Chapter 2

REVIEW OF LITERATURE

Japanese encephalitis (JE) is one of the lethal viral illnesses in the world, which infects about 68,000 individuals annually and can count up to 10,000 death cases across 20 different nations[1]. According to the National Vector Borne Disease Control Programme (NVBDCP) of the Ministry of Health and Family Welfare, in India, 1,765 to 3,428 incidences and 466 to 707 cases and deaths are reported annually[2]. JE is a zoonotic disease caused by a *Flaviviridae* virus, which propagates in a cyclic pattern in mosquitoes i.e., the primary vector. Pigs and water fowl are also involved in the JE virus (JEV) life cycle[3][4]. The primary participants in the growth cycle of the JEV in most of Asian nations are aquatic birds and *Culex* mosquitoes[5]. JE is a major public health concern and the main cause of arthropod-borne viral encephalitis in several Asian countries, particularly in children. Ardeid birds, like egrets and herons, are part of virus's basic cycle and its transmission and maintenance. Domestic pigs are negatively impacted by either spontaneous abortions of pregnant sows or the infection-related death of piglets in a large JEV transmission amplification cycle. Humans and horses are significant "dead-end" hosts of JEV, which experience clinical illness. This is usually characterized by fever, altered consciousness, mobility difficulties, and behavioral changes. Thus, JE virus spreads through zoonotic cycles, with humans serving as dead end hosts and mosquitoes and various vertebrate animals as transmitting and amplifying hosts. Principal vectors are *Culex tritaeniorhyncus* and *Culex gelidus*[6]. These vectors proliferate in pools with standing water, open ditches, fish ponds, irrigation canals, and rice fields. Nearly three percent of infected mosquitoes typically attack domestic animals and birds[6]. However, occasionally, they may bite healthy individuals, who are unintentional dead end hosts and help spread the JEV infection to other people[2]. Birds and pigs act as amplifying hosts and reservoirs. Following a mosquito bite, the virus replicates in the skin before traveling to local lymph nodes[2]. Local and regional lymph nodes in humans experience the first stages of viral replication following an infection from an infected mosquito[6].

2.1. Epidemiology of JEV

According to epidemiological research on JE infestation, death rates have been high for the past 20 years and JE incidences are on the rise in Southeast Asian nations[7]. From the early 19th century, outbreaks of JE have been documented in Southeast Asian countries such as Vietnam, Japan, Myanmar, Cambodia, Nepal, India, China, Malaysia, Korea, Thailand, and Taiwan[8]. The disease has also been reported in the West, along with Pakistan, and in the north-east and southwest of India, including the east (New Guinea), and in the south (Northern Australia Ar-chipelago)[9]. Eastern and southern Asia's temperate and tropical regions are home to JEV. Its range has spread to South-east Asia and northern Australasia (Indonesia,Cambodia, Thailand, Papua New Guinea, Laos, Malaysia, and marine Siberia) as well as eastern Asia (Japan, Taiwan, Korea, the Philippines, China, and Vietnam and the northern Australia) as well as southern Asia (India, Sri Lanka Bangladesh, Myanmar, Nepal, and Bhutan)[4]. JE is mostly a disease of rural regions, notably in irrigated low land rice fields[10][4]. There is no seasonal trend when encephalitis is endemic and it is evident that, in endemic zones, virus activity and vector density peak from October to December[10]. However, epidemic activity is most prevalent in temperate and subtropical regions in Autumn and Summer[11]. JEV is continuously covering new geographical locations, as evidenced by the JEV sequencing study. The results suggest that JEV is spreading rapidly to new areas in Australia and Papua New Guinea[12]. It has also been noted that JE has rapidly spread to more recent areas of India's northern states[10][13].

There is an increasing trend of JE incidences in tropical and subtropical nations like Cambodia, Laos, Indonesia, India, Bangladesh, and Myanmar because of inadequate infrastructure for specialized surveillance systems, immunization programs, and diagnostic centers, reports on the current state[8]. While, the incidence of JE exhibits a constant and declining trend in nations like China, Japan, Nepal, South Korea, Sri Lanka, and Thailand where immunization programs are implemented and disease surveillance is routinely conducted[14]-[17]. According to the NVBDCP, India alone accounts for an average of 1714 to 6594 JE cases per year[18]. Much recent data of NVBDCP reports an annual average of 1714 to 6594 JE cases in India alone. The World Health Organization's Vaccine-Preventable Communicable Diseases, India reported 1,320 JE cases in the year 2023. The Virus Research Centre (VRC-NIV)

