A REVIEW ON FUNGUS MEDIATED NANOPARTICLES IN THE CONTROL OF DENGUE VECTOR AEDES AEGYPTI.

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Abstract - Mosquitoes are the prominent vectors of human diseases viz, malaria, yellow fever, dengue, filariasis and encephalitis, *Aedes* mosquitoes on the other hand are also painful and persistent biters *Aedes aegypti* is responsible for spreading dengue.Hence the control of Aedes aegypti is essential to check the spread of the deadly disease,dengue. A promising approach to achieve this objective is to exploit the array of biological resource in nature. Indeed, over the past several years plants, algae, fungi, bacteria and viruses have been used for production of low-cost, energy- efficient and nontoxic metallic nanoparticles.

Key words: Fungus, Nanoparticles, Aedes aegypti, larvicidal activity.

1. INTRODUCTION:

Mosquitoes which are responsible for the transmission of more diseases than any other groups of arthropods play an important role as vectors of malaria, filariasis, dengue, yellow fever, Japanese, encephalitis and other viral diseases. The mosquito *Aedes aegypti* transmits dengue and is also responsible for the transmission of other diseases,such as yellow fever, chikun gunya fever (WHO, 2015).The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people over 40% of the world's population are now at risk from dengue. WHO currently estimates there may be 50- 100 million dengue infections worldwide every year (WHO. 2012). The problem has a complex face and it has to be handled carefully. It is essential to control mosquito population so that people can be protected from mosquito borne diseases. These diseases can be controlled by targeting the causative parasites and pathogens. It is easier to control vectors than parasites. The chemical control was the most widely used conventional methods for mosquito control since chemical pesticides are relatively in expensive usually produces immediate control. Generally, the chemical control is carried out by the indoor residual spraying of insecticides such as the dichloro diphenyl trichloro ethanol, hexa chloro cyclo hexane, benzene hexa chloride, malathion and synthetic pyrothroid. But the development of resistance against these chemicals in various mosquito populations has been reported. Fungal metabolities have the greatest potential in intelligently designed and carefully applied in mosquito management programes,Expanded use of microbial larvicidal will depend heavily on the balance between production costs and ecological consideration. Fungal metabolites could be alternative source for mosquito larvicides because they constitute a potential source of bioactive compounds and generally free from harmful effects. Use of fungal metabolites in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. (Neetu Vyas*, et al.,2015).*

Nano particles (NP) are usually clusters of atoms in the size range of 1-100 nm. It is understood that the properties of a metal NP are determined by its size, shape, composition, crystallinity, and structure (Sun and Xia, 2002). In recent times, nanotechnology is being considered as an impressive technology through which various Nano systems with improved size, distribution and morphology have been developed for advanced biomedical applications especially nanostructures of noble metals, such as silver, platinum, gold and palladium have received huge attention due to their specific physical, chemical, optical, electronic, magnetic, and mechanical properties (Zhaos and Gorte 2004). Generally, materials at nanoscales level have been fabricated routinely either "top-down or "bottom-up". Most of the physical and chemical methods of nanoparticles synthesis were too expensive and involved with the utilization at toxic chemicals which is hazardous to the environment and its associated life forms. Now a new innovative route using different biologicals entities such as plants, microbes, yeast and actinomycetes have been developed as an alternative eco-friendly approach (Devi and Joshi 2014).Fungi and Fungus derived products have attracted many insect pathologists as they are highly toxic to mosquitoes, yet have low toxicity to non-target organisms and are also biodegradable.Fungi blonging to class Ascomycetes are known to produce many bioactive compounds with pharmaceutical and agriculturally importance. (Pranay Kumar, *et al.,2016).*

