al*., 2011). Biologically synthesized silver nanoparticles as a broad range of mechanism of action on targets species, which makes a complicated process for insects and microbes to develop resistance.

In this study, the formation of silver nanoparticles reduced by active properties of *Raphanus sativus* peel extract was investigated. The unaggregated silver nanoparticles appeared yellow color in the reaction vessel and the intensity was increased in direct proportion to the reaction time which was due to the excitation of a surface plasmon resonance effect and a reduction of AgNO3. Further the intensity declined after 120 h stating the saturation at the particular reaction period. The high intensity peaks are due to the decrease in particle size which increase the energy gap (Nareshkumar *et al*., 2012). Veerasamy *et al*. (2011) observed absorbance peak at 438 nm after heating the solution at 75°C for 60 min. In concordance to the present study Madhiyazhagan *et al*. (2015) reported that the final absorption intensities at 420 nm were 0.4 a.u. after 15 min, 0.4 a.u. after 30 min, 1.2 a.u. after 60 min, and 2.7 a.u. after 120 min.

X-ray diffraction spectrum recorded in the present study was compared with the standard spectrum of Ag particles conformed that they are in form of nanocrystals, as evidenced by the peaks at 2θ values. XRD results also suggest that crystallization of the bioorganic phase occurs on the surface of the AgNPs. XRD patterns of vacuumdried AgNPs synthesized using the leaf extract of *O. canum* reported by Jayaseelan & Rahuman (2012) was in concordance to the present study. Similar result was also reported by Borchert *et al*. (2005) showing intense peaks of 2θ values at 38.4°, 44.4°, and 64.2° corresponding to (111), (200), and (211) Bragg's reflection conforming the face centered cubic structure of silver