documented early evidence of JEV in 1952, and thereafter the first human transmission was recorded in Vellore, Tamil Nadu in 1955[2]. A major JE outbreak occurred in Bankura District of West Bengal in 1973, which was followed by historic epidemics in 1978 in Gorakhpur, Uttar Pradesh (UP) and Assam[18]. The initial report of JE infestation in Northeastern India was recorded in Assam in 1976[19]. According to Dev et al., (2015)[20], the first known JE epidemic occurred in Lakhimpur, Assam, in 1978. Sporadic outbreaks have become common in Northeastern India[21]. As of right now, study findings and epidemiological data make it abundantly evident that JE infestations significantly inclined in 1991, 2004 and accelerated in 2014. This is supported by multiple severe cases and a rising number of JE-induced deaths in Northeast India[20]-[23]. Growing numbers of JE infestations during the past century throughout the Indian subcontinent, particularly in Northeast India, suggest that JE vectors are almost certain to predominate in both rural and urban regions. Even though there are many reports of JE infestation in rural regions, peri-urban areas are also impacted by sporadic and asynchronous JE outbreaks[8]. NE India is a potential epicenter of JEV dissemination through widespread environmental agents, to the nearby regions and intensifies the load of global viral diseases.

2.2. **Factors affecting JEV transmission**

The environment, wild animals, domestic animals and people are all involved in the intricate JEV transmission cycle. From a conceptual standpoint, multiple factors affect JEV transmission.

2.2.1. Temperature and Rainfall

In addition to variations in illness prevalence and risk, environmental factors have a significant impact on the origin of zoonotic and vector-borne diseases. Since temperature and precipitation have a significant impact on vector density, JEV epidemiology has long only been discussed globally and mostly focused on climatic factors. The two main environmental factors that can affect JEV transmission levels are temperature and precipitation. Rainfall and temperature are believed to have a major effect on JEV transmission by affecting the life history of mosquito vectors, including population abundance and the time it takes for immature mosquito stages (i.e., larvae and pupae) to develop[24]-[29]. Mosquito population density often influences the biting

rate, with higher mosquito populations resulting in larger biting rate potential[30][31]. Furthermore, it has been discovered that temperature positively correlates with the spread of JEV in mosquitoes. Elevated temperatures boost the rate of infection and reduce the extrinsic incubation period, which is the period between an infected blood meal and the mosquito becoming infectious[32]. It is not only temperature that affects mosquito life histories, but also precipitation, which has effects on human and animal health. High temperatures and little rainfall appear to boost the threat of JEV transmission to persons, according to a pioneering Japanese study[25]. This particular mechanism was outlined in the study in this way: (1) high temperature accelerates viral reproduction in the vector, (2) low rainfall and high temperature shortens the developmental period of immature mosquitoes, increasing the vector population, and (3) low precipitation reduces aquatic stage losses due to water currents. Strong correlations among rainfall, temperature, and the incidence of JE cases have also been discovered in more recent research[33]-[37].

2.2.2. Rice Fields harboring JEV

A crucial component of the JEV transmission cycle is the physical aspects of the surrounding environment that have an impact on transmission. Its closeness to irrigated agricultural areas, especially rice fields, is the biggest environmental risk factor for the spread of JEV. Because water birds feed there and mosquitoes develop into larvae, these areas provide an environment that is favorable for the propagation of JEV. Early JE study revealed definite links between proximity to rice fields and JEV transmission[38]. Further research expanded on these conclusions and identified the precise rice farming techniques—such as planting techniques and fertilizer use—that facilitate the spread of JE vectors[39]-[41]. According to Richards et al., (2010)[42] study in Korea, it is found that there is a direct correlation between the percentage of mosquito abundance and the number of JE cases with rice field density. In order to determine risk variables linked to JE infection, a case-control study was carried out in Bali, Indonesia. The results showed that JE cases had a 2.93 times higher likelihood of residing 100 meters or less from rice fields than controls[43]. A systematic assessment has assessed how rice irrigation affects the global spread of JEVs[12]. In a review study, in rice production for Asia from 1963 to 2003, on the area of irrigated rice land, and estimates of the population-at-risk size provided by rural and irrigated land, it was found that

roughly 1.9 billion people live in earthquake-prone rural areas worldwide, 220 million people live close to rice irrigation operations [12]. According to a follow-up review, countries like Cambodia, Bangladesh, Indonesia, Laos, North Korea, Pakistan, and Myanmar, that were expected to experience amplifies in transmission of JEV included those that had increased pig rearing along with intensified rice farming, population growth, and a lack of vaccination and surveillance programs[8].