2. Metallic Nanoparticles

As an important metal, silver nanoparticles (AgNPs) have a number of application from electronics and catalysis to infections prevention and medical diagnosis. For example, AgNPs could be used as substrates for surface molecules and also use full catalysts for the oxidation of methanol to formaldehyde. AgNPs has been known as excellent antimicrobial and anti-inflammatory agents. a number of physical and chemical strategies were employed for the synthesis of AgNPs however, concern has been raised on the toxicity of chemical agents used in AgNPs synthesis, thus, it is essential to develop a green approach for AgNPs production without using hazardous substances to the human health and environment, (Guangguan et al., 2012). Among inorganic, silver nanoparticles (Ag-NPs or Nano silver), due to its novel chemical, physical and biological properties as compared to their bulk form, have attracted the attention of researchers from various academic laboratories, (Sharma et al.,2009). Ag-NPs have distinctive physical and chemical properties, for example, high thermal and electrical conductivity, surface enhanced Raman scattering, chemical stability, catalytic activity, and nonlinear optical behaviour, these properties take Ag-NPs to the top of the property list to be used in links in electronics, and medical purpose, (Krutyakov et al.,2008). Recently, the number of publications on the topic of Ag-NPs has increased rapidly and an increase of 93% in the number of published articles has been observed since 2001-2011, During this period,247 articles were published in 2001, which in 2011 increased to 3603 articles, using conventional methods for the synthesis of Ag-NPs requires, Ag precursors, reducing agent, and stabilizers /capping agent. In biological methods Ag-NPs are synthesized using plants (such as algae, yeast, fungi, and bacteria) as reducing and stabilizing agents. (Sintubin et al.,2018).

Highly stable zinc nanoparticles were synthesized extracellularly in the size range of 50-120 nm. This indicates that the metal in the media is in sulphate form the sulphate reductases are released extracellularly and reduce the compounds to sulphide but if the metal in the media is in an acetate form the enzymes in the cell wall reduce them to metal nanoparticle. Intracellular synthesis of nanoscale Pbs crystallites by Torulopsis species when exposed to aqueous Pb ions was reported by (Kowshik et al.,2002) The biogenic process in Aspergillus species open up vistas for better management of bioremediation, the biogenesis of lead nanoparticles using Aspergillus species (Pavani et al., 2012).

Biosynthesis of gold nanoparticles from fungi has been reviewed very recently. They are resistant to oxidation and dispersed nicely. The colour corresponds to the particle size in general. For instance, yellow, red and mauve refer to large, small, and fine nanoparticles respectively of varying and morphology. It is claimed that gold nanoparticles can be stabilized by substances like ascorbic acid and citrate (Andreescu. et al., 2006). Stabilization can also be achieved by polyvinyl alcohol (Pimpang, et al 2011) Enzymes are said to be responsible for the biosynthesis of gold nanoparticles. The intra-or extracellular synthesis of nanoparticles by fungi is done in a simple manner. The gold ions are trapped by the proteins and enzymes on the surface of the fungi and get reduced. They further form aggregates of large dimension. (Sanghi, et al., 2011) The gold nanoparticles synthesized from various sources. have different properties. They have been checked for their cytotoxic effects against cancer (Mishra., et al., 2011). Both the intracellular and extracellular reduction of Aucl or $Aucl_3$ follow the same pathway since $Aucl_3$ requires one electron to give gold nanoparticles, it follows one-step reduction whereas Au cl_3 requires three electron and reductions occur in three steps. (Das, et al.,2012) (Narayanan and Sakthivel 2011) have demonstrated the formation of gold nanoparticles in the presence of fungus Cyclindro Cladium floridanum. (Mukherjee et al.,2001) have reported the formation of gold nanoparticles from Verticillium sp . Which found on the surface of mycelia. Gold nanoparticles have also been produced from Verticillium fungi. Soni and Prakash have reported the green synthesis of gold nanoparticles from Aspergillus niger and identified it by a change in colour and its absorption at 530 nm. They have also suggested that broadening of the band is due to the aggregation of gold nanoparticles, they have also reported that the Au nanoparticles are toxic to Anophelesste Phensi, Culex quinque-fasciatus, and Aedes aegypti mosquito larva.

