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Soil Bacteria and their Possible Role in Mosquito Control: A Short Review

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Abstract:

Mosquitoes play an important role in vectoring pathogenic organisms to man and animal which cause major diseases in their hosts. Additionally, mosquitoes constitute a severe nuisance problem for man and domestic animals in rural, suburban and urban areas of the world. Because of their health and economic importance, control measures are carried out in practically every part of the world to reduce mosquito population.. . Vector control is an essential and effective means for controlling transmission of vector born diseases especially in area where resistance in parasite to drug is growing. Many microbial agents such as bacillus species, actinobacterial species, and fungal species are widely used for control of mosquitoes breeding in a variety of habitats. These biolarvicides are highly effective against mosquito larvae at very low dose and completely safe to other non-target organisms, environment and wildlife and are suitable for community use. In this context, the present work is designed to isolate and identify various bacteria from different soil habitats in and around Udaipur region. It also aims to assess the toxic effect of isolated bacteria against different developmental stages of Anopheline mosquitoes.

Keyword: ***Bacillus sphaericus, Bacillus thuringiensis Plasmodium falciparum***

1.0 Introduction:

Malaria is a parasitic disease confined mostly to the tropical areas, caused by parasites of the genus Plasmodium and transmitted by mosquitoes of the genus Anopheles. Annually, nearly a million human deaths, mainly of children ≤5 years of age, are registered among 500 million cases of clinical malaria, whereas 2.37 billion people are estimated to be at risk of infection by ***P. falciparum***, the most virulent among Plasmodia (Guerra *et al*., 2008). In 2007, the Bill and Melinda Gates Foundation, rapidly endorsed by the World Health Organization (WHO) and the Roll Back Malaria association, claimed for malaria eradication as the primary goal to be prosecuted (Roberts and Enserink, 2007). In order to achieve such an ambitious objective, several strategies are being adopted, involving multidisciplinary areas such as treatment, chemoprevention, vaccine research, health system assessment and of note vector control (Greenwood, 2008; Khadjavi*et al*., 2010). Mosquitoes are the disease causing vectors within almost all tropical and subtropical countries are responsible for the transmission of pathogens causing some of the most life intimidating and incapacitating diseases of man, like malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis, etc. (Chandra *et al*., 2008) which has put 55% of the world's population at risk in 124 countries (Beatty *et al*., 2007) .There is no specific treatment for these vector borne diseases. According to a study conducted by the Indian Institute of Management in Ahmadabad, mosquito-borne diseases primarily malaria costs India alone a US$ 1.3 billion every year, 95% of that due to illness (WHO, 2009) . Around the world, the medical and economic burden caused by vector-borne diseases continues to grow as current control measures fail to manage. There is an urgent need to identify new control strategies that will remain effective, even in the face of growing insecticide and drug resistance (Achs and Malaney, 2002). Vector control strategies include chemical based control measures, non - chemical based control measures and biological control agents (Poopathi and Tyagi, 2006). Repetitive use of man-made insecticides for mosquito control disrupts natural biological control systems and lead to reappearance of mosquito populations. It also resulted in the development of resistance, detrimental effects on non-target organisms and human health problems and subsequently this initiated a search for alternative control measures (Das *et al.,* 2007; Zhang *et al.,* 2011).The use of biological control agents such as predatory fish, bacteria, protozoa, fungus and nematodes had shown promising results to control mosquito populations (Murugesan *et al.*, 2009). The development of new strategies, including naturally occurring larvicides to control mosquitoes, is important in order to counter the evolution of resistance in target populations and the possible effects on non target organisms (Cetin and Yanikoglu, 2006). Vector control is a necessary and effective means for scheming transmission of vector-borne diseases, especially in areas where resistance in parasite to drugs is growing. Unlike insecticides, bio-control agents are host specific, safer to the environment, find easy application in the field, are cost-effective in production, lack infectivity and pathogenicity in mammals including man and has little evidence of resistance development in target mosquito species.