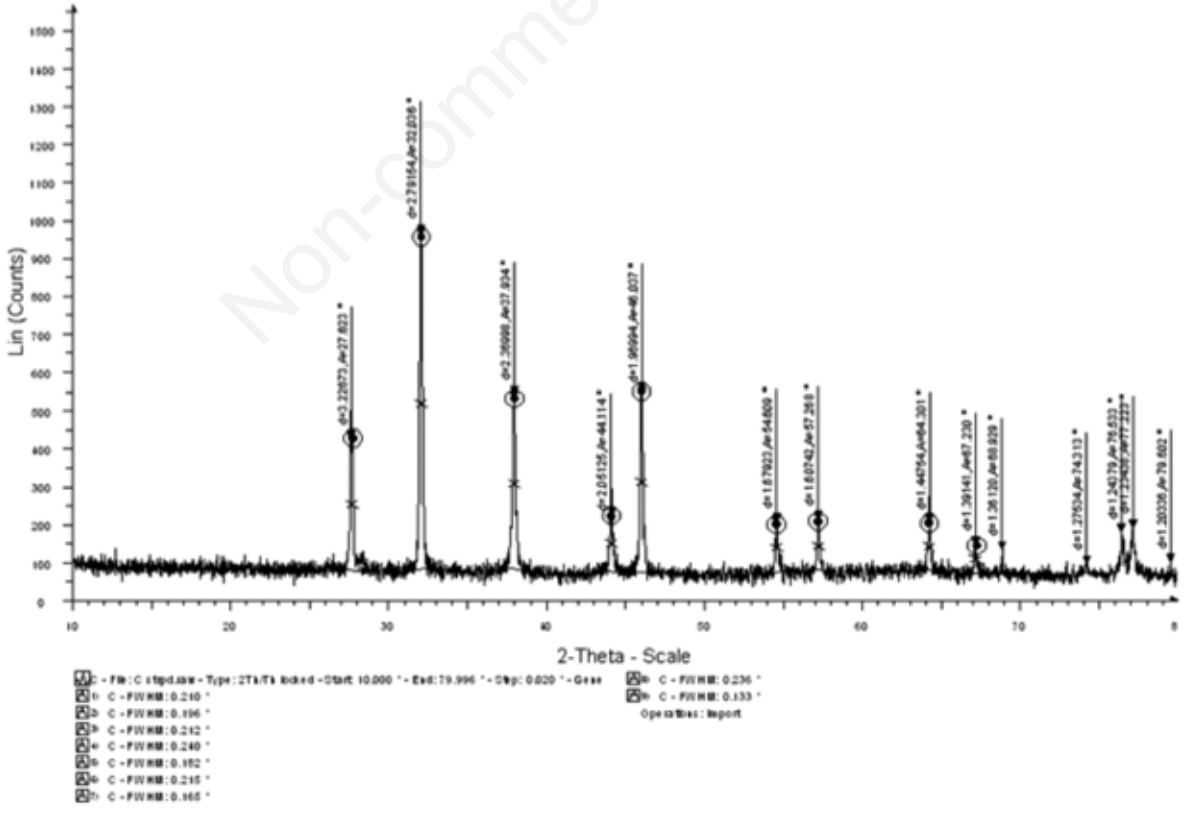

Figure 2. X-ray diffraction pattern of silver nanoparticles synthesized using *Raphanus sativus* peel extract.

nanoparticles. The Fourier transform infrared spectroscopy is used to identify the chemical composition of the surface and capping agents for the biologically synthesized of silver nanoparticles. The carbonyl group from amino acid residues and proteins has stronger ability to bind metal indicating that the proteins could possibly form metal nanoparticles (*i.e.*, capping of Ag NPs) to prevent agglomeration and thereby stabilize the medium, thus suggesting for dual role of formation and stabilization of AgNPs in aqueous medium by the biological molecules (Sundaravadivelan *et al*., 2013). Santhoshkumar *et al*. (2011) reported sharp and strong absorption band at 1.631 cm^{-1} assigned to the stretching vibration of (NH) C=O group.

Scanning Electron Microscopic analyses of the synthesized AgNPs was irregular with a large spherical composition, poly-dispersed and having an average size of 35 nm with interparticle distance. An approximate matching range of particle size and shape to the silver nanoparticles of the present study were reported by few recent researchers (Marimuthu *et al*., 2012; Subarani *et al*., 2013). The Energy-dispersive X-ray spectroscopy analysis provided information on the chemical constituents and composition of the final yield. In addition to silver other organic compounds were recorded indicating the presence of active secondary metabolites, which is naturally occur in the peel extract of *Raphanus sativus* thus the analysis confirms the bioreduction of silver which is also supported by biosynthesis of metal nanoparticles by Kamaraj *et al*. (2012).

Problems due to insect pest in agriculture were increased in recent years due to ecosystem and technological changes. Direct and indirect damage caused by the insect pests are the main reason for destroying one fifth of the world's total crop production annually. Uses of chemicals are not appreciated due to the concerns increased with respect to public health and environmental security, hence, requiring detection of natural products that may be used against insect pests (Amer & Mehlhorn, 2006). In concern, use of biologically synthesized nanomaterial products in various sectors of science including health increased during the last decade. In the present study the biomediated silver nanoparticles showed significant effect on the adult mango leafhopper, *Amritodus brevistylus* which may be due to the penetration of nanoparticles to gut system through food materials and interaction with the gut epithelial cell. Adulticidal and larvicidal activities of plant extracts (LC50-250

Table 2. Insecticidal activity of silver nanoparticles on the mango leafhopper, *Amritodus brevistylus*.

No.	Hrs	Mortality (mean±SE)					LC_{50} (LL/UL)	LC_{90} (LL/UL)	χ^2 value
		5 ppm	10 ppm	20 ppm	50 ppm	100 ppm			
1.	12	1.5±0.28^{a}	2.0±0.40^{b}	2.3±0.25^{bc}	2.8±0.25^{c}	3.3±0.25^{d}	181.83 (122.11/468.14)	436.02 (276.5/1228.6)	1.57*
2	24	2.8±0.25^{a}	3.0±0.40^{ab}	3.5±0.28^{b}	4.0±0.00^{c}	4.3±0.25^{cd}	135.75 (86.94/555.02)	461.26 (270.82/2258.8)	1.32*
3	36	3.5±0.28^{a}	4.0±0.57^{b}	4.5±0.28^{c}	5.5±0.28^{d}	6.7±0.25^{e}	40.894 (27.30/56.03)	191.84 (147.53/288.78)	0.78*
4	48	4.0±0.40^{a}	5.5±0.28^{b}	6.0±0.57^{c}	7.0±0.40^{d}	8.3±0.25^{e}	7.609 (3.645/19.016)	125.3 (100.89/171.30)	5. 11*

Means±standard deviation (SD) followed by same letter within rows indicate no significant difference (Duncan's multiple range test, P<0.05). LC50, LC90, lethal concentration; LCL, lower confidence limits; UCL, upper confidence limits. *Significant at P<0.001 (heterogeneity factor used in calculation of confidence limits).