The production of Copper nanoparticles by microorganisms e.g., bacteria, fungi, and algae) is relatively a novel approach. There is a wide variation in the production of metallic nanoparticles by living cells e.g., organelles and compounds responsible for production, shape and size of nanoparticles, which depends on the mechanisms of metal ions bio reduction (Singh,2015) Microorganisms act as a bio factory and can also be synthesized intracellularly or extracellularly which have been used (Fusarium oxysporium) to synthesize copper nanoparticles (93-115nm) at ambient temperature (Majumber 2012). Pavani et al., used Aspergillus species of fungus for extracellular synthesis of Cu nanoparticles. (Ramanathan et al., 2011) used a biological method to synthesize copper nanoparticles using Morganella bacteria and under aqueous physiological conditions. Copper as a metal or oxides exhibit broad-spectrum biocidal activity, and several studies during the last two years found that copper demonstrates remarkable antibacterial activity at the nanoscale (Duranand and Seabra 2012). Copper is an essential element for living organisms and may be suitable for biomedical application (Rubilar 2013). An important aspect in copper nanotechnology is the production of nanostructures through eco-friendly and safe process. One of the process that fulfil these requirements is the biogenic synthesis of nanostructures (Jia et al.,2012). In this context, a limited number of studies have been published, and these evaluated different fungal strains for the biosynthesis of copper nanoparticles, Fungi, such as Penicillium sp. And Fusarium oxysporum strains, have been reported to biosynthesize Copper oxide and Cu_2S nanoparticles (Honary and Hosseini et al.,2012). The synthesis of Copper or Copper Oxide Nanoparticles can present different surface plasmon resonance, formed by the strong coupling between incident electromagnetic radiation and surface plasmons in metal nanoparticles (Noguez 2007). In the case of copper nanoparticles, the peak absorbance between 580 and 590 nm indicates formation of Cu nanoparticles (Soomro 2014). The use of Copper or Copper derivatives for the biosynthesis of nanoparticles using fungal strains has only been reported $CuSo_4$ Copper salts, and no comparative studies have been reported using other copper salts, such as $CuCl_2$ or Cu $(No_3)_2$, with other fungal strains Moreover, it is important to note that biosynthesis of metal nanoparticles using different fungal extracts is really clean and environmentally friendly (Duran 2010) and (Ahmad 2003) (Honary, et al 2012).

3. Fungi in nanoparticle synthesis; (Aspergillus sp)

Use of microorganisms in the green synthesis of metal nanoparticles with special reference to the precious metal using fungi has been done. Since fungi contain enzymes and proteins as reducing agents, they can be invariable used for the synthesis of metal nanoparticles from their salts. Since some fungi are pathogenic, one has to be cautious while working with them during experiment. Fungus) biomass normally grows faster than those of bacteria under the same conditions. Although synthesis of metal nanoparticles by bacteria is prevalent, their synthesis by fungi is more advantageous because their mycelia offer a large surface area for interaction, Also, the fungi secrete fairly large amount of protein than bacteria, therefore the conversion of metal salts to metal nanoparticles is very fast (Husen and Siddiqi 2014).The extracellular biosynthesis of silver nanoparticles using four Aspergillus species including A.fumigatus, A.clavatus,A.niger and A.flavus.In order to determine the probable role of nitrate reductase in the formation of silver nanoparticles, the quality and quantity of biosynthesized silver nanoparticles by Aspergillus species and their nitrate reductase activity (Kamiar Zomorodian,et al.,2016). (Mukherjee et al., 2001) studied the synthesis of intracellular AgNPs using the fungus Verticillium The authors observed that exposure of the fungal biomass to aqueous Ag^+ ions result in the intracellular reduction of the metal ions and formation of 25+ 12 nm Ag NPs.Electron microscopy analyses of thin sections of the fungal cells revealed that the Ag NPs were formed below the cell wall surface, possibly due to the reduction of the metal ions by the enzymes present in the cell wall membrane. The authors speculated that trapping of $AuCl_4$ ions on the surface of fungal cells could occur by electrostatic interaction with positively charged groups such as lysin residues in enzymes that existed in the mycelial cell wall, (Mukherjee, et al.,2008). In addition to primary metabolites many fungi produce low-molecular weight, often biologically active compounds known as secondary metabolites. Although these compounds may be chemically diverse, they are usually produced via common biosynthetic pathways, often related to morphological development. (Bhard, et al.,2006).

3.1. Aspergillus Niger

Aspergillus niger is a filamentous ascomycete fungus and group of saprophytic molds, Generally, A.niger can reproduce by means of conidia It can grow at 6-47 and Ph 1.4-9.8 It is an important industrial fungus used for producing citric acids, amylases, lipases, cellulases, xylanases, proteases production and for removal of heavy metal ions from waste waters(Khan et al.,2016) previously, metal nanoparticles synthesized using A.niger culture filtrate. Fungal filtrate contains enzymes and anthraquinone compounds are more responsible for reducing and capping processes. The synthesized metal nanoparticles showed better antibacterial and larvicidal activities, (Vigneshwaran et al2006). A.niger is filamentous Keratinophilic fungi with compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. This fungus secretes some reducing agents which convert silver nitrate into silver nanoparticles, therefore, it can be a useful green exercise to invent and discover new fungal Nano larvicide for respective ecological and environmental management system (Namita Soni and Soam prakas 2013). Fungi (A.niger) are well known to secrete large amounts of proteins enzymes, toxins, and other components that play a major role in their life cycle. The process of synthesis occurs in the presence of reductase enzymes, which may be present in the cell-free extracts of A.niger. These enzymes are supposed to reduce the silver ions to Ag NPs, However, the interaction between protein and nanoparticles, (Namita Soni & Soam Prakash 2012). The A.niger is the best producer of extracellular lipase(Demain and Fang 2000). A.niger purified fungal culture filtrates have enhanced their lethal effects against An.stephensi, Cx.quinquefasciatus, and Ae.aegypti, Moreover the presence of mycotoxin “ochratoxin” in A.niger can be fast acting metabolites for control of adult mosquitoes. Ideally, all the these new findings could be implemented with a time application with its fast acting impact against An.stephensi, Cx.quinquefasciatus, and Ae.aegypti population.(Gavendra sing and Soam Prakash,2012)