Two bacterial agents such as the *Bacillus thuringiensis* and *Bacillus sphaericus* are being widely used for control of mosquitoes breeding in a variety of habitats (Geetha and Manonmani 2010).These bio-control agents are targeted against larval stages of mosquitoes as they are ingested and act as stomach poison. However, there are some recent reports indicating development of resistance in mosquitoes against microbial agents too (Mir *et al.*, 2011).These reports have prompted many workers to look for new microorganisms and their metabolites with mosquito control potential.
Soil is an excellent source for unknown microorganisms. Soil contains a variety of microorganisms included bacteria that can be found in any natural ecosystem. Few reports from the East Coast of India, suggests that soil is a major source of Actinomycetes (Sivakumar *et al.*, 2005; Vijayakumar *et al.*, 2007; Dhanasekaran *et al.*, 2008). Recently, the soil derived actinomycetes have become recognized as a source of novel antibiotic and anticancer agent with unusual structure and properties (Jensen *et al.*, 2005). It is well known fact that the actinobacteria are the potential source of antibiotics, which could profitably develop in the pharmaceutical industries and the best known example is the product of streptomyces. There is an increasing demand for the new type of antibiotic to control mosquito. The purpose of the present study is to explore the larvicidal activitiesof natural microbes isolated from soil in and around Udaipur of South Rajasthan (India) against the life threatened disease causing mosquito vector anophiline. In our study we have isolated different types of bacteria from various soil samples to isolate potent bacteria that will be effective against larvae of mosquitoes.

1.1 *Insecticides used for malaria vector control*

The most prominent classes of insecticides are organochlorines (OCs), organophosphates(OPs), carbamates (Cs), and pyrethroids (PYs). In general, they act by poisoning the nervous system of insects, which is fundamentally similar to that of mammals. A small amount of pesticide can be fatal for an insect, primarily because of its small size and high rate of metabolism. Such an amount is not fatal for humans, but it may still harm. Since the similarities between the nervous system structures make it nearly impossible to design insecticides affecting only insect pests, insecticides may affect non-pest insects, people, wildlife, and pets. Some insecticides harm water quality or affect organisms in other ways; for example, the insecticide carbaryl is listed as a carcinogen by the state of California. In insects DTT opens sodium ion channels in neurons, causing them to burn spontaneously. This effect leads to spasms and eventual death. For this reason, insects with certain mutations in their sodium channel gene are resistant to DDT and other similar insecticides. DDT resistance is also conferred by up-regulation of genes expressing cytochrome P450 in some insect species (Denholm*et al*., 2002).Moreover, based on the results of animal studies, DDT is suspected to cause cancer. By epidemiological studies it is worth demonstrated that DDT causes liver, pancreas and breast cancers(Eskenazi, 2009).At present, DDT resistance is thought to be triggered further by the use of synthetic PYs(Diabate*et al*., 2002). Indeed, DDT and PYs share a common target, thus facilitating the development of a cross-resistance mechanism. In addition, evidence of increased frequency of resistance genes due to IRS or ITN programs is quite alarming (Karunaratne and Hemingway 2001; Stump *et al*., 2004). PYs, the only class approved for use on ITNs (Zaim M *et al.,* 2000), are being increasingly deployed in IRS programmes in Africa and there has been a dramatic increase in reports of PY resistance in malaria vectors over the past decade (Santolamazza*et al*., 2008) moreover, PYs are also widely used in the control of agricultural pest's worldwide (Ranson*et al.,* 2011).Unfortunately, although these approaches are potentially promising, they remain a complex approach with a limited use (Coutinho-Abreu *et al*., 2010). The newer insecticides are designed to be more specificand less persistent in the environment (Toxipedia, 2011).