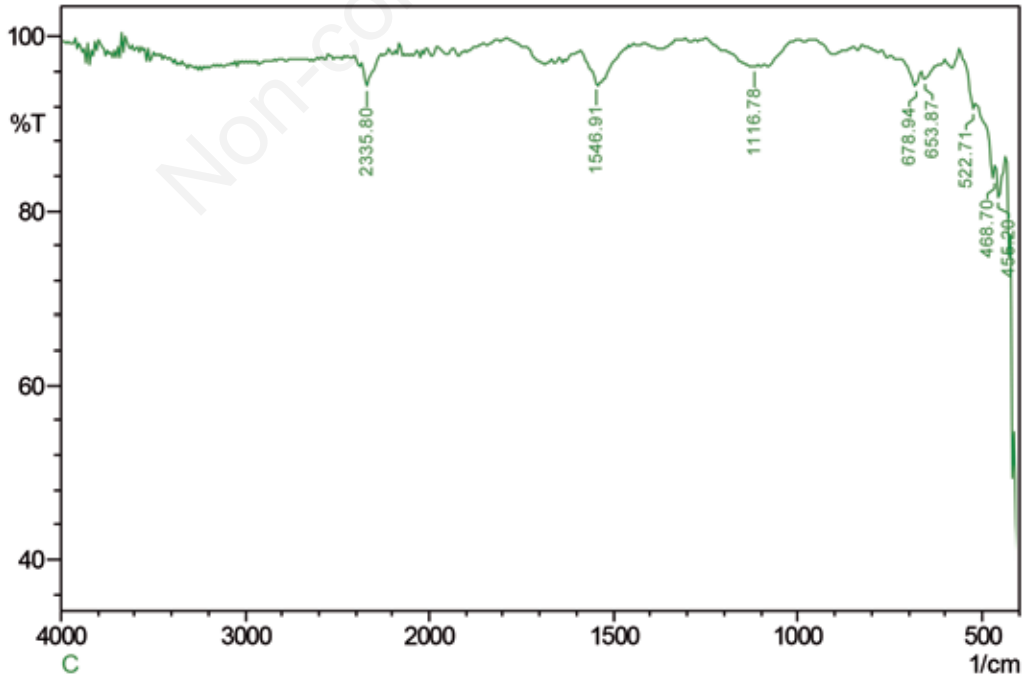

Figure 3. Fourier Transform infrared spectrum of silver nanoparticles synthesized using *Raphanus sativus* peel extract.

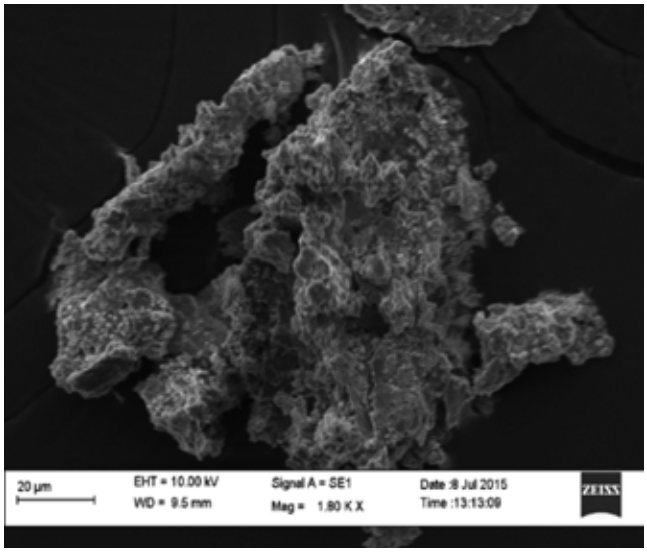

Figure 4. Scanning Electron Microscope image of silver nanoparticles (20 µm view) synthesized using *Raphanus sativus* peel extract.