3.2. Aspergillus flavus:

A.flavus is a sporophyte and a haploid filamentous fungus. It is found all over the world, mainly at the warm places and is also abundant in areas with temperate climates during warm drought years. It grows at temperature of 25-42^{oC} and the optimum temperature for its growth is 37^{o}c. It is yellow-green mold with distinctive conidiophore composed of a long stalk supporting an inflated vesicle (Sweta Bhan et al 2015). The synthetic insecticide temephos and the fungus A.flavus act onto mosquito larvae in a different mode of action A.flavus with the most potent Phyto extract of Cuscuta reflexa against the larvae, An.Stephensi and Cx.quinquefasciatus, (Anderson, et al., 2013). (Bhan, et al., 2013). Nanoparticles can be synthesized extracellularlarly (or) intracellularly. The present study involves the mycosynthesis and characterization of silver nanoparticles from both cell free and mycelial extracts of filamentous fungi Aspergillus flavus (Manimozhi and Anitha 2014). The extracellular synthesis of stable silver nanoparticles using the fungus Aspergillus flavus has also reported, (Vigneshwaran et al., 2007). The Ag NPs was synthesised from Aspergillus flavus by green method and were characterized, Ag NPs were prepared using silver nitrate as silver precursor and Aspergillus flavus as reducing agent and stabilized, (Amin Bhat et al., 2014). Silver nanoparticle synthesis for A.flavus occurs initially by a 33kDa protein followed by a protein,(cysteine and free amine groups) electrostatic attraction which stabilizes the nanoparticle by forming capping agent, (Jain, et al., 2011).

3.3. Aspergillus fumigatous:

Synthesis of nanoparticles in higher amounts from fungal species shows beneficial aspects like environmentally friendly and amiability. Extra cellular enzymes are potentially and easily produced from fungi in large amounts. The main feasibility of using fungi in synthesis of nanoparticles include easy of handling and economically possible, For synthesis of nanoparticles filamentous fungi plays an important role silver ions are extracellularly reduced by filamentous fungi like A.fumigatous reports these species produced biosynthesis of nanoparticles rapidly is a good candidate for rapid biosynthesis of silver nanoparticles (Souza et al., 2004),(Saravanan and Nanda 2010). The filamentous fungus, A.fumigatous has shown potential for extracellular synthesis Ag-NPs, Synthesis of Ag-NPs using the cell free filtrate is rapid, this indicates nanoparticle synthesis from biological process is quick suitable for larger scale production (Ratnasri and Hemalatha 2014).Nitrate reductase activity and the efficiency of studied Aspergillus species in the production of silver nanoparticles A. fumigatous as the most efficient species, highest nitrate reductase activity, it produced greater amount of silver nanoparticles with smaller size and higher monodispersed in comparison with other species, (Kamiar et al 2016). The biosynthesis of Zinc oxide nanoparticles using Aspergillus fumigatous and characterization of synthesized nanoparticles, the reduction of aqueous Zinc sulphate solution with cell free filtrates of fungus A.fumigatus (Arya Rajan, et al., 2016). A majority of the filamentous fungi (e.g. Aspergillus fumigatous) that have reportedly been used for the purpose of extracellular biomass free synthesis of Ag NPs are pathogenic to plants and/or humans. This makes handling and disposal of the biomass a major inconvenience toward commercialization of the process, thus there is a need for developing a newer/novel approach of testing a non-pathogenic fungus for the successful synthesis and capping of nanosized silver particles, (Mukherjee, et al., 2011)