1.2 *Non-chemical alternatives for malaria vector control: Biological Control*

Biological control is a method of controlling pest and vector by using natural enemies – predators, parasites and pathogens i.e. reduction of pest population by natural enemies. Successful biological control reduces the density of the target species (Source reduction).Microbial biopesticides are more valuable as far as toxicity to non target organisms and human beings are concerned.Since most of the chemicals synthesized have proved lethal for non target organisms. There are many microorganisms such as fungi, actinomycetes, and bacteria which have proved their potential against many insect pests and vectors including mosquitoes. *Bacillus thuringiensis, Bacillus sphericus* are already in used as a good substitute for synthetic pesticides. Since last two decades they are very effective against anopheles, culex, and aedes species although *Bacillus sphericus* is very effective against culex. These bacterial biopesticides appears to persist in the environment for longer period especially in polluted water hence they can be good candidate for the long lasting control of mosquito population. +. Biological control is long lasting, inexpensive, and harmless to living organisms and the ecosystem; it neither eliminates the pathogen nor the disease, but brings them into natural balance (Ramanathan*et al.,* 2002). At present, microbial insecticides are the main component of the bio-pesticide industry (Shi, 2000). Most of the pesticidal micro-organisms, however, have been isolated from entomopathogens and the terrestrial environment (Leonard and Julius 2000).Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. Microorganisms are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents.

Table 1: Advantages of Mosquitocidal Bacteria and Synthetic Insecticides (Porter *et al*., 1993).

S.NO	Mosquitocidal Bacteria	Synthetic Insecticide
1	Safe for human and animals	Rapidly killing the mosquitoes
2	Safe to handle	Wide range of mosquito species controlled by these insecticides
3	Persistence in environment is less	Chemicals remain to be there in mosquito larval feeding zone
4	Resistance development is very slow process	Chemicals not rapidly inactivated by UV-light
5	Recycling potential is higher	Few synthetic chemicals eventually degrade to harmless products
6	Can able to withstand polluted water as well as high	Effective under varied environmental conditions
7	Toxicity level is very low	Easy to use
8	Less intensive toxicological testing is require	Long lasting effective against mosquito

Table 2: Bacteria used as insecticides

S.No.	Pathogen	Product name	Host range
1	*Bacillus thuringiensis* var. kurstaki (Bt)	Bactur®,Bactospeine®,Bioworm®,Caterpillar Killer®, Dipel®, Futura®, Javelin®, SOK-Bt®, Thuricide®, Topside®, Tribactur®, Worthy Attack®	Caterpillars (larvae of moths and butterflies)
2	*Bacillus thuringiensis* var. israelensis (Bt)	Aquabee®, Bactimos®, Gnatrol®, LarvX®, Mosquito Attack®, Skeetal®, Teknar®, Vectobac®	larvae of Aedes and Psorophora mosquitoes,black flies.
3	*Bacillus thuringiensis* var. tenebrinos	Foil®,M-One®,M-Track®,Novardo®,Trident®	larvae of Colorado potato beetle, elm leaf beetle adults
4	*Bacillus thuringiensis* var. aizawai	Certan®	Waxmoth caterpillars
5	*Bacillus popilliae* and *Bacillus lentimorbus*	Doom¨, Japidemic¨,® Milky Spore Disease, Grub Attack®	Larvae (grubs) of Japanese beetle
6	*Bacillus sphaericus*	Vectolex CG®,Vectolex WDG®	Larvae of Culex,Psorophora, and Culiseta mosquito

Table 3: Bacteria already reported as a mosquito larvicidal

S.No	Name of Microorganism	Effective against mosquito species	Effect on pupa/ larvae
1	*Bacillus thuringiensis*	Culex and Anopheles	Larvae
2	*Bacillus sphericus*	Culex and certain species of Anopheles	Larvae
3	*Bacillus alvei and Bacillus brevis*	*Culexfatigans, Anopheles stephensi, Aedesaegypti*	Larvae
4	*Bacillus circulans*	*Cx. quinquefasciatus* and *Anopheles gambiae* and 107 times more toxic to *Aedesaegypti*	Larvae
5	*Brevibacilluslaterosporus*	*Cx. Quinquefasciatus, Aedesaegypti*	Larvae
6	*Bacillus subtulis*	*Cx. Quinquefasciatus*	Larvae
7	*Clostridium bifermentans*	*Anopheles maculates*	Larvae
8	*Pseudomonas fluorescens*	*Anopheles stephensiliston, cx. Quinquefasciatus, Aedesaegypti*	Toxic to Larvae and pupa

1.3 *Bacterial infection in mosquito*

Although several bacteria are known to be pathogenic to mosquito larvae, relatively few of them occur naturally in an insectary setting. Infections in the rearing containers are often caused by the inadvertent introduction of bacteria from the skin of an insectary employee or through the addition of contaminated water or food. In Africa, *Enterobacteria*infections were seen in the haemolymph of insect larvae.In advanced stages of disease, black spores were visible inside the larvae and eventually the larvae displayed a “milky” coloration and swollen appearance.