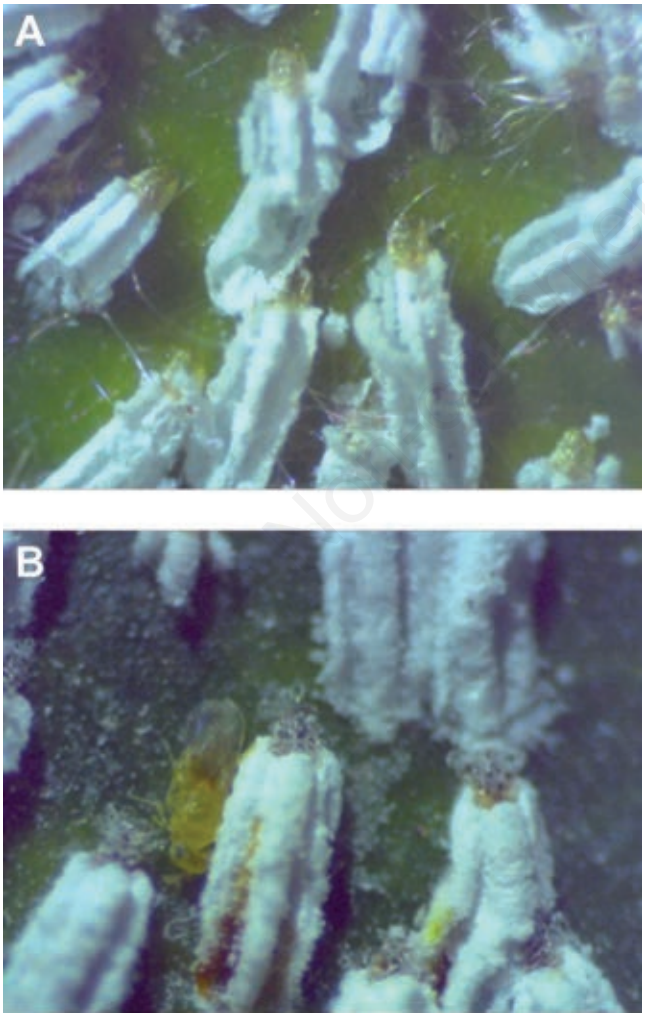

Figure 5. The hatching process of mango leaf hopper, *Amritodus brevistylis*.

ppm) are very low (Panneerselvam & Murugan, 2013) when compared to that of Nanoparticles (LC_{50}=16.72 and 3.44 mg/L) synthesized using plant materials (Rajakumar & Abdulrahuman, 2012). The present study was also in concordance to the earlier with maximum effect at low concentration (LC50=7.61 ppm). Nanomaterials are beneficialto human health, energy, defense, catalysis, agriculture and environment (Barik *et al*., 2012).

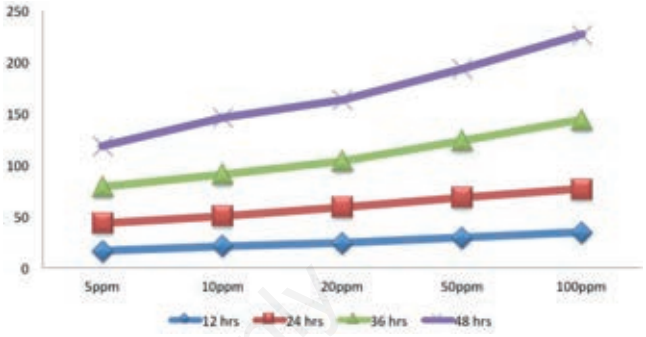

Figure 6. Insecticidal activity of silver nanoparticles on the mango leafhopper, *Amritodus brevistylus*.

Table 3. Inhibitory activity of silver nanoparticle against *Aspergillus niger*.