2.2.3. Mosquito as Vectors of Transmission

The primary risk factors for mosquito-borne diseases are the presence and quantity of virus-carrying mosquito vectors; hence, the lack of mosquito vectors stops the spread of pathogens carried by mosquitoes. Therefore, it is critical that mosquito vectors are correctly implicated as disease pathogen vectors, as this has implications for comprehending patterns of transmission and management. In order to ascertain a vector's involvement in the spread of a pathogen, it is necessary to verify the vector's abundance, biting behavior, and competence[44]. Numerous mosquito vectors have been found to be associated with the spread of JEV. Still, owing to its high abundance, its biting adaptation, its capability to transmit JEV, and proximity of its preferred larval development sites to reservoir hosts, *Culex tritaeniorhynchus* is generally established as the major vector for transmission of JEV all over the Asian region[45][46]. In addition to *Culex tritaeniorhynchus*, other vector populations may function as regional or secondary vectors, or they may be the primary vectors of JEV transmission[47][48]. For instance, *Culex annulirostris* is identified as the primary vector of transmission in Australia[2], Simpson et al., (1976)[49] identified *Culex gelidus* as a major vector of JEV transmission in pigs in Malaysia, with "spill-over" transmission to human and equine hosts, and Arunachalam et al., (2004)[47] identified *Mansonia* spp. as significant secondary vectors in India. Other mosquitoes have also been linked to the transmission of the disease[46]-[51]. Since the most of JEV vectors are opportunistic blood feeders, the feeding behaviors of these vectors are determined by host availability. But main feeding habit displayed by JE vectors is zoophilic (eating on animals). According to multiple studies[52]-[55], these mosquitoes preferably feed on pigs, cattle, and goats. They also show relatively modest feeding rates on people and birds[53]. Nonetheless, opportunistic eating behavior, complete abundance, and the JE vectors' preferred larval development habitats encourage the spread of JEV from birds to other mammals.

Anthropogenic activities have been proposed as a major way for humans to become exposed to JEV. For instance, it has been demonstrated that sleeping outside more often at night during hot seasons increases the risk of JE transmission by JE vector bites[53]. Khan et al., (1996[54]) and Schultz and Hayes, (1993)[55][56] reported that *Culex tritaeniorhynchus* bites more often at night. Peak biting behavior, however, varies and can be either bimodal, as shown in Thailand and Japan[57][58] or generally consistent, as shown in Malaysia[59]. Like other JE vectors, *Culex tritaeniorhynchus* primarily feeds and rests outdoors, as do *Culex annulus*, *Culex vishnui*, and *Culex pseudovishnui*[60][52][61]. However, according to Kanojia and Geevarghese, 2004[61] certain populations might engage in indoor resting behavior. In fact, even indoors, interaction with human hosts may become substantial due to the high concentration of JE vectors present during JE epidemic seasons[52]. The choice of vector control techniques is affected by these phenomena. For instance, an investigation conducted in Pakistan discovered that applying organophosphorous insecticides to homes and cattle sheds was ineffectual against mosquito species that exhibited exophilic (i.e., partially or totally ecologically independent of human) resting habits [62]. There has been an assessment of mosquito vectors' JEV competency[51][52].

There have been reports of JEV transmission efficiencies varying from high to low for various mosquito species. The transmission dynamics of *Culex tritaeniorhynchus* are well-documented because it is thought to be the primary vector[63][64][32]. Depending on the ambient temperature, *Culex tritaeniorhynchus* can spread JEV as early as five days after infection in laboratory experiments including Japanese encephalitis. Highly skilled vectors, such *Culex tritaeniorhychus*, can contract the virus at low concentrations; between 101.0 and 3.5 suckling mouse intracerebral (SMIC) LD50 (lethal dose 50%)/0.03 ml of blood are the range at which this can happen. As much as 104.2 SMIC-LD50/1 ml of saliva and viral diluents can be found in the virus concentration in mosquito saliva[32]. In temperate regions, hibernating adult female infected *Culex sp.* or *Aedes sp.* at hibernating egg stages are believed to contribute to the continuation of JEV during non-transmission seasons. The first phase in the overwintering process is assumed to involve upright JEV transmission in mosquito vectors. A female mosquito becomes a carrier of the virus through biting and transfers the virus to her offspring, this is known as vertical transmission[50].