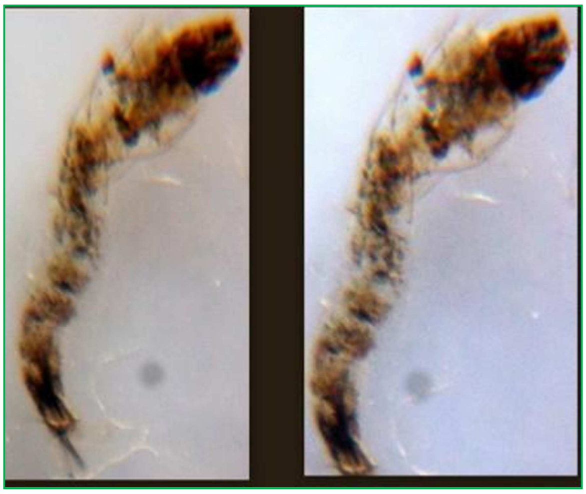

Fig 1 : Enterobacteria infection in mosquito larvae *Escherichia coli* common human fauna *E. coli* bacteria have been found to be pathogenic to early instar *Culex*mosquitoes(Jenkins, 1964).

Serratiamarcescens commonly found growing in standing water. This bacterium has not been reported to be lethal, but in poor rearing conditions it grows quickly forming a reddish film on the bottom of the rearing pans.

Pseudomonas fluorescens ubiquitous flagellated bacterium. *P. fluorescens*is a commonly isolated bacterium from soil and water sourcesand it has been shown to be lethal to mosquito larvae. Pseudomonas is known to cause extensive larval mortality due to their production of toxic substances (Jenkins, 1964).

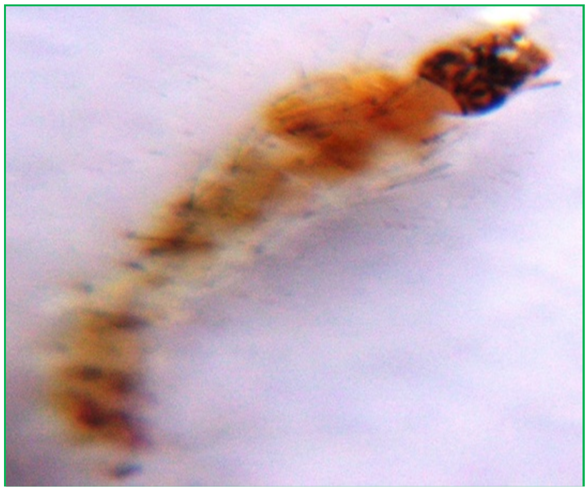

Fig 2: Pseudomonas infection in mosquito larvae

Leptothrixbuccaliscommon water bacterium isolated in fresh and polluted water sources and found to be highly lethal to *An. maculipennis*. With this infection, the larva maintains the disease through eclosion, but death does not occur until sometime after emergence.

Streptococcus species common human fauna,these bacteria can rapidly grow in the warm insectary conditions and will attach to larvae in large numbers. The bacteria invade the integument (insect's hard outer coat) and cause internal damage leading to mortality in the L3 or L4 stage (Kramer, 1964).

1.4 *Significant gaps*:
Problem of controlling malaria is associated with inadequate health infrastructures, deteriorating social and economic conditions, global climate change and mass movement of refugees. The situation has become more complex due to emergence of multi drug resistant strains of parasite.Hence malaria control program rely significantly towards vector control rather than parasite control. Controlling vector is easier, cheap, and ecosafe. Since mosquitoes have become resistant to chemicals and drugs, microorganisms can open a new gate of success to control the important vector of malaria.