Organism	Zone of inhibition (mm)			
Concentration (mg/mL)	Control	50 µL	100 µL	250 µL
Aspergillus niger	30 mm	50 mm	60 mm	80 mm

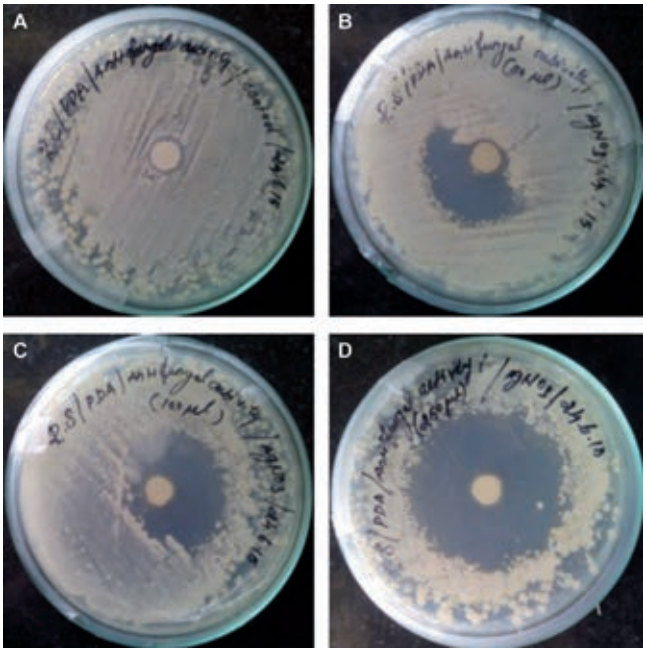

Figure 7. Antifunal activity of silver nanoparticle at different concentrations (50 µL, 100 µL and 250 µL) against *Aspergillus niger*.

The most important problem caused by the chemical antimicrobial agents is multidrug resistance. The silver nanoparticles possess good antimicrobial property due to the Surface Plasmon Resonance and extremely large surface area, which provides better contact with microorganisms. Thus, nanotechnology has become one of the most promising new approaches for the control of insect pests and pathogenic microbes in the recent years (Nareshkumar *et al*., 2014). The antifungal activity of plant mediated silver nanoparticles clearly reveals that the growth of *Aspergillus niger* was inhibited at different concentration of silver nanoparticles. Soni & Prakash (2015) reported the antifungal activity of bacteria mediated silver nanoparticles against the entomopathogenic fungus *Chrysosporium keratinophilum.*

The current investigation showed that the biologically synthesized silver nanoparticles with low toxicity to environment and a wide range of antifungal activity are very effective for reducing plant diseases caused by spore producing phytopathogenic fungi in an eco-friendly manner. The present study also suggested that silver nanoparticles was more effective for the control of mango leafhopper, *Amritodus brevistylus* and for the plant pathogenic fungi *Aspergillus niger* thus, silver nanoparticles prepared by a cost effective method has great promise as a pesticide and antimicrobial agent.

Conclusions

From the present study we conform that the peel extracts of *Raphanus sativus* are a good reducing and capping agent in nanoparticle preparation. Silver nanoparticles synthesized using the peel extract of *Raphanus sativus* were stable for more than a month. Further studies on identification of active compounds are need to find out the key compound involved in actual reduction and capping process which ensures the mono dispersion of nanoparticles. Further, the green synthesized sliver nanoparticles are much effective against the insect pest, *Amritodus brevistylus* and plant pathogenic fungi, *Aspergillus niger*.

References

ABBOTT W.S., 1925 - A method of computing the effectiveness of insecticides. - J. Econ. Entomol. 18: 265-267.

ABDOU I.A., 1972 - Antimicrobial activities of *Allium sativum, Allium cepa, Raphanussativus, Capsicum frutescens, Eruca sativa, Allium kurrat*on bacteria. - Qualitas Plantarum Plant. Foods. Hum. Nutr. 29-35.

AMER A., MEHLHORN H., 2006 - Larvicidal effects of various essential oils against *Aedes, Anopheles,* and *Culex* larvae (Diptera, Culicidae). - Parasitol. Res. 99: 466-472.

BARIK T.K., KAMARAJU R., GOWSWAMI A., 2012 - Silica nanoparticle: a potential new insecticide for mosquito vector control. - Parasitol. Res. 111: 1075-1083.