An overwintering-infected newly emerged female mosquito, mates and feeds for sugar to build up fat bodies, then goes straight into hibernation in resting sites without taking a blood meal during winter periods[50]. These winter phases are characterized by reduced period of light and harsh temperature drops. Infected post-overwintering females may re-initiate transmission if they consume a blood meal in the spring and summer. On the other hand, laboratory research has shown that egg stages of *Aedes albopictus* and *Aedes togoi*, that carry the virus may harbor the virus for two months at room temperature, which results in infected larval stages. The offspring may transmit this virus to more hosts. Venereal transmission, which occurs when female mosquitoes contract the virus from male mosquitoes carrying it, has also been demonstrated to be effective and may help explain the virus's survival. However these topics are receiving less attention[67]. Though intriguing, the above suggested method of transmission of JEV in mosquito vectors raises some fundamental questions about whether such mechanisms are enough to sustain virus transmission in the wild[50].

2.2.4. **Immunization against JE**

One economical method for preventing and controlling JE is immunization. Global reports have shown that endemic areas where high immunization rates are implemented have decreased JE incidence rates. The three most significant JE vaccine types currently being used are: mouse brain derived, purified, inactivated vaccine derived from the Beijing or Nakayama strains of the JE virus; inactivated vaccine obtained from cell culture based on the Beijing P3 strain; and live attenuated vaccine derived from cell culture based on the SA 14-14-2 strain of the JE virus [66]. Chemically inactivated vaccines (INV) and a live attenuated vaccine (LAV) are the two vaccines against JE that are currently on the market. BIKEN's mouse brain-derived INV, which has been used globally since the 1960s, was the only vaccination to receive international approval. However, there were reports of serious side effects, such as acute disseminated encephalomyelitis (ADEM), in recipients of the vaccination. Vero cell-derived INVs made by BIKEN (Japan) and Intercell (Austria) were licensed in the beginning of 2009 and since 2014, JENVAC was produced and licensed in India[9]. While these INVs are beneficial in industrialized markets, their requirement for repeated doses to induce long-lasting immunity makes them unsuitable for widespread vaccination campaigns in many endemic nations. Thus, LAVs are a practical substitute

and been employed for many years in Asian countries including China, however, their production methods are not approved in other markets, which is the drawback to this vaccine[67]. INVs and LAV are potentially effective against flaviviral disease, as well as they are cost-effective also, but sometimes they may not be suitably effective in immune compromised patients. INVs are safer, but are more expensive to produce and less potent. Hence there is an immense need of devising improved products. Therefore, there is an urgent requirement to develop innovative and enhanced products. In addition to boosting immune responses, type I IFNs are essential for limiting infections caused by harmful viruses. As a result, their influence on the efficacy of live-attenuated vaccines entails striking a equilibrium between promoting the increase of adaptive immune responses and reducing viral antigen expression. The impact of type I IFNs on such factors has been investigated subsequent to vaccination with the single-cycle flavivirus vaccine RepliVAX WN (SCFV). Mice inoculated with Repli-172 Encephalitis VAX WN generated IFN-α and showed enhanced transcription of IFN-stimulated genes[68]. There are several vaccinations available to prevent JE, but they all have drawbacks. However, a novel flavivirus vaccine called RepliVAX WN, which is a pseudo-infectious virus that conveyes the JEV prM and E proteins, can prevent flaviviral disease. Second generation engineered RepliVAX (RepliVAX JE.2) gave experimental mice complete protection against a lethal challenge with JEV and produced neutralizing anti-bodies[69]. Even though there has been a licensed vaccination to prevent JE for more than 40 years, each year over 20,000 cases are reported, of which 6,000 cases ending in death[6] while, in India, between 2017 and 2021, there was an markedly elevated rate of JEV infection among local residents, with almost 2500 cases per year in 2019[9]. Regretfully, the likelihood of JE is also likely to be far higher than re-ported because of gaps in surveillance[6]. There are several vaccines available to prevent JE, and throughout their history, they have proven to be incredibly effective. The INV made from mouse brains infected with JEV is the vaccination that has been in use the longest. This vaccine, whose protective efficacy is estimated to be 80–90% in JEV endemic regions, has been used extensively in East Asia to manage JE since the 1960s and is frequently used globally to immunize visitors to endemic areas[6]. However, there is still a problem to the product: in order to create protective immunity, it requires a three-dose vaccination regimen. Additionally, booster shots are advised every two to three years, which can be costly and time-consuming for patients from low-income countries. However its license was terminated in 2005 as among those