2.0 Experimental Protocol:

2.1 *Origin and laboratory maintenance of mosquito colonies*:
Anopheles stephensi mosquitoes reared for several generations at temperature 27±2˚C relative humidity 70±10% and 12-12 light dark regime. Adult mosquito were kept in (30×30×30) cages provided daily cotton pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period females were allowed to make a blood meal, necessary for laying eggs (anautogeny).Plastic cup containing tap water was in the cages for egg laying. The obtained eggs rafts picked up from the plastic dishes. The hatching larvae were provided daily with yeast powder as a diet. This diet was found to be the most preferable for food for the larval development and a well female fecundity.

2.2 *Isolation of bacteria from soil*:
The samples were brought to the laboratory and 1 gm of soil was weighed and transferred aseptically to a vial containing 10 ml of sterile water and kept on a rotary shaker at 100 rpm for 30 min to dislodge the bacterial cells from the soil particles. The supernatant was diluted and 0.1 ml was spread on pre-solidified nutrient agar. The plates were incubated at 30°C for 48h and bacterial colonies, which appeared, were purified on nutrient agar. Each of the purified colonies was then sub-cultured on nutrient slants, allowed to grow for 72h and stored at 4ºC.

3.0 Results and Discussion:

In the present study, the soil is collected from different parts of Udaipur, Rajasthan India., showed the presence of *Bacillus, Staphylococcus,Pseudomonas* and *Enterobacter, Actinobacteria*species. Bacterial groups are identified by Gram Staining, Catalase-Oxidase test and HLA test. Amongst isolated bacterial groups some bacteria were found active against mosquito larvae .From different soil samples we could isolate Bacillus, Staphylococcus, Enterobacter, and Actinobacteria by adopting standered protocol. Almost all bacterial strain depicted larvicidal activity but actinobacterial isolates were potent against anopheline larvae. The present investigation is a first hand observation on the efficacy of bacterial insecticides from soil samples. Further detail studies are being taken up. The efficacy were adjudge on their basis of morphogenetic changes.