BEEVI S.S., MANGAMOORI L.N., ANABROLU N., 2009 - Comparative activity against pathogenic bacteria of the root, stem, and leaf of *Raphanussativus* grown in India. - World J. Microb. Biot. 25: 465-473.

BENEVIDES S.D., RAMOS A.M., STRINGHETA P.C., CASTRO V.C., 2008 - Quality of the fruits and pulp of Ubamango. - Cienc. Tecnol. Aliment. 28: 571-578.

BORCHERT H., SHEVCHENKO E.V., ROBERT A., MEKIS I., KORNOWSKI A., GRÜBEL G., WELLER H., 2005 - Determination of nanocrystal sizes: a comparison of TEM, SAXS, and XRD studies of highly monodisperse CoPt3 particles. - Langmuir. 21: 1931-1936.

FLETCHER J., BENDER C., BUDOWLE B., COBB W.T., GOLD S.E., ISHIMARU C.A., LUSTER D., MELCHER U., MURCH R., SCHERM H., SEEM R.C., SHERWOOD J.L., SOBRAL B.W., TOLIN S.A., 2006 - Plant pathogen forensics: capabilities, needs, and recommendations. - Microbiol. Mol. Biol. Rev. 70: 450-471.

GNANESWARAN R., HEMACHANDRA K.S., VRAKAMATH C.A., AHANGAMA D., WIJAYAGUNASEKARA H.N.P., WAHUNDENIYA I., 2007 - *Idioscopus nagpurensis* (Pruthi) (Hemiptera: Cicadellidae: Idiocerinae): a new member of mango leafhopper complex in Sri Lanka. - Trop. Agricult. Res. 19: 78-90.

GUNDAPPA P.D., JAYANTHI K., VERGHESE A., 2014 - Migratory behaviour of mango hoppers, *Idioscopus*sp. in relation to host plant flowering phenology a synchronous shift. - Bioscan. 9: 639-641.

JAGESSAR R.C., MARS A., GOMES G., 2008 - Selective Antimicrobial properties of *Phyllanthusacidus* leaf extract against *Candida albicans, Escherichia coli* and *Staphylococcus aureus* using stokes disc diffusion, well diffusion, streak plate and a dilution method. - Nat. Sci. 6: 24-38.

JAYASEELAN C., RAHUMAN A.A., 2012 - Acaricidal efficacy of synthesized silver nanoparticles using aqueous leaf extract of *Ocimumcanum* against *Hyalommaanatolicumanatolicum* and *Hyalommamarginatumisaaci* (Acari: Ixodidae). - Parasitol. Res. 111: 1369-1378.

JIA Y., 2009 - A user-friendly method to isolate and single spore the fungi *Magnaporthe oryzae* and *Magnaporthe grisea* obtained from diseased field samples. - Plant Health Progr. doi:10.1094/PHP-2009-1215-01-BR.

KAMARAJ C., RAJAKUMAR G., RAHUMAN A.A., VELAYUTHAM K., BAGAVAN A., ZAHIR A.A., ELANGO G., 2012 - Feeding deterrent activity of synthesized silver nanoparticles using *Manilkarazapota* leaf extract against the house fly, *Muscadomestica* (Diptera: Muscidae). - Parasitol. Res. 111: 2439-2448.

KOSZEGI T., POOR M., 2016 - Ochratoxin A: molecular interactions, mechanisms of toxicity and prevention at the molecular level. - Toxins. 8: 111-136

MADHIYAZHAGAN P., MURUGAN K., NARESHKUMAR A., NATARAJ T., DINESH D., PANNEERSELVAM C., SUBRAMANIAM J., MAHESHKUMAR P., SURESH U., RONI M., NICOLETTI M., ALARFAJ A.A., HIGUCHI A., MUNUSAMY M.A., BENELLI G., 2015 - *Sargassummuticum* synthesized silver nanoparticles an effective control tool against mosquito vectors and bacterial pathogens. - Parasitol. Res. 114: 4305-4317.

MARIMUTHU S., RAHUMAN A.A., RAJAKUMAR G., SANTHOSHKUMAR T., KIRTHI A.V., JAYASEELAN C., BAGAVAN A., ZAHIR A.A., ELANGO G., KAMARAJ C., 2011 - Evaluation of green synthesized silver nanoparticles against parasites. - Parasitol. Res. 108: 1541-1549.