3.4. Aspergillus terrus:

Aspergillus terreus is a fungus, that is widespread throughout the world and found in warm arable soils and found more commonly in cultivated soil than the forest, it is rarely found in the acidic forest soils from the colder temperate zone, (Steinbach et al.,2004). A.terreus played an important roles as reducing agents and capping agents in the reaction fungi strain for synthesizing Ag NPs based on the biodiversity, More importantly, it could also facilitate the deeper understanding of molecular mechanism for Ag NPs biosynthesis, the biosynthesis of Ag NPs using Aspergillus terreus(.White et al.,2011).(Guangq Uan Li et al.,2012, Reported that silver nanoparticles (Ag NP) synthesized using a reduction of aqueous Ag ion with the culture supernatants of Aspergillus terrus. In organism NADH is a widespread reduced coenzyme involved in redox reaction and can be used as a reducing agent many enzymes in vivo. Thus NADH dependent reductase released by A.terrus might account for the synthesis of Ag NPs In the process, NADH acted as an electron carrier, and the silver ions obtained electrons from NADH via The NADH dependent reductase and then reduced to Ag These results indicated that NADH might be a key factor for the synthesis of Ag NPs by Aspergillus terrus (Guangquan Li et al.,2012).

4. Larvicidal Activity:

Fungi and fungus-derived products are highly toxic to mosquitoes yet have low toxicity to non-target organism. Accordingly, the use of fungi and their derived products may be a promising approach for biological control of mosquitoes (Kirscbaum,1985). Extracellular secondary metabolites from many fungi have been screened for larvicidal activity against mosquitoes (Vijayan & Balaraman,1991). The larvicidal potential of silver nanoparticles synthesized using fungus cochliobolus lunatus against two mosquitoes Aedes aegypt and Anopheles stephensi (Salunkhe et al., 2011). The efficacy of fungus-mediated silver and gold nanoparticles against Aedes aegypti larvae has been evaluated (Soni and Prakash 2012) The larvicidal activities of mycosynthesized silver nanoparticles against vectors : Ae.aegypti and An. Stephensi responsible for diseases of public health importance have been evaluated (Salunkhe et al 2011) .The silver and gold nanoparticles synthesized with C.tropicum have been tested as a larvicide against the mosquito larvae (Soni et al., 2012) The silver nanoparticles synthesized by using the fungi, have also been tested against adult mosquito (Soni et al 2012). The larvicidal activities of mycosynthesized silver nanoparticles against vectors: Ae.aegypti and An.stephensi responsible for diseases of public health importance have been evaluated (.Salunkhe et al., 2011). The silver and gold nanoparticles synthesized with C.tropicum have been tested as a larvicide against the mosquito larvae (Soni and Prakash 2012).The present communication describes the larvicidal and pupicidal effect of extracellular synthesized silver nanoparticles by using the soil fungi A.niger 2587 against the An.stephensi, Ae.aegypti and Cx,.quinguefasciatus mosquitoes. The fungal species like F.oxysporum Fusarium,sp is a keratinophilic fungus it is being used for the first time to evaluate the larvicidal and pupicidal effect of AgNps and AuNps against the larvae and pupae of An.tephensi,Cx.quinquefasciatus and Ae.aegypti larvae,(Namita Soni and Soam Prakash.,2013). The extracellular biosynthesis of silvernanoparticles (Ag NPs) by using a fungus named Trichoderma Reesei, (Vahabi et al.,2011). The larvicidal potential of silver nanoparticles synthesized using fungus Cochliobolus lunatus, against Ae.egypti and An.stephensi has already been tested,(Salunkhe et al.,2011).

The larvicidal effect of four fungal aflatoxins against fourth instar larvae of the mosquito culex quinguefasciatus (Culicidae; Diptera). The electronic microscopic studies revealed the association of two fungi aflatoxins extracted from A.terrus and A.niger effect on different parts, legs, antennae and whole body surface of fourth larvae of Cx.quinguefasciatus. (Hussaini and Hergian.2014). The extracellular secondary metabolities of Metarhizium anisopliae were found effective against the all larvae of Ae Aegypti and Cx-quinguefasciatus.(Vyas., et al,2015)

5. Conclusion

The biological synthesis of metallic nanoparticles has the potential to be utilized as a good, rapid, eco-friendly approach for the control of mosquito population. It is totally a new pathway but, can be effectively utilized for the efficient killing of mosquitoes. Therefore, biological control can thus provide an effective and environmental friendly approach, which can be used as an alternative to minimize the mosquito population. To understand the current research trends of nanoparticles in mosquito control, research papers on NPs synthesised using biological organism such as fungi, bacteria and plant extracts, fungi and bacteria were thoroughly analysed and discussed in terms of the type of Nanoparticles.

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