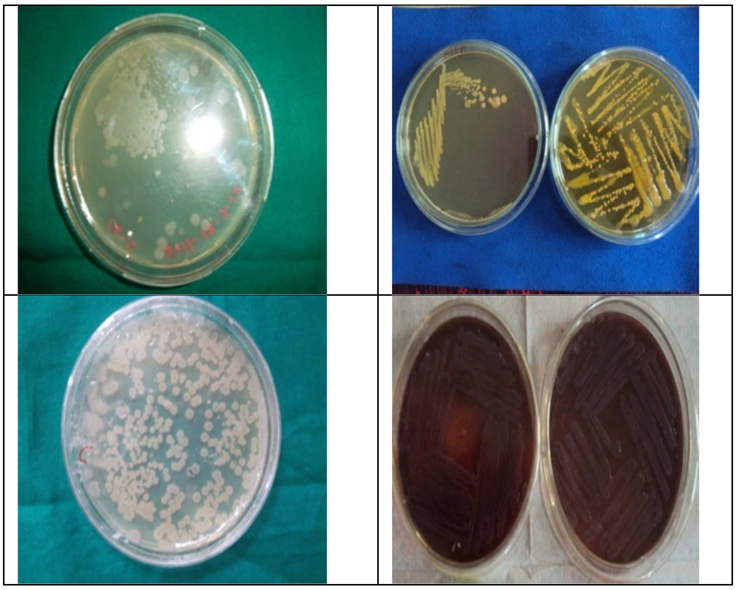

Fig 2: Bacterial Colonies

Table 4: Bacterial fauna found in different types of soil in Udaipur region

S.No	Station	Culture code	Colony appearance	Size	Grams staining	Catalase test	Oxidase test	HLA test	Identified group
1	Garden soil	Gs1	Grey	3	+ve	+ve	+ve	+ve	Bacillus
2	Garden soil	Gs2	Grey	5	+ve	+ve	+ve	+ve	Bacillus
3	Garden soil	Gs3	Light creamy	3	+ve	+ve	+ve	+ve	Bacillus
4	Sewage soil	Ss1	Yellow	4	+ve	+ve	+ve	+ve	Staphylococcus
5	Sewage soil	Ss2	Dark pink	2	-ve	-ve	+ve	+ve	Enterobacter
6	Sewage soil	Ss3	White to grey violet	3	+ve	+ve	+ve	+ve	Bacillus
7	Nursery soil	Ns1	Grey with pink	2	-ve	+ve	+ve	+ve	Pseudomonas
8	Nursery soil	Ns2	Creamy	6	+ve	+ve	+ve	+ve	Actinobacteria
9	Nursery soil	Ns3	White creamy	5	+ve	+ve	+ve	+ve	Bacillus

Natural soil sample is an excellent residence for plentiful microbes have ability to produce secondary metabolites applied in industrial production process and biocontrole activitiesBcteial ha preparaions *B.sphericus* is a spore forming aerobic bacterium several strains of which are pathogenic for mosquito larvae. *B.sphericus* able to produce two crystal proteins 51.4 KDa and 41.9 KDa which were encoded by highly conserved chromosomal genes (Charles *etal*.,1996).previous study proved that *B.thuringiensis* subspecies *israelensis* (*Bti*) and *B.sphericus* (*Bsp*) were entomopathogenic bacteria that have ability to control the larvae of anopheline mosquitoes(Das and Amalraj,1997). Monnerat *et al.,* (2004) found that the effective strains of *B. sphaericus* were isolated from the soil sample against *A. aegypti.* Baumann et al. (1987) had found that cell suspension containing 174 ng (dry weight) of the more toxic recombinant *B. sphaericus* 2362 strain per ml killed 50% of the larvae during sporulation, produce a parasporal crystalline protein which is toxic for the larvae of a number of mosquito species. *B. subtilis* is a ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue. The bacterium produces an endospore that allows it to endure extreme conditions of heat and desiccation in the environment. The culture supernatant of a strain of *B. subtilis* subsp. *subtilis* isolated from mangrove forests was found to kill larval and pupal stages of mosquitoes through their secondary metabolite surfactin (Geetha *et al*., 2010). *B. cereus* also a gram positive, spore forming rod shaped bacteria used for biological control agent widely available in soil environment. *B. cereus* is a natural facultative mosquito pathogen (Chaterjee *et al*., 2008). *B. cereus* strains are able to colonize in the guts of the mosquito larvae (Plearnpis *et al*., 2001). *Acinetobactor sp.* is common soil borne coccoid bacteria that also give 97 ± 5% mortality rates. Sezen, and Demyrbag (2007) found that *Acinetobactor sp*. active against summer cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae) is one of the pests. Furthermore, *Acinetobactor spp.* can be potent for the control of
mosquito larvae. For the basis of these concern we isolate microbial species from soil and explore their larvicidal activity against mosquitoes**.**

4.0 Conclusion:

(1) Mosquito borne diseases are influencing the global economy now due to large scale loss of human health.
(2) Bacterial insecticides are safer easily available and non hazardous insecticides which can be introduced in the mosquito control programme. (Lacey, 2007).
(3) Using entomopathogenic bacteria to control mosquitoes is a promising environmentally friendly alternative to chemical insecticides (Park and Federici, 2009).
(4) Bacteria could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these microbial insecticides in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution.

4.1 *Future Prospects*:

By combining the genes from a variety of micro-organisms, it should ultimately be possible to design smart' bacteria that will seek out and kill larvae of specific vector mosquitoes.Recently, recombinant DNA techniques have been used to improve bacterial insecticide efficacy by markedly increasing the synthesis of mosquitocidal proteins and by enabling new endotoxin combinations from different bacteria to be produced within single strains. Finally, the availability of Bin toxins of B. sphaericus and newly discovered mosquitocidal protein offers the potential for constructing recombinant bacterial insecticides for more effective biopesticides for the biological control of mosquito vectors. Thus, recombinant bacteria show excellent promise for development and use in operational vector control programs.

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