MARIMUTHU S., RAHUMAN A.A., SANTHOSHKUMAR T., JAYASEELAN C., KIRTHI A.V., BAGAVAN A., KAMARAJ C., ELANGO G., ZAHIR A.A., RAJAKUMAR G., VELAYUTHAM K., 2012 - Lousicidalactivity of synthesized silver nanoparticles using *Lawsoniainermis* leaf aqueous extract against *Pediculushumanuscapitis* and *Bovicolaovis*. - Parasitol. Res. 111: 2023-2033.

NARESHKUMAR A., JEYALALITHA T., MURUGAN K., MADHIYAZHAGAN P., 2013 - Bioefficacy of plant mediated

gold nanoparticles and *Anthocepholuscadamba* on filarial vector, *Culexquinquefasciatus* (Insecta: Diptera: Culicidae). - Parasitol. Res. 112: 1053-1063.

NARESHKUMAR A., MURUGAN K., MADHIYAZHAGAN P., NATARAJ T., SHOBANA K., 2014 - Impact of climate change on filarial vector, *Culexquinquefasciatus* and control using bacterial pesticide, spinosad. - Asian Pac. J. Trop. Dis. 4: 87-91.

NARESHKUMAR A., MURUGAN K., REJEETH C., MADHIYAZHAGAN P., BARNARD D.R., 2012 - Green synthesis of silver nanoparticles for the control of mosquito vectors of malaria, filariasis, and dengue. - Vector Borne Zoon. Dis. 12: 262-268.

NEGAHBAN M., MOHARRAMIPOUR S., 2007 - Fumigant toxicity of *Eucalyptus intertexta, Eucalyptus sargentii* and *Eucalyptus camaldulensis* against stored product beetles. - J. Appl. Entomol. 131: 256- 261.

PANACEK A., KOLAR M., VECEROVA R., PRUCEK R., SOUKUPOVA J., KRYSTOF V., HAMAL P., ZBORIL R., KVITEK L., 2009 - Antifungal activity of silver nanoparticles against *Candida* sp. - Biomaterials 30: 6333-6340.

PANNEERSELVAM C., MURUGAN K., 2013 - Adulticidal, repellent, and ovicidal properties of indigenous plant extracts against the malarial vector, *Anopheles stephensi* (Diptera: Culicidae). - Parasitol. Res. 112: 679-692.

PULIT-PROCIAK M., 2016 - Silver nanoparticles - a material of the future…?. - Open Chem. 14: 76-91.

RAJAKUMAR G., ABDULRAHUMAN A., 2012 - Acaricidal activity of aqueous extract and synthesized silver nanoparticles from *Manilkarazapota* against *Rhipicephalus* (*Boophilus*) *microplus*. - Res. Vet. Sci. 93: 303-309.

ROGERS J.V., PARKINSON C.V., CHOI Y.W., SPESHOCK J.L., HUSSAIN S.M., 2008 - A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. - Nanoscale Res. Lett. 3: 129-133.

RONALD M.A., 1990 - Microbiologia. Compania Editorial Continendal S.A. de C.V., Mexico: p.505.

RONI M., MURUGAN K., PANNEERSELVAM C., SUBRAMANIAM J., HWANG J.S., 2013 - Evaluation of leaf aqueous extract and synthesized silver nanoparticles using *Nerium oleander* against *Anopheles stephensi* (Diptera: Culicidae). - Parasitol. Res. 112: 981-990.

SAHAB A.F., WALY A.I., SABBOUR M.M., NAWAR L.S., 2015 - Synthesis, antifungal and insecticidal potential of Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles against some seed borne fungi and insects of soybean. - Int. J. Chem. Tech. Res. 8: 589-598.

SALUNKHE R.B., PATIL S.V., PATIL C.D., SALUNKE B.K., 2011 - Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedesaegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera; Culicidae). - Parasitol. Res. 109: 823-831.

SANTHOSHKUMAR T., RAHUMAN A. A., RAJAKUMAR G., MARIMUTHU S., BAGAVAN A., JAYASEELAN C., ZAHIR A.A., ELANGO G., KAMARAJ C., 2011 - Synthesis of silver nanoparticles using *Nelumbonucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. - Parasitol. Res. 108: 693-702.