receiving the vaccine, have been known to induce allergic reactions and more serious side effects include complications, such as acute disseminated encephalomyelitis and other severe neurological disorders[9][70]. In China, Japan, Taiwan, and Korea, JE has been successfully controlled and eradicated with vaccination for the past five years[71]-[73]. Additionally, genes encoding prM and E proteins are being packed onto vectors to create second generation recombinant vaccines. Clinical trials are underway for DNA-based JEV vaccines, which show promise as effective vaccinations against the virus. When administered intra-cerebrally to JE-infected mice, DNA zymes virtually entirely cleave the RNA sequence of the 3'-NCR of the JEV genome[74], preventing the virus from replicating in the brain. Neutralizing bodies may be used to aid in the vaccine design process.

2.3 **AWDI as a tool for mosquito control**

Since vaccination rates are very low in vector-endemic rural areas with limited government accessibility, integrated approaches may prove advantageous. In addition to being environmentally beneficial, smart agricultural technologies such as alternate wet and dry irrigation can lower the prevalence of vector-borne diseases in their natural habitat. To implement realistically, evaluation of such technique is crucial. According to Van der et al., (2001)[75], AWDI has been found to be successful in reducing the spread of some water-borne diseases in places where paddy fields serve as mosquito breeding grounds[75].

The presence of water bodies, high temperatures, humidity, and irrigation techniques used in paddy agriculture all have a significant influence on the number of mosquitoes that spread disease[76]. Larval growth is also significantly regulated by the cultivars' tillering tendencies and the characteristics of the standing water, such as temperature, dissolved oxygen, ammoniacal-N, and nitrate-N[41]. The AWDI process's repeated drying of rice fields, which keeps the water column at a lower level, might be partly responsible for the decline in the number of immature mosquitoes. Intermittent irrigation has been studied as a potential strategy for malaria control in a number of experimental experiments dating back to the l920s, conducted in various ecological and epidemiological situations. The primary idea is to periodically restrict the rice field of water in order to disrupt the mosquito's reproductive cycle. Multiple mosquito life cycles can be completed in traditional rice fields because they are kept flooded for the

duration of the cropping season—and in some cases, for up to two or three crops[77]. Antoine, (1936)[79] stated that intermittent irrigation resulted in a complete reduction of larvae. Antoine, (1936)[79] and Mutero et al., (2000)[80] reported 9% to 48% reduction of *Anopheles arabiensis* in Mwea, Kenya. In a study conducted in Portugal, Hill, (1941)[78] reported that intermittent watering resulted in an 80% reduction of *An. Atroparvus*[78]. Among different species of JE vectors, *Culex tritaeniorrhynchus, Culex vishnui* and *Culex pseudovishnui* are identified as major vector in JE transmission[81]. Report shows that these vectors prefer to breed mainly in paddy field[82]. Rajendran et al., (1995)[83] found to reduce the growth of mosquito larvae *Culex tritaeniorrhynchus* by 75% to 88% with 4% increase of yield by applying 3–5 days of interval in irrigation. Similar result was reported by Lu, (1988)[84], in *Culex tritaeniorrhynchus*. They found that intermittent irrigation of 5 days interval can reduce the JE vector by 81% −91% with 13% increase in yield with 50% reduced water input[84]. However, little information is available on the time length of survival of pupae on dried soil. There are reports which indicates that soil with 20% moisture content can act as an antagonistic environment for growth and development of mosquito larvae and due to shortening of life span, larvae become unable to complete their life cycle to attain maturity[86]. It is established that AWDI with several days of intervals in water input in the rice field can reduce the production of *Culex tritaeniorrhynchus*[85][86] found that AWDI together with drying of field in the mid of the season was able to reduce the fourth instars larvae by 14.3%–48.2%[77]. However, because it requires well-managed irrigation infrastructure and farmers are unwilling to alter their agricultural methods, AWDI is not a common practice in India[41].