SAUCO V.G., 2004 - Mango production and world market: current situation and future prospects. - Acta Hort. 645: 107-116.

SCHUSTER E., DUNNCOLEMAN N., FRISVAD J.C., VAN DIJCK P.W.M., 2002 - On the safety of *Aspergillus niger*: a review. - Appl. Microbiol. Biotechnol. 59: 426-435.

SHARMA B., SHARMA S., 2011 - Seasonal abundance of mango hopper, *Amritodusatkinsoni*(Leth.) (Jassidae:Homoptera) in different environmental condition of Jammu region. - J. Env. Bio. Sci. 25: 81-83.

SHOBANA K., RAJALAKSHMI K., 2010 - Quantitative analysis of primary metabolites in *Mangifera indica* (unripe mango). - Rasayan J. Chem. 3: 597-599.

SONI N., PRAKASH S., 2015 - Antimicrobial and mosquitocidal activity of microbial synthesized silver nanoparticles. - Parasitol. Res. 114: 1023-30.

SUBARANI S., SABHANAYAKAM S., KAMARAJ C., 2013 - Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against *Anopheles stephensi* Liston and *Culexquinquefasciatus* Say (Diptera: Culicidae). - Parasitol. Res. 112: 487-499.

SUGANYA G., KARTHI S., SHIVAKUMAR M.S., 2014 - Larvicidal potential of silver nanoparticles synthesized from *Leucasaspera* leaf extracts against dengue vector *Aedesaegypti*. - Parasitol. Res. 113: 875-880.

SUNDARAVADIVELAN C., NALINIPADMANABHAN M., NALINIPADMANABHAN M., SIVAPRASATH P., KISHMU L., 2013 - Biosynthesized silver nanoparticles from *Pedilanthustithymaloides* leaf extract with anti-developmental activity against larval instars of *Aedesaegypti* L. (Diptera; Culicidae). - Parasitol Res. 112: 303-311.

SURESH U., MURUGAN K., BENELLI G., NICOLETTI M., BARNARD D.R., PANNEERSELVAM C., MAHESHKUMAR P., SUBRAMANIAM J., DINESH D., CHANDRAMOHAN B., 2015 - Tackling the growing threat of dengue: *Phyllanthusniruri* mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedesaegypti* (Diptera: Culicidae). - Parasitol. Res. 114: 1551-1562.

VEERAKUMAR K., GOVINDARAJAN M., RAJESWARY M., 2013 - Green synthesis of silver nanoparticles using *Sidaacuta* (Malvaceae) leaf extract against *Culexquinquefasciatus, Anopheles stephensi,* and *Aedesaegypti* (Diptera: Culicidae). - Parasitol. Res. 112: 4073-4085.

VEERASAMY R., XIN T.Z., GUNASAGARAN S., XIANG T.F.W., YANG E.F.C., JEYAKUMAR N., DHANARAJ S.A., 2011 - Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. - J. Saudi Chem. Soc. 15: 113-120.

VELAYUTHAM K., RAHUMAN A.A., RAJAKUMAR G., SANTHOSHKUMAR T., MARIMUTHU S., JAYASEELAN C., BAGAVAN A., KIRTHI A.V., KAMARAJ C., ZAHIR A.A., ELANGO G., 2012 - Evaluation of *Catharanthusroseus* leaf extract mediated biosynthesis of titanium dioxide nanoparticles against *Hippoboscamaculata* and *Bovicolaovis*. - Parasitol. Res. 111: 2329-2337.

VERGHESE A., JAYANTHI K.P.D., 2001 - Integrated pest management in fruits. - In Pest Management in Horticultural Ecosystems. Capital Publishing Company, New Delhi, India. p. 1-21.

YEUNG H.C., 1985 - Handbook of Chinese Herbs and Formulas, Institute of Chinese Medicine, Los Angeles.

YILDIUM Z., JOHNSON M.G., 1998 - Detection and characterization of a bacteriocin produced by *Lactococcuslactis* subsp. isolated from radish. - Lett. Appl. Microbiol. 26: 297-304.