Table 2.1: Irrigation levels influencing the growth and development of JE vector larvae[8]

Place	Vector	Irrigation interval	Change in mosquito larvae	Change in grain yield	Reference
	An atroparvus	10 days wet and 7 days dry	-80	+6 to+8	Hill and Carmbourance,1941
Henan, China	*Ansinesis* and *Cxtritaeniorrhynchus*	3 to 5 days irrigation interval	-81 to -91	+13	Lu Baolin, 1988

Tamil Nadu, India	*Cxtritaeniorrhynchus*	3 to 5 days irrigation interval	-75 to -88	0 to +4	Rajendran et al.,1995
Mwea, Kenya	*An arabiensis*	3 days wet	-9to -48	-2 to -9	Mutero et al., 2000
Mwea, Kenya	*An arabiensis*	3 days wet	-9to -48	-2 to -9	Mutero et al., 2000

The loads of mosquito larvae and their development, in its breeding habitat, are found to be influenced by the physico-chemical parameters of water. To study the factors influencing growth and development of larvae, detailed study was conducted in rice fields in Tamil Nadu, India, by Sunish and Reuben, (2001)[41] and Rao, (1984)[87]. For easy understanding, parameters like water temperature, plant height, water column, pH, alkalinity, acidity, dissolve oxygen, ammonium nitrogen, sulphate, phosphate, and nitrate nitrogen were divided into three separate groups. At the end of their study, they concluded that height of paddy inversely affects the rate of growth and maturity of larvae by reducing the reach of sunlight and exerting cooling effect which also effects the growth of microorganisms on which mosquito larvae feed. On the other hand, height of water column directly influenced the immature population, as water column increases the immature larval population also increases and vice versa[88]. Senior and White, (1991)[88] in their study on influence of physical factors in mosquito ecology, pointed out that *Culex tritaenorrhynchus* larvae was abundant in high oxygenated water. On the Instead, low oxygenated habitat is preferred by *Culex vishnui*[89]. Cates, (1968)[90] in his study on effect of improved rice farming techniques on mosquito populations in central Taiwan in 1968, reported that *Culex vishnui* were found in abundance in the habitat with pH 8.6 while *Cx tritaenorrhynchus* prefer it's habitat with pH. 8.6 while *Culex tritaenorrhynchus* prefer it's habitat with pH<6. Takagi et al., (1997)[86] also reported that abundance *Culex tritaeniorrhynchus* larvae in habitat with lower pH value. Ikemoto and Sakaki, (1976)[87] in their study found that increase in ammonium nitrogen increases the larval abundance in rice field. Inorganic nitrogenous fertilizer increases the abundance of *Culex vishnui* in rice field whereas organic fertilizer lowers the breeding of *Culex vishnui*. Ammonium nitrogen attracts the adult mosquito for oviposition. However to best of our knowledge no authentic research evidence is available on change of water and soil environment due to application

AWDI in rice field. Hence it would be novel and interesting to appreciate the role of AWDI technique in regulating je mosquito larval growth.

A thorough analysis of AWDI will assist in creating baseline data for technical advancements in agricultural research for upcoming projects. It will also inspire policymakers to support research and development in the areas of vector-borne disease management and water management in paddy cultivation.

Table 2.2: Role of physicochemical variables in moderating JE vector propagation[91]

Physicochemical parameter	Effect on larvae	Possible explanation	Reference
Plant height	Decline in mosquito larva with immature plants showing increased height	Decline growth of micro-organism on which larva feeds, due to paucity of sunlight and decreased temperature	Rao, 1984
Maturation of paddy	Decrease in larva counts with maturation of paddy	Obstructing oviposition for *Cx vishnui*	Reuben, 1971
Temperature	Larva increases with increase in temperature	Insects are cold blooded, thus a minor change in temperature may have a potentially large biological effect on disease transmission	Sunish and Reuben,2001
pH	Larva increases with increase in pH	NA	Roger 1987, Cats 1968, Takagi,1997
Dissolve oxygen	Larva increases with increase in DO, however it depends on plant's maturity and species of mosquito	NA	Roger 1987, Cats 1968, Takagi,1997
Hardness	No influence	NA	Sunish and Reuben 2001
Salinity	No influence	NA	Sunish and Reuben 2001

Ammonium Nitrogen	Positive influence depending on species	Increases micro-organism on which the larvae feeds	Ikemoto and Sakaki, 1979
		Oviposition attractant of the JE vectors	Victor and Reuben,2000

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